

Validation of selected body fatness methods against the deuterium technique in 6-8 year old black South Africans: The BC-IT Study

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DECLARATION

Prof. MA Monyeki (Promoter of the study and co-author), and Prof HS Kruger (co-promoter and co-author) and Prof Prof. JJ Reilly hereby give permission to the candidate, Ms TL Moeng-Mahlangu, to include the articles as part of this doctoral thesis. The contribution of each co-author, both supervisory and supportive were kept within reasonable limits and included:

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This thesis serves in the fulfilment of the requirements for the PhD degree in Health Science-Human Movement Sciences within the Physical Activity, Sport and Recreation research entity within the faculty of Health Sciences at the North-West University on the Potchefstroom campus.

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SUMMARY

Various scholars report overweight and obesity as major public health problems. Though an increase in obesity, especially among children, has been observed over decades, accurate body composition measurements remain a challenge. Despite the detected increase in obesity, methods to accurately predict body fat mass (FM), fat-free mass (FFM), and total body water (TBW) in children are limited. Body mass index (BMI), and body size perception is another measure of obesity. Therefore, complex measures such as bioelectrical impedance, deuterium dilution, under water weighing (UWW) and dual energy x-ray absorptiometry (DXA) are used for accurate predictions. The first aim of the thesis was to develop and validate the prediction equation for estimating FFM and TBW determined by available bioelectrical impedance analysis (BIA) prediction equations compared to deuterium dilution method (DDM). Secondly, the research aimed to assess the level of agreement between body image perception and actual body weight determined by body mass index (BMI) z-score and %BF measured by the (DDM). The third aim was to assess the level of agreement between percentage body fat (%BF) measured by BIA and skinfold thickness in South African children.

A sub-study that followed a cross-sectional design on 299 children (predominately from the Tswana and Sotho ethnic groups) from the Body Composition using Isotopes Techniques study (BC-IT study) from the Dr JB Marks municipality (previously known as Tlokwe) of the North-West Province, South Africa. BMI z-scores were calculated relative to World Health Organization (WHO) reference data. The stable isotopes used the International Atomic Energy Agency (IAEA) protocol to analyse saliva samples using fourier transform infrared (FTIR) spectroscopy (FTIR 4500t spectrophotometer, Agilent), and BIA (Bodystat 1500®MDD) for TBW, FFM, % BF, and fat mass (FM). Body image perception was assessed by a simple questionnaire and silhouettes from Stunkard Figure rating scales (1983). Statistical analyses were performed using the statistical package for social sciences (SPSS v26.0). Paired t-test was used to determine the limits of agreement between %BF, FFM, and TBW obtained from BIA and the selected prediction equations, multiple regressions and Bland-Altman plots computed. Cohen kappa's statistic (κ) determined the magnitude of agreement between perceived and actual body size. Kendall's tau-c assessed concordance among the children's body image perception silhouette choice, BMI, and %BF determined by DDM. The level of significance was set at $p \leq 0.05$.

The results from this thesis show that some published BIA equations for TBW and FFM validated in the study demonstrated under- or over-estimations. The newly developed South African equation had higher accuracy for both TBW and FFM prediction. When the newly developed and validated BIA prediction equations were plotted on Bland Altman plots, the results showed a good level of agreement between TBW and FFM against the criterion of the DDM.

When the level of agreement was assessed between the DDM and Silhouettes, the results indicated discrepancies between fatness based on body image perception compared to the BMI z-scores classification and DDM measurement. A sample of 202 children, (32.2%, 55.1%, 8.8%, and 2.4%), perceived their body size as underweight, normal, overweight, and obese, respectively. Based on the BMI z-score, (18.8%, 72.8%, 6.9%, and 1.5%) were classified as underweight, normal, overweight, and obese, respectively. Body fatness measurement through the DDM showed that (2.5%, 48.0%, 21.8%, and 29.7%) were underweight, normal weight, overweight, and obese, respectively. The level of agreement between body size perception, body fatness, and BMI z-score was poor ($k=0.040$, $p=0.450$).

Levels of agreement differed between the BIA measurement and the three prediction equations. Significant mean differences ($p<0.001$) were found in the predicted %BF means determined by BIA, (Slaughter, Wickramasinghe and Dezenberg). The standard for evaluating prediction errors (SEE) was above 5. The magnitude of the regression lines varied from low to moderate ($r^2 = 0.46-0.61$).

The conclusion drawn from this study was that significant discrepancies between the various measurements compared to the criterion method were observed. Most of the prediction equations validated were not suitable for the sample of 6- to 8-year-old South African children. The newly developed equation demonstrates the potential for practical and accurate estimation of TBW and FFM for use in South African children aged 6- to 8-years. Still, prediction equations probably need to be population specific.

Keywords: body composition, body image perception, childhood obesity, deuterium dilution, prediction equations.

OPSOMMING

Obesiteit en oorgewig word deur verskeie '*scholars*' as groot openbare gesondheidsprobleme gerapporteer. Alhoewel toename in obesiteit veral tussen kinders gedurende dekades waargeneem is, bly akkurate metings van liggaamsamestelling 'n uitdaging. Ten spyte van hierdie waarneming is metodes om liggaamsvetmassa (LVM), vetvrye massa (VVM) en totale liggaamswater (TLW) akkuraat by kinders te voorspel beperk. Liggaams-massa-indeks (LMI) en persepsie van liggaamsgrootte is 'n ander maatstaf vir obesiteit. Daarom word komplekse metings soos bio-elektriese impedansie (BIA), deuteriumverdunning en ander gebruik vir akkurate voorspellings. Die eerste doelstelling van die tesis, was om die voorspellingsvergelyking vir die berekening van VVM en TLW te ontwikkel en te valideer, deur beskikbare bio-elektriese impedansie-analise (BIA) voorspellingsvergelykings en te vergelyk met Deuteriumverdunningsmetode (DVM). Tweedens, het die navorsing wat aangebied word gepoog om die vlak van ooreenstemming te bepaal tussen liggaamsgrootte selfpersepsie (liggaamsbeeldpersepsie) en die werklike liggaamsmassa bereken deur liggaamsmassa-indeks (LMI) z-telling en liggaamsvet (LV) gemeet aan die DVM. Die derde doelstelling was om die vlak van ooreenkoms tussen liggaamsvet persentasie (%LV) deur BIA en velvoudikte by Suid-Afrikaanse kinders te bepaal.

'n Substudie wat volg op 'n dwarsdeursnee-ontwerp op 299 kinders (hoofsaaklik van die Tswana en Sotho etniese groepe) uit die liggaamsamestelling met behulp van isotope-tegnieke studie (BC-IT-studie) van die Dr. JB Marks-munisipaliteit (voorheen bekend as Tlokwe) van die Noordwes-provinsie, Suid-Afrika. LMI z-tellings was bereken in verhouding tot die Wêreldgesondheidsorganisasie (WGO) verwysingsdata. Die stabiele isotope het die Internasionale Atomiese Energie Agentskap (IAEA) protokol gebruik om speekselmonsters te analiseer met behulp van Fourier transforme infrarooi (FTIR) spektroskopie (FTIR 4500t spektrofotometer, Agilent), en BIA (Bodystat 1500®MDD) vir TLW, VVM, %LV, en VM. Die liggaamsbeeldpersepsie was deur 'n eenvoudige vraelys en silhoeëtte van die "Stunkard Figure Ratings" skaal bepaal. Statistiese ontledings was met behulp van die Statistiese Pakket vir Sosiale Wetenskappe uitgevoer (SPSS v26.0). Gepaarde t-toets is gebruik om die omvang van ooreenkoms tussen %LV, VVM en TLW verkry van BIA en die geselekteerde voorspellingsvergelykings te bepaal, en meervoudige regressies en Bland-Altman-plotte te bereken. Cohen kappa's statistiek (κ) was gebruik om die bepaal van ooreenkoms tussen liggaamsbeeldpersepsie en werklike liggaamsgrootte te bereken. Kendall's tau-c het die ooreenstemming bepaal tussen die kinders se liggaamsbeeldpersepsie-silhoeëtkeuse, LMI en % BF deur DVM bereken. Die betekenisvlak is op $p \leq 0,05$ gestel.

Die resultate van hierdie tesis het aangedui dat sommige gepubliseerde BIA-vergelykings vir TLW en VVM wat in die studie gevalideer is, onder- of oorskating getoon het. Die nuut

ontwikkelde Suid-Afrikaanse vergelyking het hoër akkuraatheid vir beide TLW en VVM-voorspelling gehad. Toe die nuut ontwikkelde en gevalideerde BIA-voorspellingsvergelykings geplot was op “Bland Altman-plots”, het die resultate 'n goeie bepaal van ooreenkoms tussen TLW en VVM teenoor die kriterietegniek van DVM getoon.

Toe die vlak van ooreenstemming tussen die DVM en die silhoeëtte bepaal was, dui die resultate op teenstrydighede tussen vetheid gebaseer op die liggaamsbeeldpersepsie in vergelyking met die LMI z-tellings klassifikasie en DVM meting. 'n Steekproef van 202 kinders het (32,2%, 55,1%, 8,8% en 2,4%) hul liggaamsgrootte onderskeidelik as ondergewig, normaal, oorgewig en obees beskou. Op grond van die LMI z-telling is (18,8%, 72,8%, 6,9% en 1,5%) onderskeidelik as ondergewig, normaal, oorgewig en obees geklassifiseer. Meting van liggaamsvetheid (DDM) het getoon dat 2,5%, 48,0%, 21,8% en 29,7% onderskeidelik ondergewig, normale gewig, oorgewig en obees was. Die vlak van ooreenkoms tussen liggaamsbeeldpersepsie, liggaamsvetheid en LMI z-telling was swak ($k=0.040$, $p=0.450$).

Die vlak van ooreenkoms tussen die BIA-meting het verskil in die drie voorspellingsvergelykings. Statisties-betekenisvolle verskille in gemiddeldes ($p<0.001$) is gevind in die voorspelde % LV-gemiddelde wat deur BIA (Slaughter, Wickramasinghe en Dezenberg) bepaal is. Die standaardde vir die evaluering van voorspellingsfoute (SEE) was bo fyf. Die grootte van die regressielyne het gewissel van laag tot matig ($r^2 = 0,46-0,61$).

Die gevolgtrekking uit hierdie studie was dat betekenisvolle verskille tussen die verskeie metings in vergelyking met die kriteriummetode waargeneem is. Die meeste voorspellingsvergelykings wat gevalideer is, was nie geskik vir die steekproef van 6-tot 8-jarige Suid-Afrikaanse kinders nie. Die nuut ontwikkelde vergelyking toon potensiaal vir praktiese en akkurate beraming van TLW en VVM vir gebruik in Suid-Afrikaanse kinders van 6-tot 8-jaar. Voorspellingsvergelykings moet waarskynlik populasie-spesifiek wees.

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LIST OF ABBREVIATIONS

%	Percentage
%BF	Percentage body fat
2-C	Two-Component
3-C	Three-Component
4-C	Four-Component
ADP	Air displacement plethysmography
AR	Adipose rebound
BC-IT	Body composition isotope
BD	Body density
BF	Body fat
BIA	Bioelectrical impedance analysis
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
CVD	Cardiovascular diseases
DDM	Deuterium dilution method
DXA/DEXA	Dual-energy X-ray absorptiometry
ECF	Extra-cellular fluid
ECS	Extra-cellular solids
FFM	Fat-free mass
FITR	Fourier transform infrared spectrometry
FM	Fat mass

IAEA	International Atomic Energy Agency
ICF	Intra-cellular fluid
ICS	Intra-cellular solids
IRMS	Isotope ratio mass spectrometry
LoA	Level of agreement
NCD's	Non-communicable diseases
NDP	National Development Plan
<i>P</i>	Significance level
PAQ-C	Physical Activity Questionnaire for older Children
R	Bioelectrical resistance
r^2	Coefficient of determination
RLV	Residual lung volume
SEE	Standard for evaluating prediction errors
SFT	Skinfold thickness
TBW	Total body water
UNICEF	United Nations Children's Fund
UNSCN	United Nations System Standing Committee on Nutrition
UWW	Underwater weighing
WC	Waist Circumference
WHO	World Health Organisation
WHR	Waist-to-hip ratio
WHtR	Waist-to-height ratio

CHAPTER 1: INTRODUCTION

1.1 Introduction

In 2016, the World Health Organization (WHO) estimated that globally, 340 million children and adolescents were overweight or obese (WHO, 2020a). In 43 years, the prevalence among children and adolescents rose from 4% (1975) to 18% (2018). An analysis of 450 studies from 119 countries by de Onis and colleagues in 2010 estimated that there were already 43 million preschool children overweight or obese globally. However, the figure differs from the WHO estimate of 40 million overweight or obese under-fives reported in 2018. The difference could be due to the age differences, which were not specified in the 2018 report; pre-school could include children aged 5- to 6 years whilst the 2016 WHO report specifies the age group as under five-year-old children. A quarter of obese children lived in Africa, and nearly half lived in Asia (de Onis, 2010:1259; WHO/UNICEF/World Bank group, 2018:3). Overweight among African children has increased by almost 50% since 2000 (WHO, 2020a). It is further estimated that at least 2.8 million (WHO, 2017), even 3.4 million people (Biadgilign *et al.*, 2017:1) globally die annually as a result of overweight or obesity. Of great concern was the projection that globally by 2020, 9% or 60 million children under-fives will be obese (Wang, 2012:176). However, the projection was an overestimation since the 2021 WHO, Obesity fact sheet reported that 39 million children under the age of 5 were overweight or obese in 2020 (WHO, 2021). This prediction demonstrates an upward trend, compared to the 4.2% reported in 1990 and 6.7% in 2010 (de Onis *et al.*, 2010:1259).

South Africa is experiencing similar trends where the prevalence of obesity among children aged 2-5 years increased from 10.6% reported in 2005 (Department of Health, 2005:255) to 16.5% reported in 2012 (Shisana *et al.*, 2012:205). The 2016, South African Demographic Health Survey (SADHS) reported 13% of children under age five were overweight (Department of Health, 2016:179). The South African studies are also not comparable to earlier ones since the age categories are not the same. A systematic review by Adom *et al.* (2016:488) also reported an urban-rural bias in South Africa, whereby overweight and obesity was higher among urban than rural children.

The majority of studies conducted to assess overweight and obesity were primarily based on BMI measure. Such studies include, a literature review of 126 studies aimed at examining various obesity measurement and classification methods 63% of these studies used BMI to determine overweight and obesity, and 22% used methods that have been validated against the cadaver as a direct measure to assess body fatness (Sangachin *et al.*, 2018:4). Similarly, studies conducted in South Africa have determined the prevalence of obesity based mainly on BMI measures (Mamabolo *et al.*, 2014; Monyeki *et al.*, 2007:553; Monyeki *et al.*, 2009:11; Monyeki *et al.*, 2012:1;

Shisana *et al.*, 2012:204) with limited data on 'gold standard' methods (Hussain *et al.*, 2014:2; Nightingale, 2013:2; Sweeting, 2007:3). It should be emphasised that the overweight and obesity prevalence, as reflected earlier determined by BMI measure, does not provide an accurate estimate of body fatness (Bhurosy & Jeewon, 2013:72; Freeman & Sherry, 2009:23; Okorodudu *et al.*, 2010:976;) as it only reflects excess weight rather than excess body fatness. The accuracy of BMI measurement varies according to the degree of body fatness and age. Even though some authors have described BMI as a surrogate measure, there is evidence that children who have a BMI above 95th percentile do have excess fat (Freeman & Sherry, 2009:S23).

Over the years, various body composition measuring techniques have been tested and validated. Body image as a measure of fatness is gradually gaining ground, with many researchers regard it as an objective measure. Body image is viewed as a better predictor for weight management leading to positive weight-related behaviours compared to BMI and other objective measures of weight (Brenner *et al.*, 2010:2; Lemon *et al.*, 2009:90; Mchiza *et al.*, 2015:2; Pasch *et al.*, 2016:1842; Puoane *et al.*, 2010:29; Wang *et al.*, 2009:4; Xu *et al.*, 2018:1). The development of body image concerns starts as early as in preschool age (León *et al.*, 2018) and around age eight years (Birbeck & Drummond, 2006:424; Dohnt & Tiggemann, 2005:103; Knafo, 2016:4).

1.2 Problem statement

Childhood obesity has been linked to various childhood health problems, including elevated blood pressure, high cholesterol, both being risk factors for cardiovascular disease (CVD) later in life (Must & Strauss, 1999:54), impaired glucose tolerance, insulin resistance and type 2 diabetes (Caprio, 2002:487). Sleep apnoea has been identified as a major problem (Arens & Muzumdar, 2010; Mofid, 2014). Psycho-social issues such as anxiety and depression, low self-esteem have been reported among overweight children and adults (Cash, 2002; Monyeki *et al.*, 2009:12; Smolak, 2002:212; Yang *et al.*, 2017:400).

Various scholars have reported obesity and overweight to increase health risk; however, in some ethnic groups, health risks are markedly higher at any given BMI (Deurenberg *et al.*, 2002:141). Some scholars have reported that high blood pressure is more common among Blacks in a Western population, and diabetes and cardiovascular diseases are more prevalent among Indians (Teh *et al.*, 2014:1) irrespective of the BMI level. In a study by Erye and colleagues (2017:3), it was reported that South Asians had higher total body fat than white Europeans based on Bioelectrical impedance Analysis (BIA) and skinfold thickness measures. Some authors have reported lower body fat and higher lean muscle mass among blacks as compared to Caucasians at the same BMI, indicating that some ethnic groups may have a lower risk of obesity-related diseases (Aloia *et al.*, 1999:114; Rush *et al.*, 2006:1130). The importance of fatness as a measure of health risks requires an increased focus on measuring fatness per se rather than the previous focus which was just on weight.

Despite the recognised importance of measuring body composition, there are limited methods used in children in both clinical and field settings (Heyward & Stolarczyk, 1996:3). The challenge of measuring body composition has been reported as far back as the early 1940's (Behnke, 1942:451) and continues to be recognised as such (Silva *et al.*, 2013:1). Given the challenges or limitations of BMI in determining body fatness, and the increase in childhood obesity, the ability to accurately measure body fat is consequently critical (Silva *et al.*, 2013:1). The association between excess body fat and various chronic diseases of lifestyle is well-established (Steinberger *et al.*, 2005:1346; Pribyl *et al.*, 2011). Accurate measures of body composition are required to assess health and nutrition status, e.g., metabolic rate, physical fitness and fluid requirements (Goran, 1998:505; Wang *et al.*, 1992:19). These should be obtained from reliable models based on statistically sound research methods (Jackson *et al.*, 2013:S77; Silva *et al.*, 2013:476).

There have been various ventures to establish methods to measure body composition accurately. Some of the currently used methods include densitometry, dual-energy X-ray absorptiometry (DXA), air displacement plethysmography and bioelectrical impedance analysis (BIA). The methods used vary in precision and accuracy (Fields & Goran, 2000:615). Some have not been validated in children (Silva *et al.*, 2013:1) or different ethnic groups (Nightingale, 2013:7). Some methods, such as BIA, come with inbuilt equations (Wan, 2014:4) that have not been validated in a variety of populations. Other methods are based on equations that have previously been developed and validated mainly among Europeans (González-Ruíz, 2018:4) and need validation among African children.

Various researchers started developing equations that could provide accurate body composition information. Slaughter and colleagues (1988) were among the first to attempt, by developing prediction equations with a multi-compartment model of body composition as a criterion method, combining measures of total body density (BD) (from underwater weight), total body water (from deuterium dilution) and bone density (from photon absorptiometry) (Janz *et al.*, 1993:1070). These equations led to gender, race, and maturation specific equations for estimating body fat based on measurement of triceps skinfolds. The reliability of these equations for estimating fat-free mass using BIA resistance in children was > 0.99 (Goran *et al.*, 1993:1776). The accuracy of the Slaughter *et al.* (1988) equations has been examined in various studies producing different results. Even after such efforts, scientists continue to identify the need for validated reference methods with better accuracy and precision (Demerath *et al.*, 2002:394; Macias *et al.*, 2007:2). Cross-validation and testing of anthropometric prediction equations in different groups continue to be of particular importance (Wan *et al.*, 2014:9; Wells & Fretwell, 2006:613) because of the gender, ethnic and maturation-related changes in body composition (Vidulich *et al.*, 2006:433).

Studies that validate anthropometry for the diagnosis of excessive body fat among pediatric populations in Africa are limited (Ben Jemaa *et al.*, 2018:5; Cameron, 2004:70; Diouf *et al.*,

2018:1; Ndagire *et al.*, 2018:6). Most studies found were for west European and North American children (Wickramasinghe *et al.*, 2008:1237), Asian children (Kuriyan, 2018:653) and Mexican children (Pablo *et al.*, 2015). The majority of validation studies were done using the two-compartment (2C) model as the criterion method (Lohman *et al.*, 1992:10; Silva *et al.*, 2013:1). The 2C model only divides the body into lean mass and fat mass.

Various BIA equations have been developed to predict total body water from height (Ht) and bioelectrical resistance (R). However, age-specific equations have been recommended due to the age-related differences in electrolyte concentration in the extra-cellular space relative to the intra-cellular space (Heyward & Stolarczyk, 1996:44). The Kushner (1992:838) equation that includes $(Ht^2/R) + \text{water}$ for estimating total body water has been cross-validated in 4- to 6-year-old children, where the conclusion was that the Kushner (1992) equation could accurately predict total body water even in young children (Goran *et al.*, 1993:1778). Whilst the Kushner equation may be generally recommended in children, the equation still needs validation in different ethnic groups (Goran, 1998:507). Bio-impedance analysis validation studies have been conducted in other ethnic groups, including a study conducted in four European countries among 4-10-year-olds using BIA and skinfold comparing with the three-component (3C) model, which divides the body into fat mass (FM), Fat free mass (FFM) and total body water (TBW). The authors (Bammann *et al.*, 2013:525) proposed that multicentre studies with larger sample sizes are needed to create a base for international valid reference data to measure body fat in children. Another study was among 6-17-year-olds Korean children, validating BIA against DXA in which the authors concluded that BIA accuracy is dependent on the fatness level of a child (Seo *et al.*, 2018:2214). A study by Leman *et al.* (2003:65) of Nigerian 5- to 8-year-old children validating BIA against Deuterium Dilution concluded that BIA equations developed in other populations could not be generalised to Nigerian children. As such, the observed inconsistency regarding the use of body fatness assessment methods warrants further studies in South African children for comparison.

Understanding the age at which adiposity begins is important to identify stages at which to intervene. Adiposity rebound (AR) which, is an increase in body fat that occurs at certain intervals, has been found to occur as early as six years and also found to be associated with high BMI later in life (Rolland-Chachera *et al.*, 2006: S12). Adiposity rebound changes with age, increasing from birth to one year, declines until about age six and then increases through childhood (Eisenmann *et al.*, 2004:1633). Based on the AR changes in children, childhood has been viewed as representing a major 'window of opportunity and teachable moment' with respect to two of the key behavioural risk factors for NCDs: namely, an unhealthy, energy-dense diet plus a sedentary lifestyle, characterised by low levels of physical activity and an increase in sedentary behaviour (Hills *et al.*, 2014:339). Therefore, the age group included in this study is vital for the early identification of risk factors.

Though studies validating selected anthropometric measurements and BIA are available, using the deuterium dilution method (Bammann *et al.*, 2012:520; DeLozier *et al.*, 1991:238; Seo *et al.*, 2018:2207), there is still a paucity of validation studies among 6- to 8-year-old black South African children. Studies conducted in South Africa have mainly focused on older children and adults (Mchiza, 2015:1; Okop *et al.*, 2019; Pedro *et al.*, 2016:1; Puoane *et al.*, 2010:29). There are limited studies that have assessed body image perception among pre-purbety boys. Studies that could be found though not in South Africa was a study on China Health National Survey 2000–2011 on 6- to 17-year-old children in which it was reported that the majority of children kept perceiving themselves as thin, and only 2% rated themselves as fat (Min *et al.*, 2018:7). In a comparison between actual and ideal figures survey from children aged 8 – 9 years in Emilia-Romagna region (northern Italy) (Gualdi-Russo *et al.*, 2012:2), it was revealed that the Feel-Ideal Difference Index (FID) scores resulted different between the subsample with foreign-born mother in comparison to the native one (significantly lower in daughters of immigrants) (FID- F: 0.31 vs 0.57; M: 0.35 vs 0.32). More research is also needed on the perception of body image in relation to actual body fatness in children. Further validation and suitability, and accuracy of existing BIA and skinfold thickness equations South Africa are needed. This study therefore, seeks to explore answers to the following research questions:

- What will be the level of agreement between objectively determined body composition versus perceived body image outcomes in 6- to 8-year-old South African children?
- What will be the assessed level of agreement between skinfold prediction equations and BIA for estimating body fat in South African children?
- What will be the level of agreement between the newly developed and validated BIA with DDM in predicting total body water (TBW) and FFM in 6- to 8-year-old South African school children?

Answers to these questions (see Figure 1-1 below), an illustration of the links among the three objectives of the study, will contribute to the scientific knowledge on the validity of body image perception of 6- 8-year-old black South African boys and girls in predicting their actual body size. Secondly, to validate the prediction equation for estimating body fat based on skinfold thickness compared to body fatness measured by BIA and deuterium dilution in 6- 8-year-old black South African children. Also, the study could provide parents with scientific information regarding the level of body size/fatness/overweight of their children and will assist them in making better lifestyle choices for their children. Participating schools will understand the magnitude of the obesity problem, and together with the parents, use the recommendations of the study to go some way towards mitigating the problem. The validation of the equations in the study may be useful for future South African studies to get more accurate data on the prevalence of obesity and to identify children at risk of non-communicable diseases (NCD's) early in life. Furthermore, policymakers

in related fields may harness result of the study. The results may encourage other researchers to expand their research to other population groups and different ages.

1.3 Objectives

The objectives of this study are to determine the:

- level of agreement between objectively determined body composition versus perceived body image outcomes in 6- to 8-year-old South African children.
- level of agreement between skinfold prediction equations and BIA for estimating body fat in South African children.
- development and validation of BIA in predicting total body water and adiposity in 6 - to 8-year-old South African children.

1.4 Hypothesis

This study is based on the following hypotheses:

- There is a difference between perceptions of body image and objectively determined body composition and poor level of agreement in the methods for assessing 6- to 8-year-old children.
- The agreement between percentage bodyfat (%BF) obtained by skinfold prediction equations obtained by BIA in 6- to 8-year-old children will be low.
- Total Body Water (TBW) calculated from published equations will under-/overestimate TBW calculated from a newly developed equation.

1.5 Model framework for this thesis

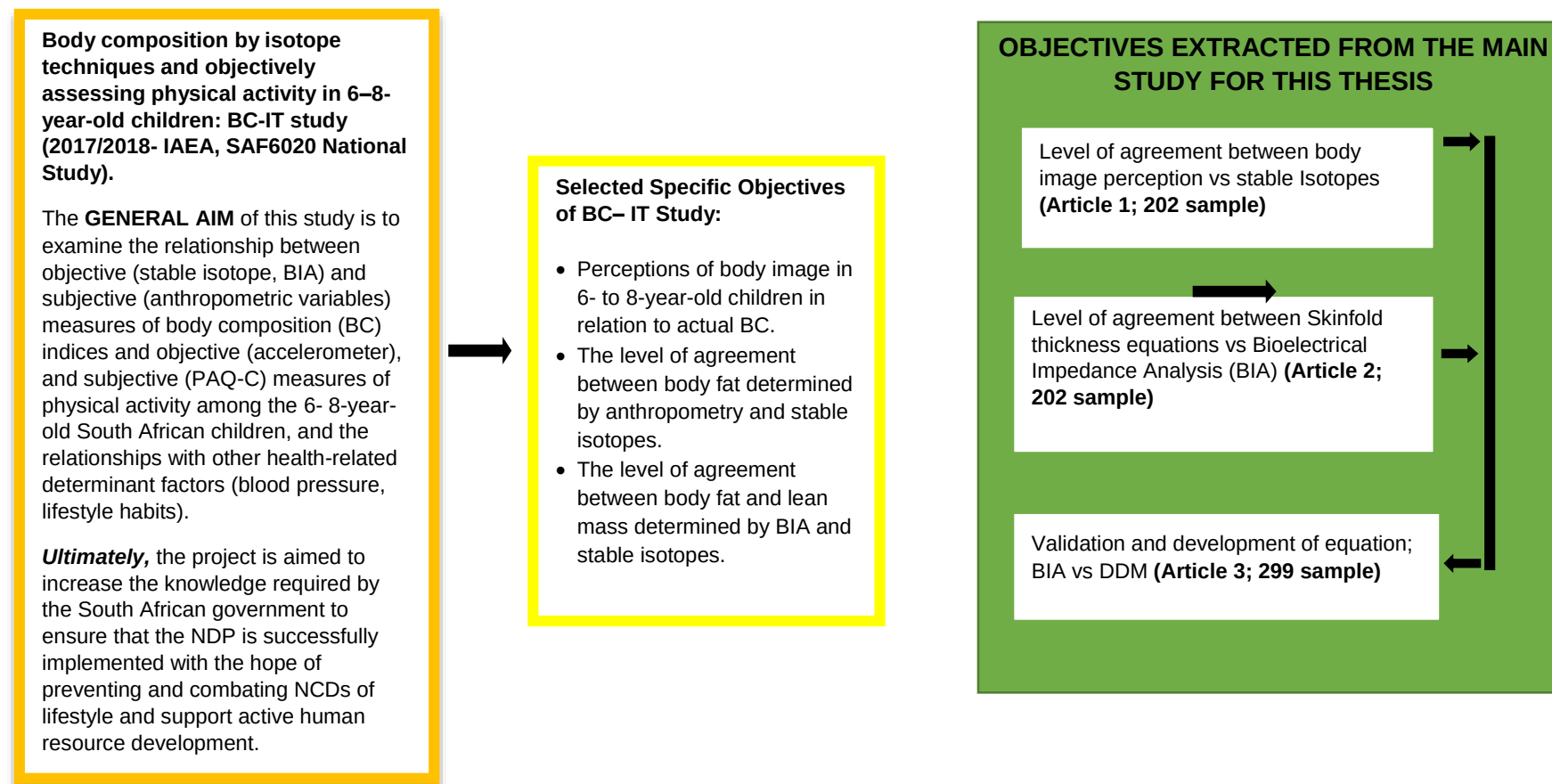


Figure 1-1: Illustration of the BC–IT study and model framework for data used in the thesis

(Data analysed for each objective in accordance with completeness of the data from the main study)

1.6 Definition of concepts used in the study

Abdominal fat -	subcutaneous and visceral fat in the abdominal region (Heyward & Stolarczyk, 1996:4). It is identified as an increase in Waist Circumference (WC) or Waist to Hip Ratio. There are no international standardized cut-off points for children and adolescents to classify abdominal adiposity (Aranha & de Oliveira, 2020:538).
Adiposity/ Body Fatness -	a Latin word that means severe or morbid overweight (WHO, 2020a)
Adiposity rebound (AR) -	is the nadir of the BMI (Body Mass Index) curve during childhood, and has been found to predict both adult BMI and adult obesity (Eriksson <i>et al.</i> , 2003:192; Freedman <i>et al.</i> , 2001:546; Williams <i>et al.</i> , 1999:349; Williams & Goulding, 2009:338). Hughes (2014: e1355) described AR as the increase in body fatness occurring at certain intervals during child development.
Body Composition -	Body composition describes what the body is composed of, namely: FM, lean body mass (LBM) / FFM (muscle and organs), TBW and bone density/bone mineral content. These components have different functions and are determinants of a human being's health and nutritional status (Kyle <i>et al.</i> , 2015:1).
Body density (BD) -	Body density is defined as the overall thickness of the fat, water, mineral, and protein components of the human body; total body mass expressed relative to total body volume (Heyward and Stolarczyk (1996:190).
Body Mass Index (BMI) -	is a person's weight in kilograms divided by the square of height in meters. A high BMI can be an indicator of high body fatness (de Onis, 2007:663).
Body Fat -	The total amount of body fat consists of essential fat and storage fat. Fat in the bone marrow, heart, lungs, liver, spleen, kidneys, intestines, muscles, and lipid-rich tissues throughout the central nervous system is called <i>essential</i> fat. In contrast, fat that accumulates in adipose tissue is called <i>storage</i> fat.
Body fat percentage (%BF) -	the total mass of fat divided by total body mass, multiplied by 100.

Fat Mass (FM) -	includes all extractable lipids from adipose and other tissues in the body (Heyward & Stolarczyk, 1996:4).
Fat-Free Mass (FFM)/ Fat -Free Body (FFB) -	all residual, lipid-free chemicals and tissues, including water, muscle, bone connective tissue and internal organs (Heyward & Stolarczyk, 1996:4). Due to this complexity, different techniques are needed to measure all the components accurately. For instance, body water is mainly present in FFM; therefore, obtaining an accurate TBW estimates FFM.
Lean Body Mass -	is the body's total weight, excluding the body's fat mass; however, it does include essential fats found in the bone marrow and internal organs (but not fat surrounding the organs).
Malnutrition -	the deficiency, excess or imbalance in the intake of energy and/or nutrients. It includes undernutrition, referred to as stunting, wasting, underweight and micronutrient deficiencies and over-nutrition classified as overweight and obesity (WHO, 2020).
Overweight -	bodyweight is higher than what is considered normal based on a certain height or cut off point depending on the measure used. $>+1SD$ is equivalent to BMI 25 kg/m ² at 19 years (WHO, 2020).
Obesity -	abnormal or excessive fat accumulation for a given weight (Heyward & Stolarczyk, 1996:3) that presents a risk to health (Danaei <i>et al.</i> , 2009:18). A person with 30% of excess %BF in females and 20–25% in males are classified as obese (de Onis, 2007:661; William, Going, Lohman <i>et al.</i> , 1992:358). For children, the following reference data is recommended by the WHO: Obesity: $>+2SD$ (equivalent to BMI 30 kg/m ² at 19 years). When using Skinfold thickness, obesity is regarded as $\geq 97^{th}$ centile (age-specific and sex-specific percentiles) is used as cut-offs (Kromeyer-Hauschild, 2012:616).
Sub-cutaneous Fat -	adipose tissue stored underneath the skin (Mittal, 2019:571).
Total body water (TBW) -	the water content of an animal body contained in the tissues, the blood, the bones and elsewhere. The percentages of body water in various fluid compartments add up to TBW (IAEA, 2010b:60). TBW encompasses both intracellular fluid and extracellular fluid in the body. Total body water is used to estimate the amount of FFM and Body FM. Since it forms part of the body weight, the unit of measure is the kg. (IAEA, 2010a:2).

Visceral fat - adipose tissue within and around the organs in the thoracic (e.g. heart, lungs) and abdominal (e.g. liver, kidneys) cavities (Heyward & Stolarczyk, 1996:4); associated with numerous diseases (Mittal, 2019:571).

1.7 Structure of the thesis

The thesis will be constituted partly in article format as approved by the Senate of the North-West University and will be structured as follows:

Chapter 1: Introduction, which also describes the obesity problem, aim and objectives of the study. A bibliography is provided at the end of the chapter in accordance with the North-West University guidelines.

Chapter 2: Literature review: Different methods and techniques for measuring body composition and the validation equations for assessing body composition. A bibliography will be provided at the end of the chapter in accordance with the North-West University guidelines.

Chapter 3: Article 1: Level of agreement between objectively determined body composition versus perceived body image outcomes in 6- to 8-year-old South African children: BC-IT study. The article is published in *PLoS ONE journal* (*PLoS ONE*, 15(8): e0237399. <https://doi.org/10.1371/journal.pone.0237399>, JIF: 2.870). Bibliography at the end of the article in accordance with the guidelines of the journal. Also, part of this article was presented at the *11th International Symposium on IN VIVO BODY COMPOSITION STUDIES*. 25th-27th June 2018, Columbia University, College of the Physicians and Surgeons, New York, USA; and on the 10 to 13 December 2018 *International Symposium on Understanding the Double Burden of Malnutrition for Effective Interventions*, held at the IAEA's Headquarters in Vienna, Austria.

Chapter 4: Article 2: Assesses the level of agreement between skinfold prediction equations and BIA for estimating body fat in South African children: BC-IT study. The article will be submitted to the *British Medical Journal Open*. The article and bibliography at the end of the article will be compiled according to the journal's guidelines. Part of the paper was presented at the HART Symposium, which was held in November 2020, NWU, Potchefstroom.

Chapter 5: Article 3: The the development and validation of bioelectrical impedance analysis in predicting total body water and adiposity in 6- to 8-year-old South African schoolchildren. The article will be submitted to the *American Journal of Clinical Nutrition*. The article and bibliography at the end of the article will be compiled in accordance with the journal guidelines.

Chapter 6: Summary, conclusions, limitations, and recommendations.

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CHAPTER 2: LITERATURE REVIEW: BODY COMPOSITION MEASUREMENTS AND THE VALIDATION EQUATIONS FOR ASSESSING OR DETERMINING BODY COMPOSITION

2.1 Introduction

A few decades ago, low to middle-income countries focused on ending undernutrition which was viewed as a threat to human development. To date, low to middle-income countries are experiencing a nutrition transition (Popkin, 2012:6) which has led to the doubling of malnutrition, i.e., undernutrition and obesity (Prentice, 2018:93). Some researchers reported a decline in the rate of undernutrition, whilst an increase in the prevalence of obesity, especially in the low or middle-income countries, was detected as a public health threat (Adom *et al.*, 2017:1; Biadgilign *et al.*, 2017:1; Karki *et al.*, 2019:2; Popkin & Slining, 2013:11).

The observed increase in the prevalence of obesity in low-income countries is, therefore, a serious public health challenge. More than 50 years ago, obesity was rare even in developed countries (Popkin, 2012:3; Prentice, 2018:50). The World Health Organisation (WHO) reported a six-fold global increase in obesity rates, from less than 1% in 1975 to 6% in 2016 among children and adolescents, and further estimates that 38.2 million under-fives were overweight or obese in 2019 (WHO, 2020). Based on the 2021 WHO, obesity fact sheet that reported that 39 million children under the age of five were overweight or obese in 2020, then the projection slightly underestimated (WHO, 2021). Arguably, the health consequences of obesity are reported to manifest mostly during adulthood, but the factors underlying the disease's conditions commonly manifest during childhood (Karki *et al.*, 2019:2; Raut *et al.*, 2014:135), and the prevalence of obesity is linked to several co-morbidities (de Menezes *et al.*, 2012:1262). Some of the co-morbidities include non-communicable diseases like type II diabetes, hypertension and certain forms of cancers (Rebello *et al.*, 2020:1810). During the current Covid-19 pandemic, increased mortality has been observed among obese patients who presented with Severe Acute Respiratory Syndrome CoronaVirus 2 (SARS-CoV-2) (Rebello *et al.*, 2020:1814).

Overweight and obesity are often determined using the proxy measurements of Body Mass Index (BMI). Body mass index has been used (Bhurosy & Jeewon, 2013:71; Mei, 2002:979; Okorodudu *et al.*, 2010:792) and is still being used globally, including in countries like South Africa, to determine the prevalence of obesity (Jacobs & De Ridder, 2012:43; Monyeki *et al.*, 2012:1; Shisana *et al.*, 2013:204;). However, previous scholars have stated that BMI does not distinguish between fat and lean mass, and its prediction values for children and adolescents are not clear (Buss, 2014:264; Wells & Fewtrell, 2006:613) nor does it provide the proportion of the body components, namely TBW, FFM or FM (Chung, 2017:246). Further, it has been contested that BMI in children changes considerably during growth and development (McCarthy *et al.*,

2006:598), which may be influenced by pre-pubertal growth hormones, which differ in boys and girls at different stages of development (Chung, 2017:246). Sex differences may not be visible when BMI is used as a measure (Buss, 2014:264; Wang *et al.*, 2018:6). As discussed above, it is clear that BMI does not reflect true body fatness. Using methods that measure fatness are, therefore, the best way to assess actual adiposity.

Body composition, including Fat Mass (FM) and Fat-free Mass (FFM), is regarded as a sensitive indicator of current health and nutritional status in children (Ghosh *et al.*, 2009:1516). Previous studies have observed the importance of body composition in monitoring health and nutrition status (da Silva *et al.*, 2015:2; Marra *et al.*, 2019:2; Zhang *et al.*, 2019:377). The changes in FM and depletion in FFM are regarded as important indicators for energy and fluid requirements. Deficiency of these nutrients could be a signal of ill-health and can also be used to assess metabolic health risk (Buss, 2014:264), undernutrition, hydration status and over-nutrition (Toomey *et al.*, 2015:17). Body composition changes are critical elements to guiding management of certain clinical conditions such as diabetes (Duren *et al.*, 2008:1139), cardiovascular diseases and other metabolic disorders (Wells & Shiely, 2016:4). Unfortunately, finding methods to accurately predict body components, especially %BF remains a challenge (Merrill, 2020:190). Given the significance of body composition in health and assessing obesity, it is important to find methods validated in South African children to accurately predict body fat, a significant factor in metabolic health risk.

Different techniques have been developed and validated in different populations, each with its advantages and disadvantages. Different scholars have argued that the best way to measure body composition is by applying multicomponent models to assess FFM (Elia, 1992:114; Essa'a *et al.*, 2017:1). However, the multiple component model is not always feasible due to the specialised skills, equipment cost required. The choice of a technique is based on various aspects outlined later in this document.

The reviewed literature serves as the framework for the research described in this thesis. An electronic and manual literature search was conducted to identify the literature on the various facets of the topic and to select relevant resources for the review. The search was performed using various electronic databases: Pubmed, Medline and Science direct. The Google Scholar search engine was also used. Keywords used were: *body composition, body fat, fat-free mass, fat mass, body composition methods, bioelectrical impedance, stable isotopes for body composition, validation of body fat equations and outcomes research*. The search was limited to resources in the English language, and was conducted over the period January 2017 to October 2020. The researcher also searched the reference lists of the relevant articles, and in particular, resources that could subsequently be found online, through an inter-library loan and in the archives of the Health Sciences Library of the North-West University.

The literature review is presented according to the following non-exhaustive sub-topics derived from the research objectives, and which encapsulate the essence of the study. The literature appraised the causes of malnutrition and obesity, and look at body composition assessment methods and the Advantages and disadvantages of body composition methods. The literature reviews further reviewed studies validating body composition equations studies developed from low to middle-income countries and among different ethnic groups. Measures to prevent and control underweight, overweight and obesity were also reviewed. Lastly the chapter summary is also included.

2.2 Obesity and undernutrition

2.2.1 Causes of obesity and undernutrition

Poor diets, lifestyle habits, rapid urbanisation, increased industrialisation (Biadgilign *et al.*, 2017:1), access to information and health services during the early years of a child's life (Redsell *et al.*, 2011:2) are important determinants of the child's health and developmental outcomes. These factors play a role in the manifestation of the double burden of nutritional deficiencies (wasting, stunting, and micronutrient deficiencies), overweight and obesity. Co-existence of both under- and over-nutrition may be experienced within the same country, community, or household (Tzioumi & Adair, 2014:2), especially in developing countries (Abdulla, 2015:337). The burden of malnutrition threatens the survival, development of children and eventually the economy of affected countries. In the following sub-sections, undernutrition, overweight and obesity are described, as well as their causes.

2.2.1.1 Causes of undernutrition

Multiple factors cause undernutrition, the basic being inadequate food and nutrient intakes. Inadequate food intake may occur because of illness, infections, poor feeding practices (Cuanalo de la Cerda *et al.*, 2012:46), and other factors such as preconception maternal health and nutrition status, intra-uterine growth deprivation, and poverty (Hashmi *et al.*, 2019:2; Perez-Escamilla, 2018:1). Additionally, factors such as social inequities and psychosocial deprivation, poor hygiene and sanitation and lastly, food insecurity, which may be due to markets and land access, also contribute to undernutrition (Perez-Escamilla, 2018:3). Tackling stunting and overweight or obesity has been identified as a challenging public health problem, especially in low to middle-income countries, since they have common origins entrenched in the social determinants of health (Perez-Escamilla, 2018:4).

2.2.1.2 Causes of overweight and obesity

Obesity is a complex health problem caused by a myriad of factors which differ from one situation to another, the basic factor being the imbalance between energy intake and expenditure (Hill *et*

al., 2013:27). The imbalance is influenced by cultural, social factors (Gualdi-Russo *et al.*, 2012:1; WHO, 2016:6), and level of education of the parents (Lamerz, 2005:376). Early infant feeding practices, childhood stunting (Hoffman *et al.*, 2000:706; Martins *et al.*, 2004:819) and genetics (Albuquerque, 2017:160) are also linked to growth and development of obesity in older children. The influence of the changing food environment coupled with increased supply and access to energy-dense foods (Golan, 2006:66; Sheikh, 2017:3; Taha & Maran, 2015:1) is another contributory factor. The physical environment includes changing modes of transportation which increases the risk of physical inactivity (Draper *et al.*, 2018: s407). In South Africa, factors relating to physical environment that have been cited include; urbanisation, fear of playing traditional outdoor games due to high crime rate (Kruger *et al.*, 2005:4), road traffic and high speed on the road prevent children from playing outdoor (Malambo *et al.*, 2018:5). Furthermore, the entertainment preferences of children are reflected in numerous studies (Lee *et al.*, 2019:11; Nurwanti, 2019:4; Poskitt, 2014:239). Recent studies have highlighted the presence of a TV in the bedroom as a contributory factor to overweight and obesity (Adachi-Mejia *et al.*, 2007:647; Wethington *et al.*, 2013:7). The family environment also plays an important role, whereby overweight mothers and single-parent families (Gibson, 2007:591), or both parents being obese (Bahreynian, 2017:116) are associated with an increased likelihood of a child being overweight or obese.

Adipose rebound (AR) has been linked to critical obesity development stages, such as in intra-uterine, infancy, ages 3- to 7-years and during adolescence (Hughes *et al.*, 2019: e1354; Koyama *et al.*, 2014:114). Adiposity rebound is described as the nadir of the BMI (Body Mass Index) curve during childhood, and has been found to predict both adult BMI and adult obesity (Williams & Goulding, 2009:338). The early occurrence of AR is linked to a higher risk of obesity (Koyama, 2014:114) and increased incidence of Type 2 diabetes (Eriksson, 2003:190). Scholars hypothesized that the early onset of AR predisposes a person to the development of obesity (Freedman *et al.*, 1987:588; Prokopec & Bellisle, 1993:517; Rolland-Cachera, 2006: s11), and early AR is reported to account for increased obesity in adulthood (Rolland-Cachera *et al.*, 2006: S12; Kang, 2018:375).

In South Africa, obesity has occurred in conjunction with urbanisation (Schutte *et al.*, 2003:339), linked with increased sales of sugar-sweetened beverages and high caloric, energy-dense foods (Tugendhaft & Hofman, 2014:6). Therefore, understanding the development and appropriate techniques to measure and categorise of malnutrition is the first step towards developing appropriate interventions to address obesity.

2.3 Body composition assessment methods

2.3.1 Body image

Body image perception refers to the various mental representations that individuals form about their bodies at different life cycle stages. It may also be based on how an individual is perceived by others (Zaccagni *et al.*, 2014:5). Gupta and colleagues described the important role that body image plays in self-esteem, anxiety, and depression (Gupta *et al.*, 2016:490).

Body image is viewed as a subjective measure for assessing body size since it is influenced by various factors, including cultural beliefs, social norms (Mchiza *et al.*, 2005:510), socio-economic status (Dinsa *et al.*, 2012:1063), geographic setting (urban/rural) (Okop *et al.*, 2016:10), peers, mass media (Shoraka *et al.*, 2019:19), parents and maturation stage (Zalilah *et al.*, 2003:743). Even though bodyweight perception has been regarded as a subjective measure, some scholars regard it as a better predictor for weight management and related behaviour change compared to BMI and other objective measures (Mchiza *et al.*, 2015:8; Okop, 2019:11; Xu *et al.*, 2018:30). Body image perception has been reported as an influential factor in the development of eating disorders, especially in women (Artoni, 2020:1; Manaf, 2016:2; Schuck, 2018:2), whilst Michels and Amenyah (2017:66) reported no relationship between body size dissatisfaction and eating disorders.

Ethnic differences are linked to misclassification and dissatisfaction with body image perception. Among Africans and African Americans, larger figures have been considered desirable (Jamy, 2013:5) and a sign of happiness and wealth (Puoane *et al.*, 2010:29). In some African countries (e.g., in North Africa), larger figures are viewed as a sign of social status and symbolising beauty, fertility and prosperity (Mokhtar *et al.*, 2001:888S). From a United Kingdom (UK) multi-ethnic study of adolescents, it was revealed that whites were more likely to overestimate their weights than blacks, who are more likely to underestimate their weights. However, misclassification was common in all ethnic groups (Standley *et al.*, 2009:58).

Gender preferences have been documented where boys in Western societies have demonstrated a preference for muscular and lean bodies whilst girls prefer thin bodies, often influenced by media and peers (Tatagelo, 2013:592). In a Minneapolis study conducted among children 9-18-year-old, both boys and girls overestimated their weights by 2kg and 2.2kg, respectively. On the other hand, studies found that boys underestimate their body size (Edwards *et al.*, 2010:e456; Yao & Hillemeier, 2012:2) and girls more likely to overestimate their weight (Wang *et al.*, 2018:4).

The level of fatness also contributes to the misclassification; both boys and girls at the extreme end of the weight spectrum (either thin or overweight) tend to misclassify their weights.

Apparently, the higher the BMI, the more likely a child would correctly interpret their weight (Aloufi, 2019:121; Freeman *et al.*, 2009:5)

Another factor which influences misclassification of body size is the child's age, where younger children, more than older ones tend to underestimate their weights (Mulasi-Pokhriyal, 2010:347). The notion of body image dissatisfaction has been observed in children as young as five years (Pallan, 2011:5). At this early age, children begin to develop higher levels of self-awareness (Rochat, 2003:727).

Some of the discrepancies found in the literature on body image perception are associated with the lack of consistency in the measuring instrument (Pallan, 2011). In some studies, the focus was only on overweight participants; different measurement tools such as figural pictorial scales, questionnaires were used (Cuesta-Zamora & Navas, 2017:1674), face to face interviews, mailing or online visual selection of figural pictures scales (González-Carrascosa, 2015:22). The number of figural pictures scales to select from seem to differ from one study to another (Cuesta-Zamora & Navas, 2017:1669) and may contribute to the noted discrepancies. A study on Pakistani children by Hussin and coworkers observed similar results when using either visual or verbal methods among children (Hussin *et al.*, 2011:369). Other studies have also relied on participants' self-reported weights, rather than actual measurements (Sarrar *et al.*, 2020:2). Hence, a significant bias has been reported with self-reports compared with actual BMI measures (Shiely *et al.*, 2013:2). The major challenge is that misclassification of body weight influences future weight management behaviour (Wang *et al.*, 2009:7).

The use of body image silhouettes designed and validated by Stunkard and colleagues (1983) have been found to be reliable and can be used for different age groups and among different ethnic groups (González-Zapata *et al.*, 2019:989). The silhouettes are simple, trustworthy, and an effective instrument to classify subjects' perceptions of being obese or thin and does not require participants to be literate. The silhouettes

There has been an interest for some time on the relationship between obesity and body image perception (González-Zapata *et al.*, 2019:989). However, most studies focused on how body image perception influences behaviour change related to weight loss (Alwan *et al.*, 2011:5; Radwan *et al.*, 2019:7). Relatively few studies in Africa and South Africa have assessed the accuracy of weight and body image perception in young children. Most studies are conducted among older children and adolescents (Alwan *et al.*, 2011:2, Gitau *et al.*, 2014:469; Mchiza *et al.*, 2015:509,) hence the identified gap in data on younger children.

Pedro and colleagues (2015:3) excluded young children in their study because they felt that children have a poor comprehension of their body image. However, in a systematic review focusing on children aged 3 to 6 years, the authors concluded that age does not influence

perception, but rather that the instrument selected should be appropriate for the age (León *et al.*, 2018:182). The study identified 22 studies, and 18 of these studies were conducted in the West and the remaining studies from the Far East; none of the studies was conducted in Africa (León *et al.*, 2018:180). It is unclear whether in Africa there may be an underestimation of the young child's ability to accurately perceive their body image.

The positive fact about the silhouettes is that it has been validated in children in which classification corresponding with the BMI was made; the thin silhouettes (1) is equated with a BMI less than 18.5, the next three silhouettes (2-4) BMI 18.5 to less than 25 kg/m² (Bulik *et al.*, 2001:1521). Silhouette 5 equates to overweight at a BMI of more than 25 and less than 30 kg/m², and lastly, silhouettes 6 and above equate with BMI equal to and more than 30kg/m² indicate obesity (Bulik *et al.*, 2001:1521).

The majority of studies have compared body image perception with BMI measures (Canpolat *et al.*, 2015:151; González-Carrascosa, 2015:23; Pasch *et al.*, 2016:4; Prabhu & D'Cunha, 2018:146). However, limited studies have reported inconsistencies between body image perception and other commonly used methods to assessing overweight and obesity (Zaccagni *et al.*, 2020:6) found inconsistencies when comparing perception of participant's size with actual measure determined by BIA.

2.3.2 Anthropometry measurements

Anthropometric measurements are a sequence of quantitative measurements of the different components of the body (muscle, bone, and adipose tissue) used for assessing body composition (Casadei & Kiel, 2020). The most common anthropometric measurements are: body weight/mass, height/length, skinfold thickness (triceps and subscapular), abdominal circumference and waist to hip ratio is a better predictor of cardiovascular diseases (Hassan *et al.*, 2021:6). Anthropometric measures alone are not able to accurately measure body fatness and the total body composition. For this purpose, other complex measures such as BIA, dual-energy X-ray absorptiometry, body density, and underwater weighing, deuterium dilution and others are used. In the next section, descriptions of the methods are discussed.

2.3.2.1 Measuring weight

Body weight consists of all body components, which include water, FM, minerals and FFM (Fogelholm & Lichtenbelt, 1997:495) weight refers to the heaviness of an object and only measures the quantity of the object without differentiating the content. The unit for measuring weight is kilogram, used by more than 90% of countries or a pound, used by some countries including the UK and the United States (US).

It is important to note that body mass taken without other measures of body size can be misleading since weight is highly related to stature (Duren, 2008:1140). Body weight is a somewhat poor indicator of health and nutritional status (IAEA, 2010a:2). However, a rapid change in weight alone should be a cause for concern and should trigger assessment through other measures (Gaddey & Holder, 2014:718).

There are various scales for measuring body mass; different scales are used for measuring young children (below two years) and for adults (WHO, 2008:18). In clinical settings there are, dedicated such as the detachable, scales that are build- in on the legs of hospital beds and integrated bed scales that are more suitable for very sick, immobile patients (Manoj *et al.*, 2019:1).

Scales used to measure weight have been found to be inconsistent and inaccurate. In a study conducted by Stein and Colleagues (2005:266) in England aimed at assessing scale precision, 223 body scales were tested. Relatively large inaccuracies were found at all weight levels and in all locations. For the higher weight levels, nearly 21% of the scales were off by more than 2.7 kg. Stein *et al.* (2005:269) recommend that scales be calibrated as directed by the manufacturer to improve accuracy. Weight should always be measured to the nearest 0.1kg with light clothing. Furthermore, measurement accuracy is improved if the scale is placed on a flat, hard and even surface (WHO, 2008:17). Electronic scales are recommended for research purposes (Kumar *et al.*, 2014:595; WHO, 2008:15). This may be supported by Yorkin *et al.*, (2013:4) findings, in a study comparing different scales, the study concluded that that digital scale were more accurate than manual scales.

2.3.2.2 Measuring height/length

Measuring height is important for measuring growth. The height measurement is also used for calculating BMI, which is essential in tracking a child's growth. A decision on how to measure length or height depends on the child's age and the ability to stand. Length is measured lying down (recumbent). Length is used for children younger than two years. Height is measured standing upright for children older than two years and able to stand (WHO, 2008:19).

Standing height is reported to be about 0.7 cm less than recumbent length. Therefore, it is important to adjust the measurements if length is taken instead of height, and vice versa. Height/length should be measured to the nearest 0.1 centimetre. Length is measured with a board (sometimes called an infantometer), placed on a flat, stable surface. Height can be easily measured with a height board (sometimes called a stadiometer) often mounted on the wall, placed on a smooth, level surface (WHO, 2008:22). Accurate recording of height and weight is vital as inaccurate measures may result in misclassification if used to calculate BMI.

2.3.3 Body Mass Index

Body Mass Index was first described in Belgium in the 19th century by a mathematician who observed that an individual considered 'normal frame', based on how proportional their weight was proportional to their height (Freedman & Sherry, 2009: S24; Okorodudu *et al.*, 2010:791). Body mass index is measured as Body Mass (kg) divided by the square of the height (kg/m^2) and regarded as a ratio of body weight for height.

The BMI is used globally to assess underweight, overweight and obesity and may predict clinical outcomes such as type 2 diabetes in adults (Gutin, 2018:9). In the late 80's and early 90's that the unreliability of BMI as a predictor of obesity was raised (Ashwell, 2011:80). Since then, researchers have constantly outlined its limitations, indicating that it should mainly be used as a screening tool (Bhurosy & Jeewon, 2013:75). Freedman *et al.* (2007:184) indicated that BMI had been characterized as a proxy for percentage of body fat in normal-weight children. However, Freedman *et al.* (2007:184) also state that evidence indicates that in children and adults, a high BMI for age (is likely to be an indicator for excess body fatness and adverse risk factors including diabetes, cardiovascular diseases and cancer (Freedman, 2009:6).

Some studies have questioned the use of BMI in children due to its moderate association with height during growth and development. Height correlates with body fatness, and higher BMI's of taller children correctly identifies their increased fatness (Freedman *et al.*, 2009:S23).

The advantage of using BMI, is that it has well established international reference points, making it easier to categorise body fatness (Cole *et al.*, 2000:1242). It is useful in measuring changes in body mass and also used to determine morbidity risks and mortality (Ghanta *et al.*, 2017:5; Henderson, 2005:2). The accuracy of BMI varies according to the degree of fatness and the cut-off points used to categorise (Freedman & Sherry, 2009:S23). When using BMI to measure a change in weight status, a change of about 3.5 kg is required to reflect a change in BMI (Duren *et al.*, 2008:1140).

While BMI may be the frequently used measure to assess health risk, it should be noted that relying on BMI to identify individuals at risk, may result in delayed response since the risk profile may be population specific (Wang *et al.*, 2018:7). A study by Liu *et al.* (2011:5), aimed at assessing fat distribution among 8-10 year old children from different ethnic groups namely Chinese, Lebanese, Malaysian and children of Thai revealed that even at such an early age, children of Asian origin vary in fat distribution, this finding may explain the paradox where some populations may have higher rates of Cardiovascular diseases with lower BMI values. During growth, children tend to increase FFM more than the FM, and therefore the change in BMI cannot always be attributed to changes in adiposity. Growth spurts and muscles changes occur at different stages during childhood in the first two years of life. This growth is followed by rapid

increases in mass and height during pre-puberty and puberty, characterised by substantial increases in BMI. The changes represent changes in body composition and appearance of initial gender characteristics (Chang *et al.*, 2018:3).

Though BMI is regarded as an unreliable measure for fatness, some authors believe that very high BMI in children can still be used as a proxy for obesity in children and still a valuable measure of adiposity at a population level (Gómez-Ambrosi, 2012:292). In early childhood, BMI is a good health indicator linked to diet and nutritional status (Freedman & Sherry, 2009:S24). Lastly, knowing how fat is distributed in the body is important since abdominal fat is linked with an increased risk of developing cardiovascular disease independent of BMI levels (Freedman & Sherry, 2009: S24). Therefore, using BMI alone to screen for health risks could result in an underestimation of 35% of men and 14% of women who may be classified in the normal BMI categories compared with WtHR – waist to height ratio (Ashwell, 2009:97).

2.3.4 Waist circumference and waist-to-hip ratio

Waist circumference (WC) measure central fatness and is more predictive of a better predictor of health risk such as cardiovascular disease and type 2 diabetes than BMI and diagnostic criterion for risks associated with the metabolic syndrome (Kalk *et al.*, 2011 491; Mederico, 2012:236; Pereira *et al.*, 2010:665). In adults, WC is associated with morbidity and better estimate risk of morbidity than weight alone. Whilst WC is used widely, its main challenge is the lack of uniformly accepted measurement protocols, resulting in a variety of techniques used as reflected in published literature (Mason & Katzmarzyk, 2009:1789). Further, WC lacks acceptable cut off points for different ethnic groups, making its use in public health limited (Cameron *et al.*, 2010:2043), especially in children.

Waist circumference (WC) (Bassali *et al.*, 2010:2), waist to hip ratio (WHR) and the waist:height ratio (WtHR) (Liu *et al.*, 2011:2) are currently used to assess risk factors. The WtHR is reported to be a better predictor of mortality than BMI (Ashwell *et al.*, 2014:2). WC and WtHR are recommended as tools for identifying central obesity in children (Fredriksen, 2018:17 Central obesity, known as increased intra-abdominal adipose tissue, reflects central adiposity, has higher sensitivity and specificity (Huxley *et al.*, 2010:2). Further WC is a simple and effective anthropometric, surrogate measure of abdominal obesity (Li *et al.*, 2006:e1396)

Different cut-off points are used for men and women. Central obesity (Kalk *et al.*, 2011 491; Mederico, 2012:236; WHO, 2020) is defined clinically by waist circumference of 102 and 88 centimetres in men and women, respectively (WHO, 2011:19). However, for children, WC above 90th percentile is regarded as reflecting abdominal overweight, and 95th percentile is regarded as obese (Mederico, 2012:237) or 97th (Liu *et al.*, 2010:4). Various other measurements are used to determine including skinfold thickness.

2.3.5 Skinfold Thickness

The use of skinfold thickness dates as far back as the 70's (Brook, 1971:182). It is based on the principle that fat is of a known density and that total and regional fat can be estimated if all subcutaneous fat thickness measurements from the whole body could be added (Tanner & Whitehouse, 1975:142; Wells & Fewtrell, 2006:612). The measures are taken to assess skinfold thickness, which is used to predict body density and use appropriate population-specific conversion formula to predict percentage fat. However, prediction of the fat percentage may be affected by the variances in skinfold compressibility at different sites, which affects the reading of the calliper (Martin *et al.*, 1992:453).

Studies have reported a Standard Error of Estimate (SEE) ranging from 3% to up to 11 % (Wang *et al.*, 2000:323), approximately 5% depending on the prediction equation, the population being studied (Lustig & Strauss, 2003:4181), and the age of children being assessed (Heyward & Stolarczyk, 1996:95). It should be noted that subcutaneous fat varies considerably by sex and reflect changes in the underlying triceps muscle rather than actual change in body fatness (Hattori *et al.*, 1991:54). However, skinfold thickness is more predictive of fatness than BMI (Freedman *et al.*, 2015:7).

The triceps skinfold is usually the site recommended and widely used. It is better than the subscapular skinfold to predict %BF (Rolland-Cachera, 1993:25). However, various studies use a sum of two or more sites; some scholars find these sites to be better predictors of fatness since they may provide better information on the distribution of subcutaneous fat (Kehoe, 2011:1265; Ozturk, 2009:280; Slaughter *et al.*, 1988:712). However, globally there is no agreement as to which skinfold sites best represent %BF. In a study by Ripka and colleagues (2017:6), different sites were used to determine the best fit prediction equation, and all thickness of the subcutaneous sites used to develop prediction equation provided an ideal SEE (1.78-1.87) (and $r = 0.85-0.94$), compared with DXA; indicating that in the study all sites were interchangeable. Even though the correlation was based on Lohman's SEE reference standard, the equation tended to overestimate %BF. The most commonly used equation for children is the Slaughter *et al.* (1988) prediction equation. This skinfold prediction equation has been validated in different age- and race groups and in populations that differ in terms of fatness (Hastuti *et al.*, 2019:923).

Available skinfold prediction equations are developed based on the sum of different sites (Dezenberg, 1999:254); each equation is developed using different variables such as age, height, weight or ethnicity to produce the closest prediction to reference methods. Skinfold thickness provides better predictions where raw skinfold values are used (Wells & Fewtrell, 2006:613). Further skinfold thickness prediction equations have been validated against different reference methods namely DXA, producing inconsistent results (Peterson, 2003:1186). These are the factors that may contribute to the variances in %BF in different populations groups.

Skinfold thickness measurement is simple, portable, convenient and less expensive than most laboratory-based techniques (Mei *et al.*, 2007:978; Mooney *et al.*, 2011:3), thus may be used in field studies. Previous studies had cited different views about the validity of skinfold thickness at varied BMI levels. Some scholars argue that skinfold thickness is an important and valid anthropometric indicator of the amount of subcutaneous fat tissue and regional fatness (Liem *et al.*, 2009:839; Sarria *et al.*, 1998:573). Opposing views were presented by Freedman (2007:184) who indicated skinfolds vary substantially according to the BMI level. While Freedman and Sherry (2009: S25) found skinfold thickness to be more precise in predicting adiposity in children (5- to 18-year-old) with normal weight, but less precise among overweight children. Furthermore, two expert panels from the US have suggested that skinfolds could be used for individuals with increased BMI as part of in-depth medical assessments and adolescents with increased BMI (Himes & Dietz, 1994: S4). However other measures such as BIA have been found to have better predictions of body composition in children.

2.3.6 Bioelectrical Impedance Analysis (BIA)

The principle behind BIA dates as far back as 1786 when Galvani, an Italian physicist, who was experimenting with a dead frog, observed the influence an electrical current had on it. Nevertheless, it was only in 1962 that Thomasset discovered that electrical resistance could measure human body fluid. In the same year, Thomasset and colleagues developed the first impedance analyzers to measure biological tissues (Thomasset, 1962:15). Further analysis by Hoffer in 1969 concluded that a whole-body impedance measurement could be used to predict Total Body Water (TBW) (Hoffer, 1969:534). Around 1970, Nyboer (1950:820) was finally able to prove that impedance values can assist in understanding body composition and validated its use for measuring body composition in 1983 using densitometry to determine FFM and skinfold thickness and hydro densitometry to validate % BF. Other scholars have accepted it as a tool for assessing body composition and it has been validated across many populations, including children (Schaefer *et al.*, 1994:619; Sun *et al.*, 2003:333).

The BIA initially relied on a single frequency based on a two-compartment model and is used to assess TBW and FFM. Currently, BIA measurements use equipment with different frequencies: a single 50 kHz and multiple frequencies up to 300 kHz equipment. The limitation of the single frequency BIA is its inability to detect variations in body composition in the trunk region (Fogelholm & van Marken Lichtenbelt, 1997:500; Wan *et al.*, 2014:2) and inability to differentiate between intra- and extra-cellular fluids (Kyle *et al.*, 2015:2). The recent BIA technology, which includes multiple frequencies, can be placed in different body segments, and evaluate limbs, the trunk and skeletal muscle, thus improving BIA accuracy. The multiple frequency BIA equipment can differentiate between intra- and extra-cellular fluid and measure TBW and extra-cellular water in individuals with normal weight (Dasgupta *et al.*, 2018:1602).

While it may be argued that BIA is a useful tool, various authors agree that BIA equations for determining %BF and FFM may need to be validated in different ethnic groups (Diouf *et al.*, 2018:2; Newton *et al.*, 2005:601; Wang *et al.*, 2014:2299) since most published BIA equations for children and adolescents have been based on Caucasian populations (Wang *et al.*, 2014:2299). For example, in a US study conducted among adolescent girls based on seven different BIA equations, the majority of BIA equations overestimated %BF by only 2% in normal weight and overweight African-American adolescents in all the fat levels (Normal weight, overweight, obese and severely obese) (Newton *et al.*, 2005:595). In a different study by Wang and colleagues, three different BIA equations used for Asian children and adolescents were not suitable for Chinese children (Wang *et al.*, 2014:2299).

Previously studies have reported that BIA underestimates %BF in obese individuals (Eisenkölbl *et al.*, 2001:428; Pimentel *et al.*, 2010:744). In a critical review of body composition methods to assess obesity, it was reported that in about six of the studies, BIA underestimated %BF in severely obese individuals (Bernhard, 2016:161). The under estimation may be since TBW and extra-cellular water is greater in obese individuals (Waki *et al.*, 1991:199). Some studies found a perfect correlation of FM and FFM measures, estimated by BIA compared to (ADP, DXA, and Isotope Dilution) in males and females (de Castro *et al.*, 2017:134).

The BIA operates by sending a harmless electrical current through the body tissue, which contains intra- and extra-cellular fluid. The BIA can be used to assess fluid distribution and changes in both healthy and unhealthy subjects (Devakumar *et al.*, 2015:2). It is relatively quick and easy, uses a portable and technologically simple method to apply non-invasive equipment, and is relatively inexpensive (Treuth *et al.*, 2000:1252). BIA is suitable for large epidemiological studies of all population groups and provides an acceptable quantification of %BF in children (Kyle *et al.*, 2015:3; Talma *et al.*, 2013). The BIA is more appealing for use in children because it is non-invasive, portable, easy to use, relatively low cost, minimal participant participation required, and safe (but not recommended for participants with a pacemaker) and is practical for large-scale studies (Lee & Ghallagher, 2008:568).

Its major drawbacks are that its validity is influenced by equipment used, age, gender (Kreissl *et al.*, 2019), dehydration (Brantlov *et al.*, 2017:27), and the equations used (Sun *et al.*, 2003:332). Furthermore, BIA predicts TBW rather than measuring it directly, resulting in variations between population groups. Another limitation of BIA is the assumption that water and fluid volume are a constant volume of the body's FFM (Brantlov *et al.*, 2017:24). In conducting BIA, Cornell recreational services developed Body Composition Protocol Guidelines to improve accuracy of BIA measurement. These include among others; participants hydration status, choice of BIA equipment, pre assessment fasting. The guidelines have been cited by Brantlov *et al.* (2017:27). In ensuring the quality of data collected with the use of BIA, the manufacturer's protocol was

followed, children were asked to refrain from exercise or taking a sauna/ playing in the sun within eight hours of the procedure. Frequency of measure was set at 50 kHz with height, gender, age and body mass entered manually. Body mass was automatically adjusted using 0.5 kg for clothing mass in all participants. Participants were asked to remove jewelry and belts containing metal or metal-rimmed glasses. Participants were asked to lie down quietly and without motion on a non-metal examination table or bed on their back with arms flexed to the side and thighs not touching. Detection electrodes were placed at the pisiform prominence of the wrist and the anterior surface of the true ankle joint after wiping with moist antiseptic towel (Right side as this is recommended) (Schoeller *et al.*, 1980). Therefore, adherence to these guidelines is important to reduce errors in predicting TBW.

The Bodystat software uses inbuilt prediction equations to produce an output specifying total body water (TBW) in litres, FFM (kilograms) BF mass (kilograms) and %BF, as well as impedance, resistance and reactance readings. The existing BIA equations developed by Luke *et al.* (1997) will also be used for estimation of TBW.

2.3.7 Isotope Dilution Method or Hydrometry

Soddy first introduced isotopes as a concept in 1913, and Thomson discovered the first stable isotope (as cited by Wilkinson, 2016:60). Later Urey and associates, Brickwedde and Murphy in 1931 as cited by Brickwedde (1982:34) discovered it and named it Deuterium. Around 1932 studies on the safety of Deuterium were conducted, which, confirmed its safety. In 1934, von Hevesy and Hofer (as cited in Schoeller *et al.*, 1980:2686) discovered the use of Stable Isotopes in assessing body composition. These initial studies were discovered through the dissection of cadavers. A total of 216 studies were published between 1934 and 1939 on the biological effect of Deuterium. The first application of this discovery was in 1946 by Moore (as cited in Schoeller *et al.*, 1980:2686). In 1955, Deuterium studies were expanded to assessing energy expenditure. However, its application in humans only started in 1982. To date, this method is known as the gold standard for assessing energy intake, expenditure and TBW (Bila *et al.*, 2013:62; IAEA, 2009:6) and %BF (Deurenberg & Deurenberg-Yap, 2002:37).

Titrated water (T_2O) and Deuterium water (D_2O) are used to measure TBW (IAEA, 2009:3; Wang, 1999:837), and was first used to measure TBW, while labelled water- $H_2^{15}O$ was used to measure energy expenditure (Westerterp, 2017:1278). These are the three different isotopes used to measure water spaces and water volumes. Deuterium is a non-radioactive stable isotope of hydrogen, with the symbol $2H^+$ (IAEA, 2009:72). It is given orally as deuterium oxide ($2H_2O$) or D_2O (IAEA, 2010:3). Total body water includes both intracellular fluid and extracellular fluid. The body water naturally contains 0.015% of all hydrogen is deuterium. Hence there is a need to collect a baseline sample before a Deuterium oxide dose is given (IAEA, 2009:50).

Deuterium Dilution is simple, non-invasive, has minimal participant burden and can be used anywhere and for different age groups (infants, children and adults) (Wells & Fewtrell, 2006:614). Furthermore, Deuterium Dilution oxide can be used to assess longitudinal changes in body composition, making it easier to compare changes pre- and post-exposure to an intervention.

Deuterium Dilution only requires administering a single dose of labelled H₂O and a few samples of urine, saliva or blood. Though minimal skill is required for administering the labelled H₂O, the mixing and the analysis of data requires a trained professional. It is reported to have a 1-2% precision and accuracy for measuring TBW (Kuriyan, 2018:652).

Enrichment of Deuterium can be measured by isotope ratio mass spectrometry (IRMS), or Fourier transformed infrared spectrometry (FTIR) spectrometry. It is important to note that FTIR spectrometry is not as sensitive as IRMS (IAEA, 2010:3). Therefore, when using FTIR, a larger dose of Deuterium oxide is required (approximately ten times more). However, FTIR is preferred because the instrumentation is easier to use, has low maintenance requirements, is less expensive to procure, and the cost of analysis is lower than the IRMS (IAEA, 2010:3).

Using stable isotopes is a direct method to determine TBW and an indirect method to estimate FM and FFM. It is a relatively accurate method in estimating %BF in healthy individuals (Ward *et al.*, 2013:126). It is based on the principle that “where the amount of TBW (solvent) can be estimated and the concentration and amount of the tracer (isotope) are known” (Kuriyan, 2018:652) then the dilution level will be known. The enriched Deuterium is distributed in all body fluids. Lukaski (1987:538) argued that the isotope exchange with non-aqueous hydrogen in the body might result in TBW being overestimated by 1% - 5%.

Deuterium oxide is a relatively safe, stable isotope of hydrogen and does not emit potentially harmful radiation; the only risk could be when there is a significantly high concentration (>15%) of Deuterium oxide in the tissues. The risk has been observed in animal studies where tissues containing >15% of Deuterium labelled water exhibited a multitude of effects, including impaired protein and nucleic acid synthesis. In mammals, concentrations of deuterium <15% have not been associated with harmful effects. In human noticeable side effects may be observed when body water is enriched above 0.2%. However, noticeable toxicity can be classified as concentration >15%. Toxic effects of Deuterium labelling are reversible; however, very high concentrations may prove fatal. Nutrition studies use a Deuterium enriched body water to a maximum of 0.1%, which is a safe level (Al-Mokbel *et al.*, 2019; Koletzko, 1997; Roberts *et al.*, 1986). Hence there is a need to have a trained laboratory technician to do measurements (IAEA, 2010:53).

2.3.8 Dual Energy X-Ray Absorptiometry (DXA/DEXA)

The DXA technology was invented by Cameron and Sorenson in 1963, this was followed by a study by Mazess *et al* in 1981 who assessed the validity of Dual-Photon Absorptiometry in measuring bone mineral contents (BMC) in different parts of the body. In the Mazess *and associate* study the BMC of the spine was correlated with the BMC from the trunk, the recommendation was that the spine status requires a direct measurement to get accurate results (Mazess, 1981:368). The Dual-Photon Absorptiometry was later replaced with DXA due to its higher radiation exposure (Lees and Stevenson, 1992:151). The DXA is used to measure bone mineral content (BMC) and bone mineral density (BMD) to assess the risk for osteoporosis (Kohrt, 1997:152). The DXA technology has evolved over decades; the new DXA instruments can measure soft tissue (Kohrt, 1995:1349; Roubenhoff *et al.*, 2012:589). The DXA is based on the principle that the composition of an object can be determined if it comprises two materials that have different mass densities (Kohrt, 1997:156). The technique uses a minimum radiation dose that passes through different tissue in the body (Damilakis *et al.*, 2010:2709; Ellis, 1994:660).

The DXA was initially regarded as a gold standard for measuring bone mineral density. More recently, some scholars recognise it as a “gold standard” even for measuring FFM and FM (Ellis *et al.*, 1993:154; Ellis *et al.*, 1994:664). It is viewed as highly reliable, accurate and can be reproduced even among children (Verduin *et al.*, 2016:6). However, its accuracy is dependent on the thickness (tissue depth), density and chemical composition of the tissue transversed (Zanini, 2015:1568), hydration status, and how the composition of soft tissue that is over- and under-lying the bone is estimated (Kohrt, 1997:159). The accuracy of DXA in measuring different body sizes has been contested (Shepherd *et al.*, 2017:101). A greater measurement error in obese than in non-obese children has been observed (Taylor *et al.*, 2002:490). Due to these discrepancies, Shypailo *et al.* (2008:461) advised researchers to be cautious when interpreting DXA results.

Earlier studies compared DXA with the Four-Compartment (4-C) models (Wells *et al.*, 2010:649), in a review of 12 studies comparing DXA with hydrodensitometry, Kohrt (1997:160), found inconsistencies ranging from a narrow to a wide (0.2 to 8.6%) %BF disagreement. On contrary, Lockner *et al.* (2000:72) found a strong correlation, but %BF by hydro densitometry was lower than DXA by 2.9%. BF lower than %BF DXA. It should be noted that both the 4-C model and hydrodensitometry are also indirect methods, and therefore, there is no absolute way of determining which one is valid. Consequently, there is no unanimously endorsed method for accurately measuring %BF (Duren, 2008:1144). In a study by Treuth and colleagues (2001:1358), where six body composition methods among pre-pubertal girls were compared, including DXA, none of the methods was interchangeable, and DXA over-estimated fat mass.

DXA is not without flaws, including exposure to radiation of subjects and individuals operating it, though a recent version has reduced radiation compared to its predecessor, the dual-photon

absorptiometry (DPA) (Kohrt, 1997:152). The low radiation may still be a concern and DXA is costly compared to other reference methods such as densitometry and deuterium dilution method. However, the advantage is that it makes fewer assumptions than other methods; namely, it does not assume that FFM is constant. Furthermore, it is quick, easy to use, and requires minimal cooperation from subjects, and can assess both bone mass, FM and FFM at the same time, making it a popular choice for assessing children (Kohrt, 1997:162; Treuth, 2001:1353).

2.3.9 Underwater weighing / Hydrostatic weighing / Hydro densitometry

Underwater weighing (UWW) was first introduced as a method of measuring body composition in the early 1940s (Behnke *et al.*, 1942 as cited in Hussey, 2017:10). Under Water Weighing was developed mainly to measure body volume and density, often used to estimate %BF (Kuriyan, 2018:651) using appropriate equations. It has also been regarded as the reference method for body composition analysis (Ellis, 2000:653). Underwater weighing is based on the 2-C model, which assumes that FFM and FM are constant (Heymsfield & Lichtman, 1990:4). The UWW reference was based on the actual determination of fat from cadavers (Warner *et al.*, 1986) and has been used to validate other methods such as DXA (Kohrt, 1997:17).

The method uses the Archimedes principle of displacement, which states that “When a body is immersed in a fluid, body volume is equal to the loss of weight in the water” (Ningthoujam, 2016:27941). Therefore, individuals with more body fat weigh less and will float underwater, whereas individuals with more muscle will weigh more and will sink underwater (Ningthoujam, 2016:27941). The method is based on the assumptions that water, minerals, and protein are the same in all age groups, across genders, for individuals with different body weights, and that the body has a fixed density of 1.10 kg/l for FFM and 0.90 kg/l for FM (Withers *et al.*, 1992:773). However, more recent evidence suggests that FFM varies between subjects and within subjects throughout the lifespan; therefore, the method cannot be unproblematically applied to children, the aged and subjects with illnesses that may affect water balance (Deurenberg, 2003:485).

The shortcoming of UWW is that while completely submerged in water, subjects are required to fully exhale to establish residual lung volume (RLV). This action is important because accurate estimation of total body volume by hydrostatic weighing depends on the ability to measure individual lung volume, and that RLV is the lung volume least affected by hydrostatic pressure (Welch and Crisp as cited in Weltman *et al.*, 1987:52). Because hydrodensitometry relies on subject responsiveness, requiring them to submerge completely in water and exhale as much as possible, it is thus difficult to use with children (Duren *et al.*, 2008:1142).

Underwater weighing is regarded as accurate in predicting %BF in population group averages, but not for individuals. However, an error could also occur if a wrong formula is used to convert total body density to %BF and if total body volume for RLV is not accurate (Heyward, 1996:147).

Katch and Katch (in Heyward, 1996:147) reported that, for example, an incorrect RLV of 600ml could result in an 8% wrong estimation of %BF. However, in another study, UWW was observed to overestimate FFM and underestimate FM, irrespective of the FFM density values used (Wells *et al.*, 1999:907).

Underwater weighing may also be affected by the hydration status of the subject, illness and medication being taken and the stage of the menstrual cycle (Heyward, 1996:147). However, some of these factors may not be relevant when measuring young children. Over the years, there has been a shift in the use of UWW due to its challenges, and other methods such as the DXA and Air displacement plethysmography (ADP) have been validated for other components and may be more accurate than UWW.

2.3.10 Air displacement plethysmography (ADP)

Air displacement plethysmography has been in use for more than a century, and at that time, it was considered a good criterion for measuring body composition in lifeless subjects (Demerath *et al.*, 2000:389). It was only in the 1990's where it was developed into a system that could be used routinely to measure body composition in humans, namely the BOD POD system. This is the device and system that is currently used (Life Measurement Instrument, Cosmed®, Rome) that can reliably measure body density (Dempster *et al.*, 2011:240) and can be an alternative to the UWW method.

The system includes the BOD POD plethysmograph, electronic weighing scale, calibration weights and cylinder, computer, and software (Fields *et al.*, 2002:454). It is based on Poisson's Law, which describes the pressure-volume relationship under adiabatic conditions (i.e. refer to conditions under which overall heat transfer across the boundary between the thermodynamic system and the surroundings is absent). It relies on the relation between pressure and volume to determine and measure the volume of air displaced by the individual. The volume is measured by displacing the air volume inside the chamber with the volume of the subject in the chamber. The actual human volume is calculated by subtracting the air volume remaining in the chamber when the human body is inside from the volume of air when the chamber is empty. The subject's mass is divided by the measured volume to yield whole-body density in grams per cubic centimetre (McCrory *et al.*, 1998:1475).

When using BOD POD, it is recommended that testing should be conducted before the subject could be involved in any form of physical activity and that the body should be dry. Subjects are also advised not to take food or liquid before the test. It is further recommended that subjects wear a swimming costume and swimming cap to minimize the isothermal air and heat from clothes and hair (Higgins, 2001:328). Some scholars have warned that the excess heat and moisture in the BOD POD chamber may lead to a slight under-estimation of %BF (Fields *et al.*, 2004:3).

Fields and Allison (2012:1735) found ADP suitable in estimating %BF in children as young as 2 to 6 years. They reported that it was accurate, precise, reliable and without bias. In another study by Lemos and Gallagher (2017:4), ADP was confirmed suitable for infants and children older than five years. Though ADP is suitable for use among children, Demerath and colleagues (2002:392) found ADP to be less reliable in children with lower FM, especially boys, and the error of measurement was estimated to be 1.7% fat mass. Also, ADP overestimated and underestimated %BF mass among children with lower and high FM and higher % FM, respectively, compared to the UWW (Demerath *et al.*, 2002:392). It can accommodate a large body volume and measure body density in obese individuals which can be used in both the 2-C and 3-C model, specifically for estimating body fat in severely obese individuals (Ginde *et al.*, 2005:1232).

Though ADP is reported to provide accurate predictions, previous authors have reported underestimation of FM and overestimation of FFM in when compared to DXA (Hames, *et al.*, 2014:6). On the contrary, in a South African study, an overestimation of FM and underestimation of FFM in infants has been reported (Wrottesley *et al.*, 2016:1256).

The ADP is quick, safe, comfortable, non-invasive, and automated; consequently, it does not require advanced skills to operate. It can accommodate different age groups and individuals with specific challenges, such as the elderly, obese people, and disabled persons. Though easy to use, it is not always feasible to use, due to the space required, the need for an operating technician and its cost (Douglas & Kennedy, 2012:24).

Heyward concluded that due to the limitations of densitometry, both UWW and ADP methods could not be considered optimum methods (Heyward, 2001:4).

2.4 Body composition validation

2.4.1 Validation studies in animals and cadavers (in vitro)

Most indirect methods used to measure body composition were validated against another indirect method; few studies were validated by direct cadaver analysis (Knight *et al.*, 1986: E179) and the dissection of human cadavers (McArdle *et al.*, 2015). Before 1945, body composition information was based on dissections of animal cadavers - the initial ones conducted around 1863 by Bischoff (Bozek, 1963: In Sheng, 1988:242). The main animals that were used were smaller mammalian species, such as rats and rabbits. This may be a challenge because of the substantial interspecies differences in water, protein, fat and mineral content between humans and mammalian species, making it difficult to extrapolate data to humans (McKellar & Hendry, 2009:1). Studies conducted in animals have shown that fat is the most variable body component. It has been stated that the best data on human body composition comes from cadaver studies and scientist (Ellis, 2000; Heymsfield, 1990; Janssens, 1994; Knight *et al.*, 1986; Wells, 2006) agree that for any method to

be regarded as the optimum standard, it should be comparable to cadaver values or techniques that have been validated against cadavers. Cadaver body composition analysis is the only accurate gold standard (Wells & Fewtrell, 2006:612).

The body composition data from cadavers is extrapolated from the dissection of few cadavers, although the few that were dissected were not dissected immediately after death. However, this is not always possible due to medical requirements following a sudden death. The other option is the dissection of a person who died in an accident (Mitchell *et al.*, 1945:627). Cadavers that have been put away for extended periods are generally embalmed, and embalming alters the fluid and the tissue weight (Clarys, 1984:466).

2.4.2 Studies on body composition methods and validation of body composition equations from developed and developing countries

Table 2-1: Selected body composition studies on children conducted in high income countries

Reference	Aim/objective	Country /population	Validation population	Method	Results /Conclusions
Reilly <i>et al.</i> (1995: 305,306)	Cross-validate the five commonly used equations for prediction of body fatness from skinfold	Glasgow Scottish (Caucasian)	64 boys (9.3±1.7 years) 34 girls 9.0±1.7 years	All equations use the Σ of 4 SFT except the Slaughter equations. Densitometry used as reference method. - Durnin and Rahaman: 12-16 yrs - Slaughter mean age 10yrs - Johnston <i>et al.</i> : 8-14 yrs - Brook; 1-11yrs - Deurenberg <i>et al.</i> : 10.5-11yrs	Distinct bias and large level of agreement (LOA) were observed between all equations compared to reference method. An example of the difference was between 5.3% fatness to 13-8% from Deurenberg equation, while 11.9% was reported from the reference method 11.9%. The Slaughter <i>et al.</i> , 1988 and Deurenberg (1989) equations overestimated body fatness and equations by Durnin and Rahaman and by Johnson underestimated fatness. The smallest bias and narrow LOA were observed with Brook equation. Different values were observed between the sum of two skinfold thickness and from the sum of four skinfold thickness.
Sarria <i>et al.</i> (1998:573)	Develop equations, anthropometric measurements to predict body density from UWW and BMI	Spain Spanish (Caucasian)	7.0-16.9 years old boys (n=175)	Σ of 4 SFT (Biceps, triceps, subscapular and suprailiac) BMI Body density by UWW	The best estimator of Body density was from a sum of 4 skinfolds and a combination of BMI and triceps skinfold. High correlation in assessing body Density by Skinfold thickness and BMI. $r^2 = 0.68$ and 0.67 for age groups 7-10.9 and 11-13.9 respectively ($p < 0.001$ in both) age groups. Prediction equations specific for each age group are necessary.
Tovar-Galvez <i>et al.</i> (2017: 424)	To estimate body fat percentage using regression equations and BIA to check for sex differences. Compare %BF by BIA and various Skinfold equations	Ceuta and Granada (Caucasian)	9-16 boys and girls (n=1518)	BIA to measure body fat percentage Brook and Durnin and Rahaman equations to calculate body Density. The %BF calculated from: Siri Brozek <i>et al.</i> Behnke <i>et al.</i> Lohman <i>et al.</i> Regression equations	Girls had higher percentage total body fat (% TBF) with all methods. Positive correlation between BIA and regression equations. The Behnke, 1942 and Lohman (1989) regression equations, combined with BIA, were found to be the most accurate methods for measuring body density in both sexes. No statistically significant differences were obtained for the Behnke ($p = 0.348$) and Lohman equations ($p = 0.383$). Equations of Siri and Brozek <i>et al.</i> slightly overestimate % TBF in both sexes. Authors proposed that equations considering sex and age should be used to estimate body density.

Reference	Aim/objective	Country /population	Validation population	Method	Results /Conclusions
Gutin et al. (1996:287;289)	Evaluated agreement of percentage fat values derived using the three techniques: BIA SFT and DXA	US African Americans and Caucasian children	9-11 years' old boys and girls (n=43)	Skinfold thickness (Triceps and calf) BIA DXA as reference method	%BF from the three methods were intercorrelated (all > 0.83). SFT showed the lowest difference with DXA measurement for FFM BIA and skinfold thickness underestimated fat mass and %BF. Authors concluded that the three methods were not interchangeable.
Atherton et al. (2013:2,8)	Compare fat mass (FM) and fat free mass (FFM) using new body composition	UK (Caucasian)	3.8–22.0 years (n=927)	FM and FFM were measured with DXA, BIA and skinfold thicknesses- 4 sites used - biceps, triceps, subscapular and suprailiac. TBW using deuterium dilution	BIA may be used to assess FFM instead of DXA BMI and SFTs can be used to measure adiposity for groups but not individuals. BMI significantly overestimated FM. DXA was the most accurate and precise indicator of FM Compared to the 4-C model (mean bias of 0.03 ($p = 0.002$) and limits of agreement of 60.62.
Bammann et al. (2013:520 & 523)	Compare different field methods for estimating body fat mass with a reference value derived by a three-component 3-C model	European (multicountry)	4–10 years boys and girls (n=78)	BIA, SFT (tricep, subscapular) WtHR WC, MUAC, Neck Circumference Used 3-C model as reference.	Anthropometry provide biased estimates as compared to reference method. Best LOA was achieved by combining circumference and SKF measurement
Lee et al. (2014:1)	Examine extent to which BIA calibration is influenced by ethnic differences in body size, proportions and composition.	UK- Multi-ethnic (caucasian, black Africa, Carribean and South Asian)	5-11 years' boys 8.5 ±1.7 years (n=698)	Fatfree mass, derived by deuterium dilution. BIA against deuterium dilution. Multiple linear regression models used to calibrate BIA	Ethnicity did not contribute significantly to the BIA calibration equation, authors concluded that ethnic-specific BIA calibration equation were not required.
Ittenbach et al. (2006:189,195)	Evaluating the validity of ADP as a body composition assessment technique for use with children	USA Caucasian, African American and Asian.	7-10 years (n=139)	ADP and anthropometry, to assess FM, FFM and %BF DXA was used as reference method.	ADP is valid and reliable for measuring FFM and FM rather %BF in children, but does not perform as well as anthropometry or DXA
%BF= percentage body fat; ADP= Air displacement plethysmography; BIA= Bioelectrical impedance analysis; BMI= Body mass index; DXA= Dual-energy X-ray absorptiometry; FM= Fat mass; FFM= Fat Free mass; LOA=Level of agreement SFT=Skinfold thickness; WHR= Waist to Hip Ratio; WC= Waist circumference. Σ =sum of;					

Table 2-2: Selected body composition studies on children conducted in developing countries

Reference	Aim/objective	Country /population	Age, Gender population size (n)	Method	Results/ Conclusions
Wickramasinghe <i>et al.</i> (2008:1238,1241)	Cross validate some prediction equations to determine the suitability in Sri Lankan children.	Colombo, Sri Lanka, Asian	Children 5-15 years' old boys and girls (n=282)	TBW assessed with D ₂ O SFT- triceps, biceps, subscapular, suprailiac and medial calf) Five equations were cross validated Deurenberg <i>et al.</i> (1990) Yuan <i>et al.</i> (2003) Slaughter <i>et al.</i> (1988) Brook, (1971) Sarria <i>et al.</i> (2001)	Brook's and Deurenberg <i>et al.</i> equations gave the lowest bias and the narrowest limits of agreement. The Deurenberg <i>et al.</i> equations using the sum of two or four sites overestimated %FM by 17.1% and 18.4% respectively. Prediction equations used were not suitable for Sri Lankan children. Need to be validated for use in different population groups because prediction equations are ethnic specific.
Resende <i>et al.</i> (2011:1167,1169)	Identify possible correlations and agreement between BIA and DDM in assessing FFM and TBW	Sao Paulo Brazilian	10-19 years (Adolescent) Overweight and obese Boys and girls (n=40)	Multi-frequency BIA and Deuterium Oxide as reference method	BIA overestimated FFM and TBW; and underestimated Fat mass compared with DDM. BIA provided a reliable estimate of the body composition in obese adolescents and compared well with DDM. BIA showed a strong correlation with FM & FFM by DDM and moderate correlation with TBW by DDM

Reference	Aim/objective	Country /population	Age, Gender population size (n)	Method	Results/ Conclusions
El Harchaoui et al. (2018:1663, 1670)	-Examine the validity of published BIA equations for assessing TBW and FFM using D ₂ O as a reference method -develop new FFM and TBW, BIA equations if needed.	Morocco Arabic	247 School children, 8-11 yrs. (n=247)	BIA, TBW equations tested: BIA TBW inbuilt Wells <i>et al</i> (2005) Davies and Gregory (1991) Wickramasinghe <i>et al</i> (2008). BIA, FFM equations: BIA FFM inbuilt Deuremberg <i>et al.</i> , (1991) Shaefer <i>et al.</i> , (1994) Lorenzo <i>et al.</i> (1998) Ruch <i>et al.</i> (2003) Wickramasinghe <i>et al.</i> , (2008) Hamrani <i>et al.</i> (2012) DDM as reference	Previously published equations showed significant bias. The Wells <i>et al.</i> , Davies and Gregory, Wickramasinghe <i>et al.</i> equations underestimated TBW in boys and girls. BIA inbuilt equation significantly overestimated TBW in girls. Deuremberg <i>et al.</i> , Shaefer <i>et al.</i> , Lorenzo <i>et al.</i> , and Ruch <i>et al.</i> significantly underestimated FFM in girls ($p < 0.05$). Wickramasinghe <i>et al.</i> and Hamrani <i>et al.</i> overestimated FFM. Authors recommended development precision equations to predict TBW and FFM for pre-pubertal Moroccan children
Ndagire et al. (2018:1, 6)	Derive predictive equations for TBW, FFM and FM by BIA and anthropometric measurements	Uganda (African)	8-19 yrs (n=203)	Anthropometry: WC, BMI, WHR, WtHRBC- BIA- single frequency D ₂ O used as reference method	Existing BIA analysis were found invalid for evaluation of TBW and FFM. Authors developed BIA predictive equations for TBW, FFM and FM and validated them for Uganda children
Ben Jemaa et al. (2018:1,4)	To propose new prediction equations to estimate body composition using impedance analysis	Tunisia (Asian) African	8 and 11 years (n=134)	BIA to assess body composition D₂O used to measure TBW and FFM	BIA underestimates fat mass and overestimates FFM and TBW. New equations applicable to Tunisia children 8 and 11 years were developed and showed good level of concordance between the TBW and FFM measured by isotope dilution.

Reference	Aim/objective	Country /population	Age, Gender population size (n)	Method	Results/ Conclusions
Wang and Hui, (2014: 2,6)	Validity of various published bioelectrical impedance analysis (BIA) equations in estimating FFM. Developing BIA equations for the estimation of fat-free mass (FFM)	China (Chinese)	9–19 years boys and girls (n=255)	24 published BIA equations DXA as reference method.	All BIA equations showed high correlations with FFM from DXA but none were statistically equivalent with DXA. Authors concluded that Different BIA devices are not interchangeable with DXA measurement in assessing individual FFM in their population.
Seo et al. (2018:2209,2214)	Validate BC analysis by BIA against DXA in obese children and to compare agreement between BIA and DXA	Korea Asians	6-17 years old boys and girls (n=316)	BIA DXA as reference method	The difference in FM, %FM and FFM by BIA and DXA was narrower with severely obese children than those who were moderately obese. BIA underestimated %BF and FM and overestimated FFM in children with mild to moderate obesity.
Diouf et al. (2018:773, 778)	Compare the WHO BMI-for-age definition of obesity against measured body fatness in African children.	8 African countries Ghana, Kenya, Mauritius, Morocco, Namibia, Senegal, Tunisia and United Rep. of Tanzania).	8-11 years (n= 2172)	WHO BMI reference values D₂O as reference method	WHO BMI-for-age underestimates excessive fatness in African children. Adiposity as measured by D ₂ O was more than 3 times the BMI-defined obesity.
Hoffman et al. (2012:157, 160)	Develop and validate new anthropometric predication equation for assessment of fat mass in children.	São Paulo, Brazilians	9.8 ±1.3 years (n=145)	SFT (bicep, tricep, subscapular, suprailiac, thigh and calf) Goran et al. (1996), Slaughter et al. (1988) Huang et al (2003) DXA as reference method	The Slaughter et al, 1988 and Goran et al 1996 Equations underestimated %BF. Existing prediction equations were not suitable for this population. New equations were developed and provided better prediction.

Reference	Aim/objective	Country /population	Age, Gender population size (n)	Method	Results/ Conclusions
Hussain <i>et al.</i> (2014:4)	Determine the correlation between DXA and skin fold thickness (SKF) equations for estimation of body fat	Pakistan Asians	9-19 years (n=99)	Skinfold thickness equations Slaughter <i>et al</i> (1988) Goran <i>et al</i> (1996) Dezenberg <i>et al</i> (1999) DXA as a reference method.	Slaughter equation accurately predicted %BF, Dezenberg <i>et al</i> 1999 and Goran <i>et al</i> 1996 equations had low level of accuracy. Authors recommend further studies for developing and validation of skinfold equations specific to Pakistani pediatric population.
van Zyl <i>et al.</i> (2019:1, 9)	Determined agreement between FFM measured by DXA and FFM calculated from published multi-frequency bioelectrical impedance prediction equations	South Africa (Black South African)	8.5 ±1.4 years (n=84)	BIA, DXA as a reference method.	The study validated 17 published BIA equations, among children aged five and ten years. Only the Clasey <i>et al</i> 2011; Deurenberg <i>et al</i> , 1991 and nielson <i>et al</i> , 2007 showed a narrow mean difference with DXA (-0.2±1.3, p value=0.264; -0.1±1.7, p value0.680 and -0.0 ±1.3, P value 0.954) respectively. Most of the published BIA equations to estimate FFM would not be suitable for use in this sample population. A new equation for this population group was developed.
%BF= percentage body fat; ADP= Air displacement plethysmography BIA= - Bioelectrical impedance analysis; BMI= Body mass index; ; DDM= deuterium dilution method; D2O- Deuterium oxide; DXA= Dual Energy X-Ray Absorptiometry; FM= Fat mass; FFM= Fat Free mass; %BF= Percentage body fat; LOA=Level of agreement; SFT=Skinfold thickness; TBW= Total Body water					

2.4.2.1 Body composition methods and validation of body composition equations from developed and developing countries.

The studies above reflect biases based on a population where the equations were validated (Tables 2.1 and 2.2). Factors that influence the prediction value when skinfold thickness is used is the site used and the sum of skinfold thickness. Most of the studies use DXA as the reference method, followed by isotope dilution. The studies have also included different age ranges with different maturity levels, and these factors may contribute to the discrepancies observed. The choice of reference method may or may not account for the discrepancies observed. Kulkarni *et al.* (2014:1151) found wide level of agreement between DXA and isotope dilution in estimating %BF, indicating poor agreement. Meanwhile, L'Abe'le *et al.* (2009:3) indicated that the differences between the two could be due to the fatness of the population being measured. In this study, the mean body fat difference measured by DXA and Isotope dilution was about 4% (21.7% and 17.5 %), respectively, among children aged 6-7 years. On the other hand, Vásquez *et al.* (2016) identified maturity as a factor in the discrepancies observed between DXA and isotope dilution compared to the 4-C model (Vásquez *et al.*, 2016:1119).

2.4.3 Summary of selected validation equations for body composition

Table 2-3: Summary of selected validation equations

BIA prediction equations	Objective	Age/ subject description	Body Component	Equation	Variables included in the equation	SEE	Results/Comment
Deurenberg et al. (1990)	The aim of the present study was to determine the relationship between densitometrically determined BF% and BMI	Age range of 7-83 years, this group is <15yrs	%BF	$BF\% = 1.51 \times BMI - 0.70 \times \text{age} - 3.6 \times \text{sex} + 1.4$	Age, gender, weight and height	In children: ($R^2 = 0.38$, (SEE) 4.4% = %BF). R^2 increased only from 0.38 to 0.39, and the SE of the estimate (SEE) of the prediction decreased only by 0.03 %	Adding age and gender to the prediction equation in children statistically improves the gap between densitometry determined %BF and BMI measures. The relationship between %BF by densitometry and BMI differs by age in children and significantly by gender in Adults In obese individual the prediction formulas slightly overestimated the BF%.
Kushner (1992:837)	We investigated the general utility of (BIA) and the implications of BIA theory in populations of various ages from infancy to adulthood	Neonates to adults 37 pre-pubertal	Compared TBW measured by BIA and deuterium dilution	$TBW \text{ (in Kg)} = 0.59 \text{ ht}^2/\text{R} + 0.065 \text{ wt} + 0.04$ (for all groups excluding children 0-60mnths)	Weight, age, ht^2/R , h^2 , $1/\text{R}$. impedance index(ht^2/R)	($r=0.995$ and SEE=1.67kg with Z. And $r=0.997$; SEE= 1.41kg with Z and wt. $r=0.996$ and SEE =1.47kg $r= 0.997$ SEE=1.24	impedance index was the strongest predictor ($r = 0.995$, SEE = 1 .67 kg) and weight as the only additional significant predictor ($r = 0.997$, SEE = 1.41 kg). The single best predictor of total body water (TBW) was height^2 , resistance (ht^2/R)
Durnin and Rahaman, (1967:686,688)	Assessment of the amount of fat in the human body from measurements of skinfold thickness	Adolescents 13-15 4 skinfold sites	Body fat Body density	Siri equation to measure %BF = $[(4*95/\text{density}) - 4.51 \times 100]$. Body density	Body density, sum of Skin fold	Correlation coefficients in boys= 0.760 girls = 0.778 with significant p-value-0.001 &	Only skin fold thickness (arm or thigh) measurement improved the prediction of Body Density. Anthropometric measurement did not improve the value of r.

BIA prediction equations	Objective	Age/ subject description	Body Component	Equation	Variables included in the equation	SEE	Results/Comment
				Male: $BD = 1.1533 - 0.0643 [\log \Sigma 4 \text{ skinfold thicknesses}]$ Female: $BD = 1.1369 - 0.0598 [\log \Sigma 4 \text{ skinfold thicknesses}]$		SEE= 3 and 3.5%.	
Brook, (1971:184)	Determine body composition of children from skinfold measurements.	Children aged 1-11years (13 obese and 10 stunted children) Population not specified	Body density	Siri's equation for % Body fat. Bd boys = $1.1690 - 0.0788 \times \log \text{sum of the skinfold thicknesses at 4 sites}$ Bd girls = $1.2063 - 0.0999 \times \log \text{sum of the skinfold thicknesses at 4 sites}$	Sum of skinfold	Correlation coefficient was 0.985 And residual sd =1.149	Over a wide range of age and physique skinfolds are a good predictor of TBW in prepubertal children. Brook found equations more appropriate for prepubertal children. Body water was predicted from skinfolds with an accuracy of $\pm 15.8\%$ in 95% of cases
Dezenberg et al. (1999:255)	Evaluate the accuracy and precision of previously published pediatric BC prediction equations. develop additional prediction equations	4-10.9 years Caucasian and African American Skinfold thickness (tricep and at skinfold sites measured)	Body fat	$FM \text{ .kg.} = .0:38 \times \text{body weight} + .0:30 \times \text{triceps} + .0:87 \times \text{gender} + .0:81 \times \text{ethnicity} - 9:42$	Weight, triceps skinfold, gender, ethnicity and abdominal skinfold estimated FM	$R^2 = 0.95$ and SEE kg was 0.46	Weight, triceps and gender improved the SEE but had little effect on R^2
Wickramasinghe et al. (2007)	Develop bioelectrical impedance analysis (BIA) equations to predict TBW and FFM of Sri Lankan children.	5-15 children Sri Lanka (South Asia)	BIA -TBW	$TBW (L) = 0.41 \text{ impedance index} + 0.17 \text{ weight} + 1.1 \text{ sex code} + 0.44$	Impedance, weight, gender	$R^2 = 86.3$ RMSE=2.1	Sex combined equation over or underestimated TBW by less than 500ml,
			BIA FFM equations	$FFM (kg) = 0.56 \text{ impedance index} + 0.22 \text{ weight} + 1.6 \text{ sex code} - 0.22$	Impedance, weight, gender	$R^2 = 86.9$ RMSE=2.7	Sex combined prediction for FFM had accuracy within 1kg
Wickramasinghe et al. (2008)	Develop a SFT prediction equation to estimate FM of Sri Lankan children	5-15 children Sri Lanka (South Asia)	FM and %FM D ₂ O used as	$FM [(0.68 \times \text{age}) + (0.246 \times \text{triceps SFT}) + (0.383 \times \text{subscapular SFT}) - (1.61 \times \text{sex code}) - 3.45]$	Age, sex code, triceps and subscapular SFT	$R^2 = 76.4$ RMSE= 3.4	CI was improved with inclusion of age, sex code, triceps and subscapular SFT

BIA prediction equations	Objective	Age/ subject description	Body Component	Equation	Variables included in the equation	SEE	Results/Comment
			reference method	% FM = (-0.28 × Age) + (0.49 × SFT-Tricep) + (0.34 × SFTSS) - (7.94 × Sex Code) - 26.8		R ² =46.9 RMSE = 8.6	
Slaughter <i>et al.</i> (1988:717)	Predictability of body fatness as determined by three separate approaches	8 - 29 y. (Prepubertal. Pubertal, post pubertal and adults Black and white males	% BF measured from Density (PFD)	1:21*.triceps . subscapular. -0:008* (triceps+ subscapular) ² 1:7 for white males;	Gender, race and maturation and two Skinfolts	SEE= 4.3 R ² =66	In the pre-pubertal group %BF was 23.5 Sum of triceps and calf and sum of triceps and calf squared plus were lower in all groups
			% BF measured from Density and water (PFDW)	1:21*.triceps . subscapular. - 0:008* (triceps+ subscapular) ² - 3:2 for black males		SEE =3.7 R ² =80	In the pre-pubertal group %BF was 20.4 Sum of triceps and calf and sum of triceps and calf squared plus were lower in all groups
			%BF measured from Body density, water, and bone mineral (PFDWB).	1:33*. triceps. subscapular. 0:013* (.triceps + Subscapular) ² -2:5 for all females		SEE= 3.6 R ² =82	In the pre-pubertal group %BF was 19.0 Sum of 2 skinfolds in combination with the addition of bone density and weight increased prediction accuracy, R ² increased from 62% to 77% and the SEE dropped from 4.5 to 3.9%.
BC= Body composition; h ² = height squared; ht ² /R= impedance index; FFM =Fat Free Mass; FM=Fat mass; R ² = regression analysis/the coefficient of determination; PFD=Body fat with Density; PFDW=Percentage Body fat with density and water; PFDWB= Percentage Body fat with Water and bone mineral; RMSE= Root Mean Square Error; SEE=Standard Error of Estimate SE=Standard Error; 1/R= correlation.; Wt=weight; y=year/s							

2.4.3.1 Discussion of a summary of selected validation equations for body composition (Table 2-3)

Body composition equations are sensitive to ethnicity, age, and gender (Table 2.3). Different equations have been developed for measuring different components (TBW, %BF, FFM and Density). The equations discussed above are the commonly used ones and have been validated in different populations (Caucasians, Asian and African Americans), but none have been developed for African children. Also, the equations used different predictors and varied in the outcomes: An equation developed by Deurenberg *et al.* (1990) used age and gender as predictors and demonstrated that in obese individual the prediction formulae slightly overestimated the BF%. Kushner (1992:837) equations reported height² and resistance (ht²IR) as the single predictor of TBW. In the Durnin and Rahaman (1967:686,688) equation, the arm and thigh skinfold thickness improved the prediction of body density. Brook (1971:184) reported skinfolds with an accuracy of $\pm 15.5\%$ in 95% of cases as predictor of body water. Wickramsighen *et al.* (2007) revealed sex combined equations to over and underestimating TBW by less than 500 ml. An equation by Slaughter and colleagues (1988:717) reported the sum of two skinfolds in contribution with the addition of bone density and weight increases the prediction accuracy, R² increased from 62% to 77% and SEE dropped from 4.5% to 3.9%.

2.5 Body composition

2.5.1 Components of Body composition

Measuring body components have proved to be important in assessing nutritional status in health and disease (Marra *et al.*, 2019:1), and it plays a significant role in monitoring and evaluating the effectiveness of nutritional interventions or treatment (Kuriyan, 2018:648).

Attempts to better understand human body composition have been made (Heymsfield *et al.*, 1989:1287), and various techniques have been developed to measure the different body composition in living humans throughout the life span (Kuriyan, 2018:648). However, finding an accurate measure has been a major challenge, Scientists acknowledge that the most accurate way to measure body composition is through direct chemical analysis of cadavers (Heymsfield *et al.*, 2015:2; Wells and Fewtrell, 2006:615). To measure all body components, different techniques that vary in sensitivity, cost, precision, invasiveness, and technical complexity are used (Lee & Gallagher, 2008:567; Kuriyan, 2018:648), since none of the existing techniques adequately measure all components (Duren *et al.*, 2008:1144).

2.5.2 Models for body composition assessment

The body weight is divided into compartments, with the 2-C model being the most basic and most commonly used (Forsum *et al.*, 2014:5889). Measuring accurately how body fat is distributed and where fat is accumulated is important to determine the related metabolic risk. Heymsfield and colleagues (2015:4) indicated that scientists had not reached a consensus on a reference approach to investigate and quantify fat mass *in vivo*. The 3-C and 4-C models use varying combinations of components, while grouping of these body components depends on the purpose of the study and the requirements of the investigators (Sheng, 1988:243). The various components are discussed in detail below. The discussion will only be limited to the 2, 3 and 4-C models due to their relevance to the current study.

2.5.2.1 The 2-Compartment (2-C) model

The 2-C model was first introduced by Behnke in 1942 and established the estimation of %BF using hydro-densitometry. Following Behnke's analysis, Brożek (1963), Keys and Brożek (1963) further classified the body into two components namely FM and FFM. Some of the assumptions on the 2-C model were based on assumptions made from the dissection of few male cadavers (Clarys, 1984:462).

The 2-C classification was based on the principle that skeletal size is directly related to FFM and that total body fat can be estimated from the weight to height index. However, the index should be highly related to body fat but be independent of height, such as in children below 15 years old. This principle was based on the wrong assumptions of stable body density and fat-free constant at all ages, genders, different development stages and different ethnic groups (Heymsfield, 1990:5), including during pregnancy (Bosaeus *et al.*, 2020:1485).

This method is also not suitable for use in certain conditions, such as in an individual experiencing weight loss and patients with unstable hydration (e.g., chronic renal failure and cirrhosis) (Morgan *et al.*, 2006:1160). Initial studies on body composition indicated that the 2-C model by Brozek *et al.* (1963), as cited by Lohman (1992), under-predicted FFM (1.5 to 2.0kg) and over-predicted %BF (3% to 4%) in children. Techniques that enable extrapolations to be made for the 2-C model include: Densitometry methods, namely ADP and UWW, whole-body counting of potassium and Hydrometry (isotope dilution). The last is the most commonly used. Recently multicomponent techniques are available that attempt to measure FFM more directly and with higher precision (Morgan *et al.*, 2006:1151).

2.5.2.2 The 3-Compartment (3-C) model

The 3-C model was defined by Siri in 1956 and reflects the density of certain components, namely water, fat and dry fat mass Siri, (1956:264). It uses the same techniques as the 2-C model but adds a different technique to measure TBW, measured by stable isotope dilution analysis or bone mineral content, measured by DXA (Marra *et al.*, 2019:2). The Siri model assumes that the fat free dry mass has a constant ratio of protein and mineral (Wells *et al.*, 1999:906)

The advantage of the 3-C model is that it measures FFM and TBW constituencies more directly, thus providing an accurate estimate of the hydration and density of FFM. In contrast to the 2-C model, it recognizes that water content of FFM differs by age groups and gender, and it provides an estimate of the hydration and density of FFM. Hence, the model is more accurate and can differentiate TBW, FM and fat-free dry tissue (Lara *et al.*, 2014:876). Most of the techniques used in the 3-C model are suitable for use in children (Kuriyan, 2018:255).

2.5.2.3 The 4-Compartment (4-C) model

Matiegka established the 4-C model in 1921 (Matiegka, 1921:223). The 4-C model can be classified into:

- a) Chemical - Fat, Water, Protein and mineral (Heyward & Stolarczyk, 1996:9; Lara *et al.*, 2014:872), which is the most commonly used;
- b) The anatomical model - muscle weight, skin plus subcutaneous adipose tissue and bone weight;
- c) Fluid model - Fat, Extracellular fluid (ECF), Intracellular fluid (ICF), Intracellular solids (ICS) and Extracellular Solids (ECS) (Heyward & Stolarczyk, 1996:9).

The model is obtained by combining several measurement techniques and is relatively vigorous in assessing differences between body components. It is based on principles similar to those of the three-component model but adds both TBW measured by stable isotope dilution analysis and bone mineral measured by DXA (Wells, 1999:904). Lastly, fat is calculated as the difference of weight from the sum of the other components subtracted from the total weight (Deurenberg, 2003:485).

The 3-C model has fewer assumptions, including the density of fat and protein (Fuller *et al.*, 1992:688), and the ratio of mineral and protein is constant (Fuller, 1992:689; Wells *et al.*, 1999:906). Some scholars regard it as the most accurate model for estimating body composition compared to other models (Wang *et al.*, 1998:335). To date, the 4-C model provides the most accurate *in vivo* measurement of FM and FFM (Atherton *et al.*, 2013:1).

2.5.3 Summary of how key body components relevant for the study are measured

2.5.3.1 Measuring fat-free-mass (FFM)

Body water is mainly present in FFM; therefore, obtaining an accurate TBW is used to estimate FFM. The most commonly used methods to measure FFM and FM include anthropometry, densitometry, BIA, isotope dilution for TBW (Weber, 2012:2), and DXA (Ellis *et al.*, 1994:660; Treuth, 2001:1353).

2.5.3.2 Measuring percentage body fat (%BF) and Fat mass (FM)

Body fat mass or percentage can be measured using various techniques; skinfold thickness measures subcutaneous fat thickness at various body regions. However, skinfold thickness is dependent on body fat distribution (Gupta, 2015:102). Skinfolds are particularly useful in monitoring changes in fatness in children. The BIA is also used to indirectly measure FM by measuring the body's resistance as a conductor to a very small alternating electrical current (Duren, 2008:1141; Gupta, 2015:101). Body fatness may be calculated using the multicompartments models, combining body density and measures of bone density and TBW. The multicompartments models also include the DXA (Fuller *et al.*, 1992:689; Wells *et al.*, 1999:904), which also reveals body fat distribution (Gupta, 2015:100). Other methods include anthropometric measurements such as mass, circumferences (i.e., neck, waist/abdomen, hip, and waist). Ultrasound measures of tissue structure is regarded as accurate measurement of subcutaneous fat thickness (Müller *et al.*, 2016:9).

2.5.3.3 Measuring Total body water (TBW)

TBW is used to estimate other body compartments such as FFM, FM, and body solids (Powers *et al.*, 2009:664). Isotope dilution is the most accurate method for measuring TBW in live subjects. The TBW volume is used to predict FFM and FM (i.e., body mass minus FFM) in normal-weight individuals. BIA is another technique used to predict TBW in healthy and sick patients and may be useful to monitor TBW changes over time (Powers *et al.*, 2009:667). Kushner and Schoeller (1986) reported that multiple regression equations by BIA are as precise as the DDM method in predicting TBW. This is useful, since cost-effective methods that can provide accurate predictions are needed, especially in poorly resourced countries.

2.5.3.4 Body density

According to Archimedes's principle:" when a body is immersed in a fluid, body volume is equal to the loss of weight in the water" (Ningthoujam, 2016:27940). The person's density is calculated as weight divided by volume, and from the example, it would be 75,000g ÷ 72, 000 cubic

centimetre (cc) or 1.0416 g cc^{-1} (McArdle *et al.*, 1986:491). McArdle *et al.* (1986:492) pointed out that once the body density is known, the next step is to convert the density value to an estimate of %BF. However, Gupta (2015:100) holds that UWW and ADP gives an actual and accurate measurement of body density based on Siri's 1956 measurements.

2.6 Strategies to manage and combat undernutrition and obesity

Numerous proposals have been made to address the double burden of malnutrition, i.e. over and undernutrition, while some proposals only focus on obesity. Seventeen years ago, at the 57th World Health Assembly (WHA), the Global Strategy on Diet, Physical Activity and Health was adopted (WHO, 2004:7). The Global Strategy stresses the need to limit consumption of saturated fats, trans-fatty acids, salt, and sugars through regulatory measures and increase consumption of fruit and vegetables and physical activity. The strategy also clarifies the role of different sectors (public and private sector, within government different departments including health, education, agriculture and trade) in achieving the objectives of the strategy. Some countries went ahead to develop country-specific plans or strategies.

Furthermore, in 2012 the World Health Assembly Resolution 65.6 endorsed a Comprehensive Implementation Plan on maternal, infant, and young child nutrition, which specified a set of six global nutrition targets aimed at addressing the double burden of malnutrition (WHO 2012:13). Following the establishment of the global nutrition targets, the World Health Assembly, as part of the WHO Global action plan for the prevention and control of non-communicable diseases 2013–2020 endorsed three diet-related global non-communicable disease targets namely; the salt reduction, halting the rise in obesity and reducing physical inactivity (UNSCN, 2017:16). The Global action plan was followed by the WHO Global Action Plan on Prevention and Control of non-communicable diseases 2013-2020, which the WHA approved as Resolution 66.10. Whilst these resolutions were approved by member states, implementation remains fragmentary due to a lack of coherence between the various stakeholders (UNSCN, 2017:16).

Many countries have focused on single approaches instead of a multipronged approach; for example, in Denmark, Austria, Hungary, Iceland, Norway and Switzerland, the unified focus was on reducing trans-fats in processed foods and salt reduction in some countries (Mouratidou *et al.*, 2014:12). The single interventions were followed by school-based interventions, which included increased intake of fruit and reducing consumption of non-alcoholic beverages in some instances (WHO: 2007:179). The various initiatives have increased uptake of the specific intervention; however, outcomes remain poor at a population level. Few countries have been able to halt or reduce obesity rates. Example of such countries include; Italy and Japan that have stabilized obesity rates (Mandal, 2017:3). Followed by a multisectoral approach implemented in Seinäjoki city in Finland that was able to achieve positive outcomes through the school health programme

and by incorporating health in all policies, changing their Health Care Act and involving various government levels, targeting children in early childhood development centres and schools. Over a seven-year period, the city was able to half the prevalence of obesity in five-year-olds (WHO, 2015). A few countries (Australia, England, France and New Zealand) have implemented multiple interventions, including labelling and education using various communication platforms to promote healthy eating and fiscal measures such as taxation of certain food products (Mandal, OECD:2017:2).

The South African National Development Plan (NDP), 2030, has identified non-communicable diseases as major threats over the next 20-30 years. The NDP targets are to reduce the prevalence of non-communicable diseases (Cardiovascular diseases, Diabetes, Cancer, Chronic respiratory diseases) by 28%. Among others, obesity and physical inactivity have been identified as risk factors that should be addressed. Strategies that have been developed to achieve the NCD targets include the Strategic Plan for the Prevention and Control of non-communicable diseases 2013-2017, the Strategy for the prevention and control of obesity in South Africa (2015-2020). These strategies have been partially implemented; Strategy for the prevention and control of obesity in South Africa, makes a recommendation of adoption of fiscal measures to address intake of unhealthy foods, South Africa became one of the countries to implement sugar sweetened beverage tax, salt reduction is one of the recommendations in the Prevention and Control of non-communicable diseases 2013-2017, South Africa has mandatory regulations for the reduction of sodium in certain foodstuffs. In partnership with Department of sports and recreation, South Africa has developed programmes to increase physical activity in various settings (Department of Sports and Recreation, 2015:63).

A renewed commitment is needed from countries considering that malnutrition is a multidimensional problem that needs a multisectoral approach, led by governments and involving various stakeholders. The new approach should be incorporated in various policies including, but not limited to, education, agriculture, health, environmental, economic and trade policies. Professor James Phillip, a London based obesity expert, has long advocated for an “All-of-government approach needed to tackle obesity” (WHO, 2013:552) involving many sectors. These strategies should be comprehensive and address drivers of obesity, barriers to healthy choices at environmental and socioeconomic levels (Chan & Woo, 2010:771). Governments should develop policies that protect consumers against the influence of industry. However, the food industry can play a role by reducing energy density by reducing fats, sugar and sodium content in food coupled with a reduction in portion size (Branca, 2007:94). Behaviour change requires an enabling environment, a supportive policy and regulatory environment that promotes a food system-reform process that can make healthy food accessible and affordable (UNSCN, 2018:10). These initiatives should be combined with strong public education, regulatory measures and

economic policy reform that prioritises investment that can promote health and increase access to healthier food options to create a balance towards economic and health benefits and promote physical activity in the school environment, workplaces and create a safe and conducive environment for the general public.

2.7 Summary

The findings from this study of the literature reveal a dearth of contextually relevant literature on the validation of body composition methods using DDM as the reference method. Furthermore, there is a sparsity of validation studies in developing countries, especially in Africa. In addition, results on body composition methods from previous studies are contradictory and inconclusive. Several studies recommend that population-specific equations should be developed and cross-validated. Based on the inconsistencies in various studies, it is not surprising that it is complicated to confidently advocate for a specific method to be the most suitable for a specific age group. Additionally, to date no study has yet validated body image perception using DDM as a reference method.

The equipment used to assess body composition in most studies is highly specialised and costly; therefore, it is not surprising that there are limited validation studies in Africa. With an increase in obesity, especially in children, validation studies are needed to accurately predict fatness in different population groups. Based on the literature, the most commonly used reference method is the DXA. There are limited studies that have validated the commonly used field techniques such as BIA and skinfold thickness with DDM. Therefore, based on the literature review, and with the assistance from the International Atomic Energy Agency (IAEA:2010) mission of helping developing countries to use stable isotopes to determine nutritional status and body composition in developing countries, the following instruments were selected for use in the current studies:

- Deuterium Dilution Methods (DDM) of a stable isotope of Fourier transform infrared (FTIR) spectroscopy (FTIR 4500t spectrophotometer, Agilent for the determination of TBW. Subsequent calculations of FM, %BF, fat free FFM. The FFM was calculated using age- and gender-specific Lohman hydration factors in children (IAEA, 2010; IAEA, 2011; Wang *et al.*, 1999). The FM was calculated by the difference between body mass and FFM: $FM (kg) = \text{body mass (kg)} - FFM (kg)$ (Moore & Boyden, 1963). Percent (%) BF was calculated: $FM (kg) / \text{body mass (kg)} \times 100$. Children were classified as underweight, normal weight, overweight and obese at the 2nd, 85th and 95th centiles based on the McCarthy *et al.* (2006) graphs for Caucasian children four to 20 years. Above 9th to below 85th was considered as normal fatness for the study. However, for the categories of overweight and obese, the cut-off points of 25% body fat in boys and 30% body fat in girls proposed by Williams *et al.* (1992) were applied. The cut-off points were established using

a skinfold thickness method previously validated against a multicomponent model to measure body fatness. The Williams *et al.* (1992 cited in Anzolin *et al.*, 2016:60) cut-off points, though relevant for African children, do not indicate underfat cut-off points. Therefore, the combination of the two sets of cut-off points could yield unbiased results. Additionally, the cut-off points set by Williams *et al.* (1992 cited in Anzolin *et al.*, 2016:60) are grounded in the findings that excessive fatness has been linked with increased cardio- and metabolic-risk profiles across wide age ranges (Chung *et al.*, 2018).

- Bioelectrical Impedance Analyses (BIA) was prepared using Bodystat 1500®MDD (MultiScan 5000, BodyStat, Ltd). The BIA was placed on the dorsal surface of the right hand and foot at the metacarpals and metatarsals, respectively. Detection electrodes were placed at the pisiform prominence of the right wrist and the anterior surface of the true ankle joint (Schoeller *et al.*, 1980:2286), which passes an imperceptible alternating current (frequency 50–60hz) from one to another, measuring the impedance. Various BIA equations have been developed to predict TBW from height and bioelectrical resistance. However, age-specific equations have been recommended due to the age-related differences in electrolyte concentration in the extra-cellular space relative to the intracellular space (Heyward & Stolarczyk, 1996:44). The BIA validated studies have been conducted in four European countries among 4-10-year-olds using BIA and skinfold compared to the 3-C model. Since validation of Bodystat 1500®MDD (MultiScan 5000, BodyStat, Ltd) instrument was mostly done in developed countries, some researchers have recommended a further validation of equations in different ethnic groups (Goran, 1998:507; Kushner *et al.*, 1992).
- Anthropometric measurements used were body mass (a portable electronic scale, Beurer Ps07 Electronic Scale®, Ulm, Germany), to the nearest 0.1 kg, and body height (Harpender portable stadiometer®, Holtain Limited, U.K.) with a perpendicular board to the nearest 0.1 cm were used to calculate BMI. From anthropometric measures of height and mass, the BMI was calculated as weight divided by height squared (Weight in kg/Height squared in metres), and BMI z-scores were calculated relative to WHO reference data (de Onis *et al.*, 2007:662). The WHO BMI z-score categories used were Standard Deviation (sd): less than -2sd for underweight; -2sd to +1sd for normal weight; greater than +1 sd for overweight and greater than +2 sd for obesity (de Onis *et al.*, 2007:1258). Also, triceps skinfold thickness was measured parallel to the long axis of the arm at the triceps-skinfolds site, and subscapular-skinfold was measured with the fold running obliquely downwards at the subscapular skinfold site. Skinfolds were taken with a Harpenden skinfold calliper (Holtain Limited®, U.K.) with a constant pressure of 10g/mm² to the nearest 0.2 mm. The sum of two skinfolds was calculated and used in the equations

such as the one developed for use in different settings by Slaughter *et al.* (1988), Wickramasinghe *et al.* (2008) and Dezenberg (1999). The skinfold prediction equations by Slaughter *et al.* (1988), Dezenberg (1999), and Wickramasinghe *et al.* (2008) were selected because of the age range and the skinfold sites used in validation studies which are similar to the study in this thesis. These equations have been validated and used in different populations and produced unpredictable BIA results (González-Ruiz, 2018:4; Janz, 1993:1070).

- Body image silhouettes: a simple questionnaire adapted from one which assessed concerns among girls and women (Mchiza, 2008) was used to assess body image perception. Additionally, eight Stunkard pictorial silhouettes of similar line drawings of girls and boys covering eight different sizes from thin to obese was incorporated to determine body image perception (Stevens *et al.*, 1999:36). The girls' silhouettes had previously been used in South African children (Mchiza *et al.*, 2015:10; Pedro *et al.*, 2016:5). The eight silhouettes for boys used, had not been tested in South Africa among 6- to 8-year-old boys. The self-perceived weight was assessed based on responses to one question, namely, "Which of the images resembles your weight?" The silhouettes used were from Stunkard Figure Ratings scales adapted for children (Mchiza, 2008). The figure scales (included in appendix G) in the current study were adapted to match the BMI classification for children older than five years such as: underweight was shown to look like silhouettes A and B, normal weight like silhouettes C and D, overweight like silhouettes E and F and obese like silhouettes G and H (Gardner *et al.*, 1999:118; Moore & Boyden, 1963; Stevens *et al.*, 1999:36).

The chapter has highlighted the importance of body composition in nutrition and health and the importance of accurately measuring various body composition components. Most studies determining obesity prevalence are based on BMI measures which, reflects a substantial underestimation of the magnitude of the problem and may delay initiation of interventions to prevent obesity and promote healthy behaviours, especially among children. It is also evident that Africa is behind with the development or validation of body composition techniques to accurately predict body composition among African populations. Lastly, there is no agreement among scholars as to the most precise method for measuring adiposity.

The following three chapters (articles which can be read independently) will use these methods in determining body composition validation and agreements of methods in determining body composition in South African children aged six to eight years, as informed by the literature review. Finally, the thesis will end with a conclusion and recommendations chapter.

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CHAPTER 3: ARTICLE 1: LEVEL OF AGREEMENT BETWEEN OBJECTIVELY DETERMINED BODY COMPOSITION AND PERCEIVED BODY IMAGE IN 6- TO 8-YEAR-OLD SOUTH AFRICAN CHILDREN: THE BODY COMPOSITION–ISOTOPE TECHNIQUE STUDY

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ABSTRACT

To assess the level of agreement between body size self-perception and actual body size determined by body mass index (BMI) z-score and body fatness measured by the deuterium dilution method (DDM) in South African children aged 6–to 8-years. A cross-sectional sample of 202 children (83 boys and 119 girls) aged 6–to 8-years from the Body Composition–Isotope Technique study (BC–IT) was taken. Subjective measures of body image (silhouettes) were compared with the objective measures of BMI z-score and body fatness measured by the DDM. The World Health Organization BMI z-scores were used to classify the children as underweight, normal, overweight, or obese. DDM-measured fatness was classified based on the McCarthy centile curves set at 2nd, 85th and 95th in conjunction with fatness cut-off points of 25% in boys and 30% in girls. Data were analyzed using SPSS v26. Of 202 children, (32.2%, 55.1%, 8.8%, and 2.4%) perceived their body size as underweight, normal, overweight, and obese, respectively. Based on BMI z-score, (18.8%, 72.8%, 6.9%, and 1.5%) were classified as underweight, normal, overweight, and obese, respectively. Body fatness measurement showed that 2.5%, 48.0%, 21.8%, and 29.7% were underweight, normal weight, overweight, and obese, respectively. The application of silhouettes and BMI z-scores resulted in either overestimation or underestimation of own body size. Overall, the levels of agreements (kappa, κ) between body size perception, body fatness, and BMI for age respectively, were small ($\kappa=0.083$, $p=0.053$ and $\kappa=0.154$, $p<0.001$). Level of agreement between body size perception, body fatness, and BMI z-score was poor. The use of silhouettes made children either overestimate their own body size while being underweight or underestimate their own body size while being overweight or obese. Given the potential health implications associated with misclassification of body size during childhood, correct self-assessment of body size is important, and may be key to the adoption of weight control strategies directed at curbing the escalating obesity epidemic in the country. Scalable measures to allow for more accurate self-assessment are urgently needed—one approach is behavior change communication at all levels.

Keywords: Perceived body image, body size, stable isotopes, body composition, South African children.

INTRODUCTION

Childhood obesity is becoming a global public health problem [1]. Being overweight or obese has been linked with a number of childhood metabolic conditions such as insulin resistance and cardiovascular disease [1-3]. Early identification of overweight and obese children should be prioritized in public health initiatives. Epidemiological studies highlight obesity as caused by a myriad of factors, including social and cultural influences [2,4], and the consumption of energy-dense diets with low nutritional benefits [5]; ultra-processed foods including refined carbohydrates and added sugar; and foods high in sodium, saturated fat, and trans fats [6].

The first South African National Health and Nutrition Examination Survey (SANHANES-1), a survey of a national representative sample of South Africans conducted in 2012, showed that the prevalence of overweight and obesity was lower among children aged 6–9 years (4.5% and 2.7%, respectively) than in children aged 2–5 years (17.5% and 4.4%, respectively) [7]. South Africa, being a developing country, is experiencing a nutrition syndemic (i.e. the coexistence of underweight and obesity, coupled with micronutrient deficiencies) across the lifespan [8]. The nutrition syndemic in children is partially related to the development of the pediatric metabolic syndrome [9-11]. Furthermore, obesity has been linked to psychological disorders that manifest as low self-esteem and body size dissatisfaction [12].

A number of scholars [13-18] suggest that compared with body mass index (BMI) and other objective weight measures, body size perception may be a better predictor of behavior change and is associated with better body size management. As such, correct self-assessment of body size is important, and may be the key to the adoption of weight control strategies directed at curbing the escalating obesity epidemic in the country. Body image perception is defined as a multidimensional construct encompassing how individuals perceive, think, and feel about their body size [19,20]. Links between body image perception and psychosocial problems, e.g. low self-esteem and depression, have been observed [21-24]. Some researchers state that body image perception is dynamic and changes at different stages of development [25]. Weight perception is reported to be highly influenced during the growth spurt, when significant changes occur to the child's body [20]. Other authors have identified culture and ethnicity [4], as well as media platforms such as television programs, cartoons, and magazines [26,27], as contributory factors to body image dissatisfaction. Among Africans, larger bodies have been considered desirable [14,28-31]. It can be argued that if not addressed, satisfaction with an oversized body may promote the development of obesity-related non-communicable diseases.

In the SANHANES-1 study [7], 84.5% of adult South Africans were shown to have a largely distorted body image, with 45.3% highly dissatisfied with what they perceived their body size to be at the time when the survey was undertaken. The findings also showed that overweight and obese South Africans underestimated their body size and had desires to be thinner. A study

conducted with 11- to 15-year-old South African children from Mpumalanga Province [31] showed that the majority of the girls (83.5%) were dissatisfied with their body size. In this case, the children desired to be thinner or fatter than what they perceived their body size to be. Additionally, girls who wanted to be fatter had significantly higher BMI ($p < 0.001$) than the girls who wanted to be thinner [31]. Findings from a prospective cohort study involving 278 adolescents aged 13 to 17 years by Assaad and colleagues [32] reported that 39.9% and 10.1% perceived themselves as obese and overweight, respectively. The study also reported a fair agreement (60.8%, kappa statistic $[\kappa] = 0.319$) between measured BMI percentile and self-perceived BMI.

Poor correlation between body size perception and BMI in children and adolescents has been reported by various scholars [31,33-36]. Some attribute this to children and adolescents possibly being unable to perceive their body size accurately, especially when the image is used twice to select current status and ideal body size [20,37]. Lombardo and colleagues argued that body size perception in children below the age of 8 years is inaccurate [38]. A study by Descamps reported that almost one-third of adolescents misperceived their body weight, with girls being more likely than boys to have body size misperceptions (27.3% of boys vs 42.2% of girls) [39]. Body image in children is frequently determined by figural stimuli such as silhouettes [40,41]. Most of these figure scales show figure contours and avoid showing race characteristics such as hair and skin color, making the instruments reliable, valid, and suitable for use among different ethnic groups [42].

To the best of our knowledge, no research has attempted to compare the level of agreement between body size perception and measured body fatness in 6- to 8-year-old children from African countries, especially South Africa. The main aim of this study, therefore, was to determine self-perceived body size among 6- to 8-year-old South African children and the level of agreement between perceived and actual body size (BMI for age), as well as body fatness objectively measured by the deuterium dilution method (DDM).

Materials and methods

Study design and participants

A cross-sectional design was used with a total of 202 children (83 boys, 41.1% and 119 girls, 58.9%) aged between 6 and 8 years with complete data on body image by questionnaire, drawn from a sample of 299 children from a larger study on body composition using an isotope technique (the Body Composition–Isotope Technique [BC–IT] study; 2018/2019). Five primary schools were randomly selected from a total of 26 primary schools in Tlokwe City in North-West Province, South Africa. The sample size was calculated using Open Epi software, Version 3 [43], in which Fleis's [44] formulae for cross-sectional studies were applied to determine the appropriate sample size for a power of 0.80 and α -level of 0.05 at a CI of 95%. The power calculation was based on the

primary hypothesis of the larger study, of a negative association between excessive fatness and physical activity and the odds of having excessive percentage body fat (%BF) in the inactive group, from which it was found that a minimum sample size of 297 was needed, based on an expected prevalence of combined overweight/obesity of 20% and physical inactivity of 30% in the children. Every third child on each class list was selected to participate in the study, but only those with signed parental informed consent forms who personally agreed to participate were finally included. The BC–IT larger study examined the relationships between more complex (using a stable isotope method and Bioelectrical Impedance Analysis (BIA)) and more indirect measures of body composition (using anthropometric variables), and objective and subjective measures of physical activity, and their relationships thereof, with other health-related determinants, among 6- to 8-year-old South African children. The parents of the children in the study as well as the children gave consent and assent to participate. The school principals and the district office of the Department of Basic Education in Dr. K. K. Kaunda District of North-West Province granted study permission. Subsequently, the Health Research Ethics Committee of the Faculty of Health Sciences (HREC) of North-West University gave ethical approval for the study (ethics no: NWU-00025-17-A1).

Anthropometry

Weight was measured with a portable electronic scale (Beurer Ps07 Electronic Scale, Ulm, Germany) to the nearest 0.1 kg and children were measured wearing light clothing, without shoes on. Stature was measured to the nearest 0.1 cm using a Harpenden portable stadiometer (Holtain Limited, UK) with a perpendicular board. BMI was calculated as weight divided by height squared (weight in kg/height squared in meters) and BMI z-scores were calculated relative to World Health Organization (WHO) reference data. The WHO BMI z-score categories used were: underweight: –2 SD from the median; normal weight: –2 SD to +1 SD; overweight: more than +1 to +2 SD; and obesity: more than +2 SD [45].

Body fat percentage by stable isotope dilution

The DDM and protocol used in our study followed the guidance provided by the International Atomic Energy Agency (IAEA) [46,47] and were also implemented as part of a multicenter study involving eight other African countries [48]. Pre-dose saliva samples were obtained from the study children after an overnight fast. Then, each child was given a dose of accurately weighed deuterium oxide-labelled water, which was prepared according to their body weight. Doses were administered under close supervision, with drinking straws to avoid potential spillage. Each child's dose bottle was rinsed twice with water (50 ml per rinse) and the rinsing water was consumed to ensure complete intake of the dose. The time of dose administration was recorded for each child. Two saliva samples were collected, at 2 hours and 3 hours post dose. From the time of dose

administration until the last saliva sample was collected, children were asked to stay in the study location and were required to avoid physical activity, to minimize water loss in breath and evaporation from the skin. Children had access to non-vigorous activities such as coloring books and storytelling to prevent boredom during the waiting period until the study protocol was completed. Children were not allowed to eat or drink anything apart from the light snack that was provided after completion of the sample collection, but they were permitted to go to the bathroom to void their bladders at any time. At each saliva sampling, the child was given a ball of cotton wool to rotate in the mouth until well soaked; the wet cotton wool was then transferred into a syringe to squeeze out the saliva into a plastic vial that was immediately capped to avoid evaporation [41]. For children who could not wet their cotton wool balls after several attempts, an alternative approach was used; such children were asked to spit directly into the vial. The plastic vial was encased in a sealable bag preventing possible losses from evaporation and cross-contamination during storage. Saliva samples were stored at -20°C in the laboratory until the analysis was performed. Saliva samples were analyzed using Fourier transform infrared (FTIR) spectroscopy (FTIR 4500t spectrophotometer, Agilent). Fat free mass (FFM) was calculated using age- and gender-specific Lohman hydration factors for children [49,47]. Fat mass (FM) was calculated by the difference between body mass and FFM: $\text{FM (kg)} = \text{body mass (kg)} - \text{FFM (kg)}$ [50]. %BF was calculated as $\text{FM (kg)} / \text{body mass (kg)} \times 100$. Children were classified as underfat, overfat, and obese at the 2nd, 85th, and 95th centiles based on the graphs of McCarthy *et al.* [51] for Caucasian children. Above 9th to below 85th centile was considered as normal fatness for this study. However, for the categories of overweight and obese the cut-off points of 25% body fat in boys and 30% body fat in girls by Williams *et al.* [52] were applied, which had been established using a skinfold thickness method previously validated against a multicomponent model to measure body fatness. The Williams [52] cut-off points, though relevant for African children, do not include under fat cut-off points. Therefore, the combination of the two sets of cut-off points would yield unbiased results. Additionally, the cut-off points by Williams [52] are grounded in the findings that excessive fatness has been linked with increased cardiometabolic risk profiles across wide age ranges [53].

Body image silhouettes

A simple questionnaire adapted from another questionnaire that assessed body image status among girls and women [54] was used to assess body image perception, together with eight pictorial silhouettes of similar line drawings of girls and boys covering eight different sizes from thin to obese [55]. The girl silhouettes had previously been used in South African children [15,31]. The eight silhouettes for boys had not been tested in South Africa among 6- to 8-year-old boys. Self-perceived body size was assessed based on responses to one question: "Which of the images resembles your weight?" A figure scale linked to this question was presented and explained to each child by a well-trained research assistant, before a child made a choice of a

figure that resembled him/her. The intra-class correlation between self-perceived body size and children's actual BMI category was 0.36, with a Cronbach's alpha value, based on standardized items, of 0.74. The body image perception of each child was compared with the BMI using the BMI z-scores and with the %BF objectively measured using the DDM.

The silhouettes used were from Stunkard Figure Ratings scales adapted for children [54]. The figure scales in our study were adapted to match the BMI classification for children older than 5 years as follows: underweight was shown to look like silhouettes A and B, normal weight like silhouettes C and D, overweight like silhouettes E and F, and obese like silhouettes G and H [42,54,55].

Statistical analysis

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS v26.0). Descriptive statistics (mean, minimum, maximum, and SDs) and the *t*-test were used to compare groups. Pearson's Chi-square (χ^2) test was used to determine significant differences for the BMI, BMI z-scores, %BF, and body weight perception categories, separately by sex. The agreement between children's own perceptions and actual body weight/fatness across the four categories was assessed using Cohen's kappa (κ) statistic. Furthermore, agreement between children's own perceptions across the four categories and actual body fatness was compared using Cohen kappa's statistic (κ) to determine the magnitude of agreement between perceived and actual body size. Categories of actual body size (fatness) were derived for each child based on their %BF measured by the DDM. Additionally, the actual difference in percentages between the perceived body image and BMI, as well as the difference between perceived body weight and body fatness, were calculated. Weighted κ coefficients (criterion validity) were interpreted according to Landis and Koch for strength of agreement: $\kappa < 0.20$: poor agreement; $\kappa = 0.21-0.40$: fair agreement; $\kappa = 0.41-0.60$: moderate agreement; $\kappa = 0.61-0.80$: good agreement; and $\kappa = 0.81-100$: perfect/very good agreement [56]. Kendall's tau-c (T_c , also called Stuart-Kendall tau-c), a non-parametric measure, was used to assess the concordance among the children's body image perception silhouette choice, BMI, and %BF determined by DDM. T_c ranges from 0 (no agreement) to 1 (complete agreement) and is more suitable than tau-b for the analysis of data based on non-square (i.e. rectangular) contingency tables [57–59]. The level of statistical significance was set at $p \leq 0.05$.

RESULTS

Table 1 represents descriptive characteristics of boys and girls. No significant gender differences ($p > 0.05$) were found with age, weight, height, BMI, and BMI for age z-score. Significant gender differences ($p < 0.001$) were observed for FM, %BF, and FFM in kg, with girls having significantly

greater FM and %BF ($p<0.001$) than boys. However, boys had significantly ($p=0.004$) more FFM (17.36 ± 2.74) than girls (16.21 ± 2.85).

Table 1. Participant characteristics on demographic, body composition and gender differences

	Boys (n=83)			Girls (n=119)		P-value for gender differences
	Mean	Minimum	Maximum	Mean $\pm$ SD	Mean $\pm$ SD	
Age (years)	7.57 $\pm$ 0.85	6.00	8.96	7.62 $\pm$ 0.79	7.54 $\pm$ 0.90	0.55
Weight (kg)	22.70 $\pm$ 4.53	14.80	43.60	22.69 $\pm$ 4.15	22.76 $\pm$ 4.80	0.91
Height (cm)	120.21 $\pm$ 7.02	103.50	139.90	120.83 $\pm$ 6.25	119.76 $\pm$ 7.50	0.28
FFM (DDM) (kg)	16.67 $\pm$ 2.84	10.71	27.12	17.36 $\pm$ 2.74	16.21 $\pm$ 2.85	0.004
FM (DDM) (kg)	6.03 $\pm$ 2.45	2.23	19.49	5.30 $\pm$ 2.06	6.56 $\pm$ 2.58	<0.001
%BF (DDM)	26.04 $\pm$ 6.17	13.04	44.71	22.02 $\pm$ 5.30	28.14 $\pm$ 5.87	<0.001
BMI (kg/m ²)	15.60 $\pm$ 1.99	12.03	26.99	15.43 $\pm$ 1.74	15.73 $\pm$ 2.15	0.29
BMI for age z-score	-0.17 $\pm$ 1.08	-2.92	3.25	-0.30 $\pm$ 1.08	-0.10 $\pm$ 1.07	0.18

%BF: Percentage body fat; BMI: body mass index; DDM: deuterium dilution method; FFM: fat free mass; FM: fat mass; SD:

standard deviation.

The results in Table 2 reflect how children viewed themselves based on their body size perception, measured using silhouettes and compared with actual weight classification as determined by BMI and the DDM. Using χ^2 for categorical variables, significant differences were found in %BF measured by the DDM and BMI for both boys and girls, and borderline significant differences in silhouettes chosen for ideal weight status for both genders.

Table 2. Perceived body sizes among children by total group and sex. This is the Table 2 legend.

	Responses	Total group (N=202)		Boys (n=83)				Girls (n=119)		χ^2	p-value
		n	%	n	%	χ^2	p-value	n	%		
Silhouette chosen for ideal weight status	Underweight	66	33.0	33	39.3	7.072	0.07	33	27.3	23.084	<0.0001
	Normal for your age	113	56.0	41	48.8			72	59.5		
	Overweight	18	9.0	6	7.1			12	9.9		
	Obese	5	2.0	3	3.6			2	1.7		
%BF measured using DDM	Under fat/thinness	5	2.5	5	6.0	51.024	<0.0001	-	-	5.765	0.056
	Normal	97	48.0	48	57.8			49	41.2		
	Overfat	42	20.8	14	16.9			28	23.5		
	Obese	58	28.7	16	19.3			42	35.3		
BMI	Underweight BMI z < -2 SD	38	19.0	19	22.9	113.964	<0.0001	19	16.0	146.714	<0.0001
	Normal BMI z -2 to +1 SD	147	73.0	61	73.5			86	72.3		
	Overweight BMI z > +1 SD	14	7.0	2	2.4			12	10.1		
	Obese BMI z > +2	3	1.5	1	1.2			2	1.7		

%BF: Percentage body fat; BMI: body mass index; DDM: deuterium dilution method.

χ^2 : Chi-square test to compare categories between boys and girls.

Table 3 shows that out of 202 children, 2.5% (n=5), 48.0% (n=97), 20.8% (n=42), and 28.7% (n=58) were under fat, normal, overfat, and obese, respectively, as determined by the DDM. When BMI z-scores were used, 19.0% (n=38), 73% (n=147), 7% (n=14), and 1.0% (n=3) were underweight, normal, overweight, and obese, respectively. Based on

the silhouettes they selected, 33.0% (n=66), 56.0% (n=113), 9.0% (n=18), and 2.4% (n=5) perceived their body size as underweight, normal, overweight, and obese, respectively.

On comparing the accuracy of body size identification between perception using silhouettes and the measures of body fatness using the DDM (Table 3), the silhouette method was shown to overestimate underweight by 92.0% and underestimate overweight and obesity by 57.0% and 91.0%, respectively. These findings suggest that measuring body size perception using silhouettes was 12 times more likely to overestimate underweight than using the DDM, while children were 2 and 11 times more likely to underestimate overweight and obesity, respectively, compared with the DDM. On comparing the accuracy of body size identification between perceptions using silhouettes and BMI, the silhouettes overestimated underweight, overweight, and obesity by 42.0%, 22.0%, and 40.0%, respectively, compared with BMI z-score category. Overall, using silhouettes for body size perception overestimates underweight and grossly underestimated obesity compared with the DDM, whilst comparison between silhouettes and BMI z-score category indicated that silhouettes overestimate underweight, overweight, and obesity.

In comparing the accuracy of body composition measurements between BMI z-score category and the DDM, BMI z-score category was seven times more likely than the DDM to overestimate underweight and more likely to underestimate overweight and obesity.

Table 3. Differences in actual absolute numbers and percentage for silhouettes, BMI, and %BF categories by DDM. This is the Table 3 legend.

Classifications	DDM	Silhouettes	Differences in absolute number and percentage, n(%)
	n(%)	n(%)	n(%)
Underweight	5(2.5)	66(33)	61(92)*
Normal weight	97(48.0)	113(56)	16(14)*
Overweight	42(20.8)	18(9)	24(57) [#]
Obese	58(28.7)	5(2)	53(91) [#]
	DDM	BMI	
Underweight	5(2.5)	38(19)	33(87)*
Normal weight	97(48)	147(73)	50(34)*
Overweight	42(20.8)	14(7)	28(67) [#]
Obese	58(28.7)	3(1)	55(95) [#]
	BMI	Silhouettes	
Underweight	38(19)	66(33)	28(42)*
Normal weight	147(73)	113(56)	34(23) [#]
Overweight	14(7)	18(9)	4(22)*
Obese	3(1)	5(2)	2(40)*

%BF: Percentage body fat; BMI: body mass index; DDM: deuterium dilution method.

** represents underestimation and * represents overestimation.

Table 4 presents categories of %BF using the DDM and κ level of agreement between the DDM and perception of body image by silhouettes. In the total group, two of the children who were actually underweight perceived themselves as underweight, 30 who were normal weight perceived themselves as normal weight, and 8 and 9 who were actually overweight and obese, respectively, perceived themselves as overweight or obese. The level of agreement between body weight perception when compared with the DDM was poor ($\kappa=0.083$, $p=0.053$) for the total group. The concordance determined by T_c between DDM and silhouette body image perception was low and significant ($T_c=0.154$, $p=0.011$) for the total group.

When κ was calculated separately for boys and girls, the level of agreement for boys was poor ($\kappa=0.023$, $p=0.702$) compared with the girls ($\kappa=0.152$, $p<0.01$). Two of the boys who were actually underweight perceived themselves as underweight, 10 who actually had normal weight perceived themselves as normal, and 1 and 3, respectively, who were overweight and obese perceived themselves as overweight and obese. The concordance determined by T_c between DDM body fatness and silhouettes (perception of body image) in boys was very low and not significant ($T_c=0.006$, $p=0.946$). No girls were actually underweight and perceived themselves as underweight, 20 girls of actual normal weight perceived themselves as having normal weight, and 7 who were actually overweight and 6 actually obese perceived themselves as overweight and obese, respectively. The concordance determined by T_c between the DDM measurement and perception among the girls was fair ($T_c=0.299$, $p<0.001$).

Table 4. Cohen's κ for percentage of body fat measured by DDM and self-perception of weight using silhouettes.

			Current weight silhouettes (count)				Total
			Underweight (Pictures 1 & 2, -3 SD to -1 SD)	Normal weight (Pictures 3 & 4, -1 SD to +1 SD)	Overweight (Pictures 5 & 6, > +1 SD)	Obesity (Pictures 7 & 8, > +2 SD)	
Males	%BF by DDM	Underfat	2	2	0	1	5
		Normal	17	10	13	8	48
		Overfat	8	3	1	2	14
		Obese	3	7	3	3	16
	Total		30	22	17	14	83
Females	%BF by DDM	Underfat	-	-	-	-	-
		Normal	22	20	7	0	49
		Overfat	10	9	7	2	28
		Obese	11	6	19	6	42
	Total		43	35	33	8	119
Total	%BF by DDM	Underfat	2	2	0	1	5
		Normal	39	30	20	8	97
		Overfat	18	12	8	4	42
		Obese	14	13	22	9	58
	Total		73	57	50	22	202
Symmetric measures							
Gender			Value	Asymptotic standard error ^a	Approximate T ^b	Approximate significance	
Males	Ordinal by ordinal	T_c	.006	.090	.068	.946	
	Measure of agreement	κ	-.023	.058	-.382	.702	
	N of valid cases		83				
	Ordinal by ordinal	T_c	.299	.075	3.940	.000	
Females	Measure of agreement	κ	.152	.063	2.530	.01	
	N of valid cases		119				
	Ordinal by ordinal	T_c	.154	.061	2.541	.011	
	Measure of agreement	κ	.083	.045	1.933	.053	
Total	N of valid cases		202				

%BF: Percentage body fat; DDM: deuterium dilution method; T_c : Kendall's tau-c.

^a Not assuming the null hypothesis. ^b Using the asymptotic standard error assuming the null hypothesis.

Table 5 presents BMI categories and κ level of agreement between BMI category and perception of body image by silhouettes. In the total group, 24 children who were actually underweight perceived themselves as underweight, 47 who were normal weight perceived themselves as normal weight, and 8 and 2, who were actually overweight or obese, respectively, perceived themselves as overweight or obese. The level of agreement between body weight perception when compared with BMI category was poor, although significant ($\kappa=0.154$; $p<0.001$) for the total group. The concordance determined by T_c between BMI category and body image perception by silhouette was low but significant ($T_c=0.231$, $p<0.001$) for the total group.

When κ was calculated separately for boys and girls, 9 of the boys who were actually underweight perceived themselves as underweight, 17 who were actually normal weight perceived themselves as normal weight, and none of the boys who were overweight and obese perceived themselves as overweight and obese. The level of agreement between body weight perception and BMI category was poor ($\kappa=0.040$; $p=0.450$). The concordance between BMI category and body image perception by silhouette was very low and not significant ($T_c=0.053$, $p=0.481$).

For the girls, 15 were actually underweight and perceived themselves as underweight, 30 who were normal weight perceived themselves as normal weight, and 8 who were overweight and 2 who were obese perceived themselves as overweight and obese, respectively. The level of agreement between body weight perception and BMI category was poor, but significant ($\kappa=0.232$; $p<0.001$). The concordance between BMI category and perception among the girls was moderate and significant ($T_c=0.378$, $p<0.001$).

Table 5. Cohen's κ for body weight by body mass index category and perception by silhouette.

BMI_Categ_ANALYSES * CURRENTWEIGHT_SILHOUETTES * SEX Cross tabulation							
			Current weight silhouettes (count)			Total	
Gender			Underweight (Pictures 1 & 2, −3 SD to −1SD)	Normal weight (Pictures 3 & 4, −1 SD to +1 SD)	Overweight (Pictures 5 & 6 > +1 SD)	Obesity (Pictures 7 & 8, > +2 SD)	
Males	BMI	Underweight	9	3	3	4	19
		Normal	21	17	14	9	61
		Overweight	0	1	0	1	2
		Obese	0	1	0	0	1
	Total	30	22	17	14	83	
Females	BMI	Underweight	15	4	0	0	19
		Normal	28	30	25	3	86
		Overweight	0	1	8	3	12
		Obese	0	0	0	2	2
	Total	43	35	33	8	119	
Total	BMI	Underweight	24	7	3	4	38
		Normal	49	47	39	12	147
		Overweight	0	2	8	4	14
		Obese	0	1	0	2	3
	Total	73	57	50	22	202	
Symmetric measures							
Gender			Value	Asymptotic standard error ^a	Approximate T ^b	Approximate significance	
Males	Ordinal by ordinal	T _c	.053	.075	.704	.481	
	Measure of agreement	κ	.040	.052	.755	.450	
	N of valid cases		83				
Females		T _c	.378	.055	6.857	.000	
	Measure of agreement	κ	.232	.056	4.888	.000	
	N of valid cases		119				
Total	Ordinal by ordinal	T _c	.231	.048	4.791	.000	
	Measure of agreement	κ	.154	.040	4.342	.000	
	N of valid cases		202				

BMI: Body mass index; T_c : Kendall's tau-c.

^aNot assuming the null hypothesis. ^bUsing the asymptotic standard error assuming the null hypothesis.

DISCUSSION

The main purpose of the study was to assess the level of agreement between self-perceived body size, BMI category, and actual body fatness determined using DDM, in 6- to 8-year-old South African school-going children. Generally, a poor to fair level of agreement between self-perceived body size, actual body fatness, and BMI category was found among the children. In summary, self-perceived weight status was generally not consistent with actual weight status (BMI for age z-score) and objectively measured body fatness.

Studies of children that have used DDM to measure body composition have been limited from African countries, including South Africa. One example is a multi-country study that generated data on body composition from 1516 children aged 8 to 11 years from eight African countries (Ghana, Kenya, Mauritius, Morocco, Namibia, Senegal, Tunisia, and the United Republic of Tanzania). The prevalence of excessive fatness, measured using DDM, was three times higher (29.1%–31.4%, $n=441$) than the prevalence of overweight or obesity (8.8%–10.1%, $n=133$) defined as WHO BMI z-score $> +2$ SD [48].

In another study, undertaken in 9727 children aged 7 to 18 years from four districts in the Jin City of China, the prevalence of underweight, normal, and overweight determined by BMI was 4.9%, 64.3%, and 30.8%, respectively, whilst approximately 19.8%, 57.8%, and 22.4%, perceived themselves as underweight, normal weight, and overweight, respectively. This study confirmed that underweight is overestimated and overweight underestimated when using body size perception as a measure [20]. The prevalence of underweight was much lower and the prevalence of overweight much higher than in our study. Many factors could have contributed to these differences. Ethnic differences in fat mass have been reported in previous studies [60]. The differences in the perception of body size in the two studies could be partly due to the use of different tools to assess body image. Wang and colleagues used questions to elicit information about children's weight status [20], whereas in our study, body size perceptions were based on a scale of eight silhouettes, which has been validated for use in South African children [54].

In our study, children's own body size perception using silhouettes was 4 times and 2 times more likely to overestimate underweight and 1.8 times and 3.8 times more likely to underestimate overweight and obesity, respectively, when compared with body fatness measured using the DDM. Our findings are somewhat similar to the findings from a study in Iran reporting that 40% of the children misperceived their body size by either overestimating underweight or underestimating obesity status [61]. In a study conducted in Lebanon using a sample of 278 adolescents aged 13–17 years, a third (30.5%) of the adolescents and more than half (56.8%) of the obese adolescents underestimated their weight [32]. In our study, BMI was compared with body fatness measured using the DDM and the results showed that children were 3 and 19 times more likely to underestimate overweight and obesity, respectively.

In Wang's study [20], a significant poor consistency between BMI and body weight perception was reported ($\kappa=0.352$, $p<0.05$), in 7-to 8-year-old Chinese children ($N=9727$) from four districts of Jilin City. In our study, the consistency of agreement between actual fatness as assessed using the DDM and perceived body size using silhouettes was very poor ($\kappa=0.083$; $p=0.053$), hence the low level of concordance between the silhouettes and the DDM. The validity of the girls' perceived body weight compared with objectively measured body fatness in our study demonstrated a fair agreement, and this finding is different from previous studies on the body weight perceptions of children's parents from Mexico [62] and South America [41]. A study from Chile [63] reported poor concordance between nutritional status, perceived with a seven-figure scale, and real BMI ($\kappa=0.031$, $T_c=0.275$), and their finding was similar to the one for boys in our study ($\kappa=0.040$, $T_c=0.53$). Interestingly, BMI category and body size perception in our study had moderate validity among overweight girls and the lowest validity among girls with obesity. This is congruent with a study conducted in Minneapolis in the United States among 9- to 18-year-old boys and girls, which indicated that overweight and obese children were more likely to underestimate their body sizes than children of normal weight [40].

The observed poor agreement between BMI category and body size perception measured by silhouettes in our study is similar to the findings of the Youth/Child Cardiovascular and Environmental (SAYCARE) multi-country study, carried out in seven South American cities (Buenos Aires, Lima, Medellín, Montevideo, Santiago, São Paulo, and Teresina) with 1035 children and adolescents aged 3 to 17 years, which reported that the correlation between children's body size self-evaluation outcome and their objectively measured BMI was lower before the age of 8 years [41]. The potential methodological errors associated with using silhouette choice in younger children as a reason for low correlation has been highlighted [62,64].

Limitation and strengths

Strengths

The strength and uniqueness of this study is that it was the first study in South Africa where a triangulation in the level of agreement between actual fatness measured using the DDM, BMI category, and body image perception using silhouettes in children aged 6 to 8 years was conducted. Additionally, 202 South African children is a relatively large sample size with adequate power for using the DDM to measure body fat, further contributing to the strength of the study.

Limitations

Our study did not consider the perception of parents and environmental factors that could influence children's body size perceptions. However, studying these parameters was beyond the study scope. The study did not look at the full spectrum of children within this development stage.

Children were selected from a homogenous environment; and therefore, the results cannot be extrapolated to other ethnic groups. The body fatness cut-offs used were developed in European children, and future research should develop cut-off points for fatness that are valid in African populations. Another limitation of the study is the lack of validation studies on body size perception in comparison to BMI and body fatness in South Africa and internationally. As such, further studies are required to develop a common standard body size perception measurement tool for proper comparison.

Conclusion

We conclude that children either overestimated being underweight or underestimated being normal weight, overweight, and obese when using silhouettes, compared with their actual measured outcomes, especially those that are measured using the DDM. Body size self-perception in South African children is generally inaccurate. Given the potential health implications associated with misclassification of body size during childhood, such as weight increase or obesity, correct self-assessment of body size is important, and may be the key to the adoption of weight control strategies directed at curbing the escalating obesity epidemic in the country. Scalable measures to allow for more accurate self-assessment are urgently needed—one approach is behavior change communication (BCC) at all levels. Demystifying “big is better” will be a good starting point.

Competing interests

The authors declare no competing interests.

Authors' contributions

TLMM supported the design, drafted the manuscript, and interpreted the data. MAM (as principal investigator in the BC–IT study) contributed to the design, analysis, and interpretation of data, and critical reviewing of the manuscript. JJR advised on the study design and commented on the manuscript. JZM commented on the manuscript and HSK contributed to the design of the study and provided comments on the manuscript. TM and CUL advised on study design and reviewed the manuscript. All authors read and approved the final manuscript.

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CHAPTER 4: ARTICLE 2: COMPARISON OF SEVERAL PREDICTION EQUATIONS USING SKINFOLD THICKNESS FOR ESTIMATING PERCENTAGE BODY FAT VS BODY FAT PERCENTAGE DETERMINED BY BIA IN 6-8-YEAR-OLD SOUTH AFRICAN CHILDREN: THE BC-IT STUDY

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ABSTRACT

Objective: Little information is available on the comparison of available anthropometry based body fat percentage (%BF) equations and %BF determined by bioelectrical impedance (BIA) in South African children. This study, therefore, compares %BF determined by BIA and calculated from available prediction equations based on skinfolds in young South African children.

Setting South Africa

Participants and methods: A cross-sectional design was used on a sample of 202 children (83 boys and 119 girls) aged 6–8 years from the Body Composition–Isotope Technique study (BC–IT). Anthropometric measures of height and weight, triceps and subscapular skinfolds were determined according to International Society for the Advancement of Kianthropometric (ISAK) standard procedures. Percentage body fat (%BF) was determined with BIA and three equations (Slaughter¹, Wickramasinghe² and Dezenberg³). SPSS was employed to analyse the data using paired samples tests, linear regression and Bland Altman plots.

Results: Significant mean differences ($p < 0.001$) were found in the predicted %BF means determined by BIA (25.12 ± 6.42), Slaughter¹ (12.76 ± 4.89), Wickramasinghe² (23.19 ± 7.42) and Dezenberg³ (17.84 ± 5.36) for the total group and by gender. Additionally, significant paired mean differences were found for BIA and Slaughter ($t_{201} = 33.896$, $p < 0.001$), Wickramasinghe ($t_{201} = 4.217$, $p < 0.001$), and Dezenberg ($t_{201} = 19.910$, $p < 0.001$). The BIA moderately and positively correlated with the Slaughter equations ($r = 0.61$, $p < 0.001$), and with Wickramasinghe equation ($r = 0.54$, $p < 0.001$), but a low correlation was observed with the Dezenberg equation ($r = 0.47$, $p < 0.001$). For all the equations, the standards for evaluating prediction errors (SEE) were above 5. The magnitude of the regression lines varied from low to moderate ($r^2 = 0.46$ – 0.61). The Bland–Altman plots show relatively large positive and negative deviations from the mean difference lines and trends of systematic under- and over-estimation of %BF across the %BF spectrum, particularly when the Slaughter equation is applied.

Conclusions: All three equations demonstrated a smaller %BF than the %BF measured by BIA, but the difference was the smallest with the Wickramasinghe equation. The three predicted equations showed a poor SEE against measured %BF using BIA. As such, age-specific %BF equation incorporating optimum methods using Deuterium Dilution Techniques are needed.

Keywords: Body composition, skinfold thickness, anthropometry, adiposity, body fat, deuterium dilution methods.

Abstract words count: 225

Article Summary

Strengths and limitations of the study

- The study was the first of its kind in the North-West Province of South Africa comparing %BF determined by skinfold thickness and BIA in 6- to 8-year old children.
- Though several available skinfold thickness predicting %BF showed smaller %BF compared to %BF by BIA; the study identified a skinfold thickness equation that can predict %BF in the absence of BIA in this sample.
- Since the study sample was from North-West Province, South Africa only, the results cannot be generalised to the entire South African population.
- The current study used in-built BIA equations; it is uncertain whether the use of equations developed by other authors in comparison with skinfold thickness would have produced a different level of agreement between BIA and the skinfold thickness.

INTRODUCTION

The rapid increase in childhood obesity is a significant public health threat in the modern world⁴⁻⁶. Over the past 40 years' obesity in the age group, 5-19 years increased in all regions; the mean BMI increase globally was 17.2 - 18.6kg/m² and 16.8 - 18.5kg/m² among girls and boys, respectively⁶. McPhee *et al.*⁷ estimate that globally 381 million children are either overweight or obese. The upsurge is observed more in low and middle-income countries⁸⁻¹², with urban areas being more affected^{13, 14}, including in South Africa^{15,12}. The relationship between excessive body fat and increased morbidity and mortality is well documented¹⁶⁻¹⁸. Obesity is also known to impact physical and psychological health^{9, 19}. Studies have identified that stunted children have a higher risk of being obese, especially in low and middle-income countries.^{20, 21}.

Early diagnosis of obesity is vital, including understanding the risk factors for obesity; a growing body of literature suggests that childhood obesity predicts obesity later in adulthood^{5,22,23}. Conversely, preventative strategies require a better understanding of contributing factors and an accurate assessment of overweight and obesity²⁴. Both overweight and obesity are associated with increased risk of coronary heart diseases, type 2 diabetes, some cancers, and their related mortality^{25,26}.

Most common, easy to use and cost-effective methods to assess overweight and obesity are based on anthropometric measures such as the body mass index (BMI): weight divided by height squared and skinfolds. Scientific evidence on the misclassification of obesity using BMI exists, especially in children²⁷⁻²⁹. BMI does not distinguish between lean muscle from fat mass²⁹⁻³¹ neither does it accurately reflect visceral fat accumulation. Visceral fat is the likely culprit leading to most of the metabolic and clinical consequences of obesity³²⁻³⁵. Despite all the criticism BMI remains an important tool to depict various health outcomes³⁶ and has previously been reported to provide a fair correlation with adiposity³⁷⁻³⁸ especially in young children³⁹. Ideally, obesity should be determined based on the percent of body fat (%BF), which is considered a better marker for assessing excess adiposity or obesity^{1,40-44}.

Body composition measurement is useful for assessing %BF and medical diagnosis, monitoring disease progression, and response to treatment⁴⁵. It is further regarded as essential in assessing nutritional status, especially in children⁴⁶. However, finding accurate and precise techniques remains a challenge⁴⁷⁶. The majority of the prediction equations used to assess body composition have been developed and cross-validated for Caucasians and may be unsuitable for other ethnic groups^{48,49}.

Numerous methods are available for determining %BF in adults and children. However, some are costly, difficult to use, and time-consuming⁵⁰, including dual-energy X-Ray absorptiometry (DEXA) and air displacement plethysmography (ADP)^{51,52}. The equipment used for such studies may be

bulky making them unsuitable for clinical settings and field research⁵³. Therefore, there is an ongoing need to investigate other methods that can accurately determine %BF in a particular population group. Bioelectrical impedance analysis (BIA) and skinfold-thickness are rapid, noninvasive, simple and relatively less costly methods for evaluating body composition in field and clinical settings⁵⁴. Both skinfold thickness⁵⁵ and BIA provide acceptable prediction %BF values in certain populations⁵⁶. Furthermore, BIA is suitable for large epidemiological studies to assess body composition and predict %BF in healthy subjects⁵⁷.

Bioelectrical impedance analysis and skinfold thickness have been used for decades in different population groups, and their prediction equations have been validated against known reference methods, producing poor to good agreement results. Nevertheless, no studies were found that validated prediction equations using skinfold thickness for estimating percentage body fat among South African children. The Slaughter *et al.*¹ equation uses the sum of triceps and subscapular skinfolds to predict %BF and has been validated in different populations and inconsistent results found⁵⁸. Nonetheless, its validation in South African children remains to be demonstrated. Therefore, an increasing number of scholars recommend the development of BIA and skinfold thickness race-specific prediction equations for %BF and FFM to improve precision^{2,46,59-62}. Hence, paucity still exists regarding predicted %BF for South African children.

Therefore, this study focused on comparing %BF derived from BIA with body fatness derived from published skinfold thickness equations (*i.e.*, (1) Slaughter *et al.*¹, (2) Wickramasinghe *et al.*² and (3) Dezenberg *et al.*³). The choice of these equations was that over the decades, various skinfold equations had been developed, with the majority of the prediction equations validated in older children and adults⁶²⁻⁶⁴; ages 10-12 years, using varying skinfold sites. The skinfold prediction equations by Slaughter *et al.*¹ Wickramasighe² and Dezenberg³, were selected because of the age range and the skinfold sites used in the validation studies, which are similar to the methodology used in our study. Also, it was noted that these three skinfolds prediction equations have been validated and used in different populations and found to produce unreliable results with BIA⁶⁵⁻⁶⁷. The use of BIA and skinfold thickness prediction equations to estimate body fat is, therefore, an acceptable alternative method instead of the complex and costly methods, especially in resource-limited communities²¹. This study, therefore, compares %BF determined by BIA and calculated from available prediction equations based on skinfolds in South African children.

METHODS

Study design and participants

A cross-sectional design was used with a total of 202 children (boys=83; 41.1% and girls=119; 58.9%) aged 6 to 8 years old with a complete skinfold thickness measurement from a sample of 299 children from a larger body composition using isotope technique study (BC-IT study). The BC-IT larger study examined the relationship between objective (stable isotope, BIA) and indirect (anthropometric variables) measures of BC indices. The current study aims to compare the two field methods included in the BC-IT study. Therefore, more details about the study have been published elsewhere⁷². The school principals and District Office, Department of Basic Education granted permission for the study to be undertaken. Subsequently, the Health Research Ethics Committee of the Faculty of Health Sciences (HREC) of the North-West University gave ethical approval of the study (ethic no: NWU-00025-17-A1). Parents of the children and the children gave consent and assent to participate in the study, respectively. Subsequently, children voluntarily participated in the study.

Anthropometry

Weight was measured with a portable electronic scale (Beurer® Ps07 Electronic Scale, Ulm, Germany) to the nearest 0.1 kg. Children were measured with light clothing and without shoes. Stature was measured by a Harpenden® portable stadiometer with a perpendicular board to the nearest 0.1 cm.

The triceps was measured parallel to the long axis of the arm at the triceps skinfolds site. The subscapular skinfold was measured with the fold running obliquely down at the subscapular skinfold site. Skinfolds was taken with a Harpenden® skinfold calliper⁷⁵ with a constant pressure of 10g/mm² to the nearest 0.2 mm.

Body fat percentage by Bioelectrical Impedance Analysis (BIA)

Body composition was assessed using BIA (Bodystat1500MDD, MultiScan 5000, BodyStat). Participants were asked to remove socks, jewellery and belts containing metal or metal-rimmed glasses. Participants were asked to lie down quietly motionless on a non-metal examination table on their back with arms flexed to the side and thighs not touching. Two BIA measurements were recorded, one with the elbows flexed at 90° and one with the elbows extended at 180°. The electrodes of the BIA were placed on the dorsal surface of the right hand and foot at the metacarpals and metatarsals, respectively. Detection electrodes were placed at the pisiform prominence of the right wrist and the anterior surface of the ankle joint⁶⁹. An imperceptible alternating current (frequency 50 kHz) was passed from one to another, measuring the impedance. Measurements were conducted at a similar time of the day (in the morning) after

overnight fasting. Participants were not allowed to eat or drink anything four hours prior to testing, no exercise within 12 hours of the test, and not urinate within 30 min of the first test. As flexing the arms to 90° lowered the impedance to obtain measurements from smaller children, this method was tested to determine if this method would produce adequate results with an acceptable level of error⁶⁹.

Existing BIA Bodystat software with inbuilt equations were used for the estimation of total body water (TBW). The Bodystat software produces an output specifying TBW in litres, FFM (fat-free mass) (kilograms), Fat mass (FM) (kilograms) and %BF, as well as impedance, resistance and reactance readings. The BIA prediction equations for TBW and FFM have been validated for use in Chinese, Lebanese, Malay, Filipino and Thai children aged 8 to 10 years (948 participants) across a wide BMI range (12.2 – 34.9 kg/m²)⁵⁷. The 8 multiple frequency BIA (BIA_{8MF}) is suitable for individuals aged 3 to 99 years old, according to the manufacturers⁴. The duration of the measurements depends on the monitor, and the body composition measurements are provided in less than 20 seconds.

Children were classified as under fat, normal, overfat or obese based on the McCarthy %BF centile curves⁵⁰. Standards for estimating prediction errors and interpretation of the effect size of a correlation coefficient are presented in Tables 1 and 2. The characteristics of skinfold equations used for deriving %BF in children are presented in Table 3.

Table 1: Standards for Estimating Prediction Errors (SEE)

SEE %BF Males and females	SEE Db* (g/cc) males and females	SEE FFM (kg)	Subjective rating	
		Male	Female	
2.0	0.0045	2.0-2.5	1.5-1.8	Ideal
2.5	0.0055	2.5	1.8	Excellent
3.0	0.0070	3.0	2.3	Very good
3.5	0.0080	3.5	2.8	Good
4.0	0.0090	4.0	2.8	Fairly good
4.5	0.0100	4.5	3.6	Fair
5.0	0.0110	>4.5	>4.0	Poor
SEE = Standard Estimate of Error, %BF = percentage body fat; Db-Body Density. g/cc= grams per cubic centimeters; kg=kilograms Adapted from Lohman (1992,) ⁸⁸ as cited by Hey and Stolarczyk ⁵⁶				

Table 2: Cutt-offs for Interpreting the Size of a Correlation Coefficient⁷¹

Size of Correlation	Interpretation
.90 to 1.00 (–.90 to –1.00)	Very high positive (negative) correlation
.70 to .90 (–.70 to –.90)	High positive (negative) correlation
.50 to .70 (–.50 to –.70)	Moderate positive (negative) correlation
.30 to .50 (–.30 to –.50)	Low positive (negative) correlation
.00 to .30 (.00 to –.30)	Negligible correlation

Hinkle, Wiersma, Jurs.⁷¹

Table 3: Characteristics of skinfold equations used for deriving body fat percentage in children

First author	Reference method	Reference method	n	Age (years)	Ethnicity
Wickramasinghe ²	FM (boys) =0.68*A+0.246*T+0.383*SS-1.61-3.45 FM (girls) =0.680*A+0.246*T+0.383*SS-3.45	D ₂ O dilution	188	5-15	South Asia
Slaughter ¹	%BF (boys) =1.21*T(T+SS) – 0.008*(T + SS) ² - 3.2 (African boys) %BF (girls) =1.33*(T+SS) - .013 (T +SS) ² – 2.5	Photo absorptiometry, D ₂ O dilution and hydrostatic weighing	310	8-29	Caucasian African American
Dezenberg ³	FM (boys) = 0.342*W + 0.256* T -6.501 FM (girls) = 0.342*W + 0.256* T -5.501	DXA	202	4 – 10.9	Caucasian African American

A = age (months); %BF= percentage Body Fat; D₂O=Deuterium oxide; T = triceps skinfold (mm); SS = subscapular skinfold (mm); FM = fat mass; DXA = dual-energy X-ray absorptiometry; w= weight (kg)

Statistical analysis

Descriptive statistics (mean and standard deviations (SD), frequencies for percentages were calculated, and independent t-test and chi-square tests were performed to determine the mean difference between gender and within age categories. Paired sample t-test, paired correlations and differences were used to evaluate the relationship and difference between %BF obtained from BIA and the prediction equations. Also, a paired t-test was used to determine the limits of agreement between %BF obtained from BIA and the selected prediction equations, and regressions, Bland and Altman plots computed. The limits of agreement (LOA) were defined as the mean $\pm$ 1.96 SD for the upper and lower limits. Analysis of variance (ANOVA) for multiple comparisons of Bonferroni post-hoc tests were performed to determine age group differences. All statistical analyses were performed using the Statistical Pacage for the Social Science (SPSS). The level of significant differences was set at $p < 0.05$.

RESULTS

Table 4 presents the descriptive characteristics of boys and girls. Significant sex differences were observed for %BF measured by BIA ($p<0.001$), triceps skinfold ($p<0.001$), and TBW ($p=0.001$). No significant ($p>0.05$) sex differences were found between age, weight, height, BMI, and BMI for age z-score. Based on BMI categories, out of 202, 15.3% ($n=31$) of the children were overweight and 6.9% ($n=14$) obese, with girls being more overweight (21.8%; $n=26$) and obese (10.1%; $n=12$) compared to the boys (overweight=6.0%; $n=5$) and obese (2.4%; $n=2$). Underweight children were 14.9% ($n=30$) for the total sample participants with more boys being underweight (22.9%; $n=19$) than the girls (9.2%; $n=11$). It was evident that there are weight differences between boys and girls (22.73 ± 4.53 and 22.69 ± 4.15 , $p=.91$) for boys and girls respectively. Similarly, fat mass in boys and girls was significantly different using both the Slaughter and the Wickramasinghe equations ($p<0.001$). Lastly, no significant sex differences ($p=0.35$) in body fatness could be found with the Dezenberg prediction equation.

Table 4: Descriptive characteristics of the total group and for boys and girls

	Total (n=202)	Boys (n=83)	Girls (n=119)	
	Mean±SD	Mean±SD	Mean±SD	p-value
Age (year)	7.57±0.85	7.62±0.78	7.54±0.90	.54
Height (cm)	120.20±7.02	120.83±6.25	119.76±7.50	.29
Weight (kg)	22.73±4.53	22.69±4.15	22.764.80	.91
BMI (kg/m²)	15.61±1.99	15.43±1.73	15.73±2.15	.29
BMI Z score	-.17±1.08	-.29±1.08	-.09±1.07	.18
FM (%) BIA	25.12±6.42	22.45±5.51	26.97±6.68	<.001
FM (kg) BIA	5.79±2.36	5.18±2.02	6.21±2.49	.002
FFM BIA	16.80±3.07	17.50±2.79	16.31±3.17	.006
Triceps (mm)	8.20±3.12	7.21±2.38	8.88±3.38	<.001
Subscapular (mm)	5.93±2.96	5.27±1.75	6.38±3.51	.01
TBW (BIA) (ℓ)	12.81±2.50	13.34±2.25	12.44±2.61	.01
%BF using Slaughter equation ¹	12.8±4.89	10.5±3.64	14.2±4.46	.001
%BF using Wickramasinghe equation ²	23.2±7.03	17.0±3.88	27.5±5.33	<.001
%BF using Dezenberg equation ³	17.8±5.36	17.3±4.69	18.2±5.77	.252
Age distribution (n (%))				
6 year	55(27.36)	19(22.9)	36(30.3)	.136
7 year	69(34.32)	34(41.0)	35(29.4)	
8 year	78(38.80)	30(36.1)	48(40.3)	
BMI categories (n (%))				
Underweight (below 18.5 kg/m²)	30(14.9)	19(22.9)	11(9.2)	<.001
Normal weight (18.5 to 25 kg/m²)	127(62.9)	57(68.7)	70(58.8)	
Overweight (larger than 25 and less than 30 kg/m²)	31(15.3)	5(6.0)	26(21.8)	
Obesity (30 kg/m² or higher ¹)	14(6.9)	2(2.4)	12(10.1)	
%BF= Percentage body fat; BIA=Bioelectrical impedance analysis; SD = standard deviation; MD = Mean differences, SD = standard deviation.				

Table 5 presents differences between %BF by BIA and several skinfold equations. Bioelectrical impedance analysis moderately and positively correlated with the Slaughter² equations ($r=0.61$, $p<0.001$), and with Wickramasinghe¹ equation ($r=0.54$, $p<0.001$) and a low correlation was observed with Dezenberg³ equation ($r=0.47$, $p<0.001$). There were significant mean differences

between BIA and Slaughter² equation ($t_{201} = 33.896$, $p < 0.001$), Wickramasinghe¹ equation ($t_{201} = 4.217$, $p < 0.001$), and Dezenberg³ equations ($t_{201} = 19.910$, $p < 0.001$).

Table 5: Difference between the percentage body fat (%BF) obtained from Bio-electrical Impedance Analysis (BIA) and the skinfold equations for the total sample

	Paired correlations	Paired differences				
	Mean±SD	r±SEE	p-value	MD±SD	t	p-value
BIA	25.12±6.42	.611±5.10	<0.001	-12.35±5.18	-33.896	<0.001
%BF from skinfolds (Slaughter <i>et al.</i> ¹)	12.76±4.89					
BIA	25.12±6.42	.540±5.42	<0.001	-1.92±6.47	-4.217	<0.001
%BF from skinfolds (Wickramasinghe <i>et al.</i> ²)	23.19±7.04					
BIA	25.12±6.42	.474±5.67	<0.001	-7.27±6.11	-16.910	<0.001
%BF from skinfolds (Dezenberg <i>et al.</i> ³)	17.84±5.36					

MD = Mean differences, SD = standard deviation, SEE = Standard Estimate of Error; r= correlation coefficient; t= t-test;

The differences and the limits of agreement between %BF obtained from BIA and various selected skinfold equations are described in Tables 6 and 7, respectively. The means of %BF were 22.45±5.1 and 26.94±6.38 in boys and girls, respectively.

Table 6: Difference between the %BFs obtained from BIA and various skinfold equations

	Paired correlations			Paired differences		
	Mean±SD	r±SEE	p-value	Mean±SD	t	p-value
Boys (n= 83)						
%BF BIA	22.45±5.51					
%BF from skinfolds (Slaughter <i>et al.</i> ¹)	10.53±3.64	.484±4.85	<.0001	-11.92±4.92	-22.08	<.0001
%BF from skinfolds (Wickramasinghe <i>et al.</i> ²)	17.02±3.88	.387±5.16	<.0001	-5.43±5.37	-9.202	<.0001
%BF from skinfolds (Dezenberg <i>et al.</i> ³)	17.32±4.69	.397±5.09	<.0001	-5.13±5.64	-8.279	<.0001
Girls (n=119)						
%BF BIA	26.94±6.38					
%BF from skinfolds (A)	14.20±4.46	.58±5.63	<.0001	-12.65±5.35	-25.78	<.0001
%BF from skinfolds (B)	27.50±5.33	.478±5.63	<.0001	0.52±6.05	0.947	.345
%BF from skinfolds (C)	18.20±5.77	.516±5.20	<.0001	8.77±6.00	-15.937	<.0001

%BF= Percentage body fat; BIA=Bio-impedance analysis; r= correlation coefficient; SEE = Standard Estimate of Error; t= t-test; SD = standard deviation.

All skinfold prediction equations showed a poor SEE above 5.0 based on the Lohnman (1992)⁸⁸ standards for evaluating prediction errors classifications (Table 1). However, %BF in boys as compared to BIA with the Dezenberg³ equation showing a smaller mean±SD difference of -5.13±5.64 compared to the Slaughter¹, which showed mean±SD of 11.92±4.92. In girls, the Wickramasinghe² equation showed the lowest mean±SD 0.52±6.05, and the largest difference was with the Slaughter equation at -12.65±5.35 (Table 7).

Table 7: Limits of agreement between the percentage body fat (%BF) obtained from Bioelectrical Impedance Analysis (BIA) and various prediction equations

	Boys (n= 83)		Girls (119)	
	Mean±limit	Lower; upper	Mean±SD	Lower; upper
BIA vs %BF from skinfolds (Slaughter <i>et al.</i> ¹)	-11.92±4.92	-12.99;-10.85	-12.65±5.35	-13.62;-11.68
BIA vs %BF from skinfolds (Wickramasinghe <i>et al.</i> ²)	-5.43±5.37	-6.60;-4.25	0.52±6.05	-0.57;1.62
BIA vs %BF from skinfolds (Dezenberg <i>et al.</i> ³)	-5.13±4.64	-6.36;-3.89	-8.77±6.00	-9.86;-7.68

%BF=Percentage Body Fat; n=sample size; sd=standard deviation

The lowest limit of agreement was observed from the Slaughter¹ equation in both boys and girls, all prediction equations under predicted %FM by different percentages ranging from 24% to 53% among boys and -1.9% to 47.4% among girls (Table 6). The Slaughter¹ prediction equation showed the largest difference of 53% and 47.4% among boys and girls, respectively.

Figures 1 a–c shows the Bland and Altman plots for the mean differences between calculated and measured %BF obtained from BIA, the Slaughter *et al.*¹, Wickramasignhe *et al.*² and Dezenberg *et al.*³ skinfold equations with upper and lower 95% Confidence Intervals (CI).

The mean difference between the calculated (Slaughter¹ equation) and measured %BF were -12.65 ± 5.35 (Table 7). The difference between the two methods is large and indicating that the Slaughter¹ equation underestimates %BF measured by BIA most ($p < 0.0001$). A slope of 0 would mean that the difference between the calculated %BF by the Slaughter¹ equation minus measured %BF by BIA stays at the same level across the range of %BF from low to high (Figure 1a). Regression analysis demonstrated a negative slope (-0.329) which implies that there is a greater positive difference between calculated %BF by Slaughter equation minus measured %BF by BIA at low %BF of the children (<20%), while the difference change to a negative difference at high %BF. The differences are the smallest between 20-28 %BF, but there are many outliers.

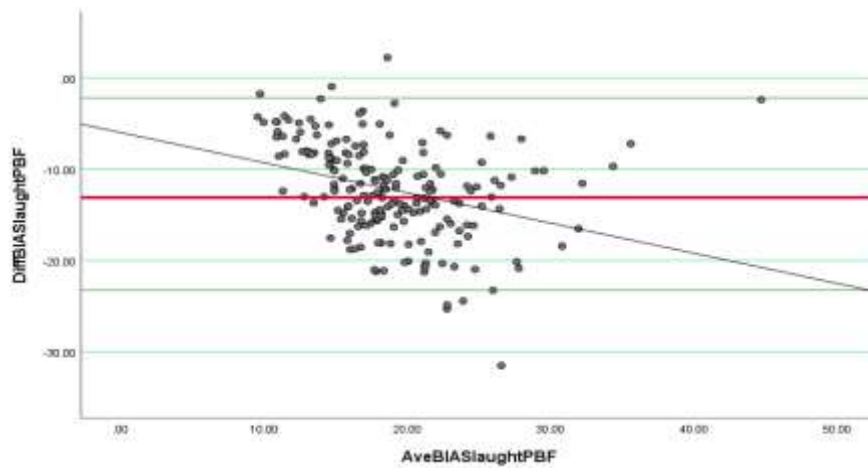
The mean difference between the calculated (Dezenberg³ equation) and measured %BF were -7.28 ± 6.12. This is a smaller difference, but still an underestimation of %BF compared to the %BF measured by BIA and significantly different from 0 ($p < 0.0001$). Figure 1b also shows a

negative slope (standardised beta) of -0.203 (Figure 1b). The smaller slope indicates a better fit across the range of %BF from low to high. However, there is also a greater positive difference between the calculated %BF by the Dezenberg³ equation minus measured %BF by BIA at low %BF of the children (<18%). The difference changes to a negative difference at high %BF. It seems the differences are the smallest, between 18-25 %BF.

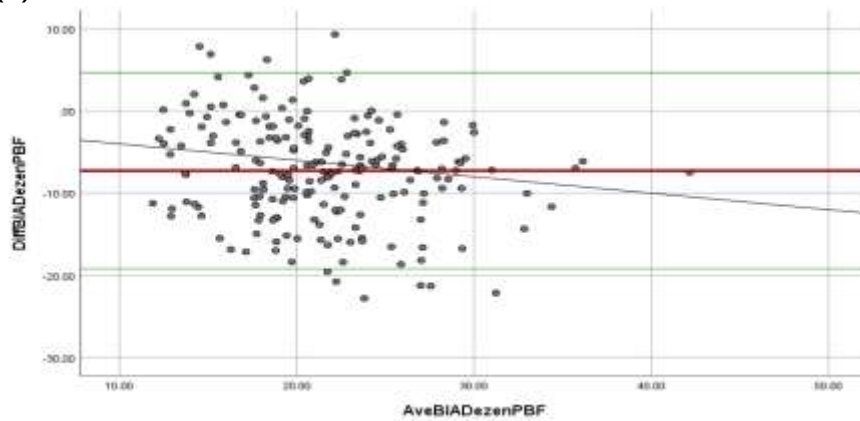
The mean difference between calculated (Wickramasinghe² equation) and measured %BF = -1.92 ± 6.48 (Table 7). This is a small difference, but still, the difference is significantly different from 0 ($p < 0.0001$). Figure 1c shows the smallest slope compared to the other two figures, indicating a better fit across the range of %BF from low to high. Due to the small positive slope (0.108), there are almost as many negative as positive differences between calculated %BF by the Wickramasinghe² equation minus measured %BF by BIA at low %BF of the children (<30%), while the difference change to more positive differences at high %BF (>30%). The differences are relatively fairly stable across the spectrum from 12-40 %BF. Therefore, this equation showed the best agreement with %BF measured by BIA in the total group across the spectrum (smallest mean difference and smallest slope of the regression line). Only six observations are outside the limits of agreement.

There are relatively large positive and negative deviations from the mean difference lines and considerable variation in all three plots and trends of differences across the %BF spectrum, particularly when the Slaughter equation is applied. With all three equations, we found a smaller %BF than the %BF measured by BIA, but the difference is negligible with the Wickramasinghe² equation in girls aged 5-to15-years old.

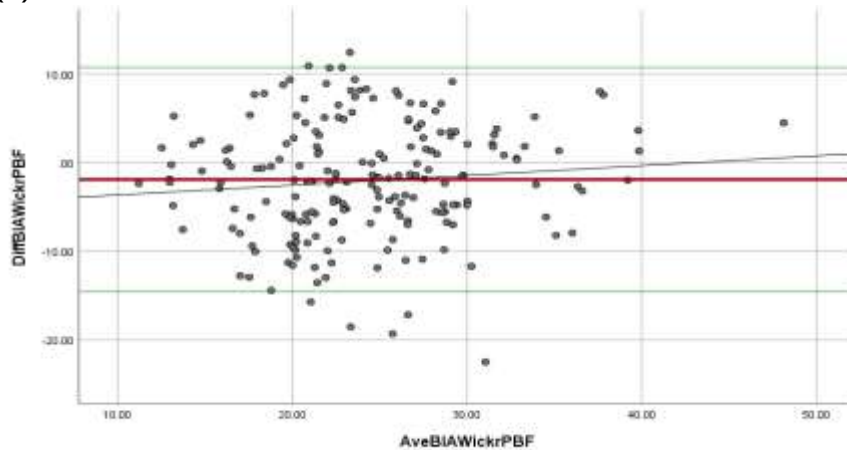
Comparatively, all the three predicted equations (Slaughter¹, Wickramasinghe² and Dezenberg³) performed poorly on Bland-Altman plots. Additionally, the prediction equations varied in the slope of the regression line and the developed standards of evaluating prediction errors of body composition estimating %BF by Lohman⁹⁴.



(a)



(b)



(c)

Figure 1(a-c): Bland Altman plots with regression lines of %BF obtained from BIA against those estimated from the skinfold equations of 1(a) Slaughter *et al.*¹, (b) Wickramasinghe *et al.*², and (c) Dezenberg *et al.*³

DISCUSSION

The purpose of this study was to compare predicted %BF derived from BIA and published equations (i.e. Slaughter et al.,¹, Wickramasinghe et al.,² and Dezenberg et al.,³). There was generally a poor agreement (above Lohman SEE⁸⁷ cut point of 5.0) between BIA measurements and the prediction equations by Slaughter¹, Wickramasinghe² and Dezenberg³. With all three equations, we found a smaller %BF than the %BF measured by BIA, but the difference is smallest with the Wickramasinghe² equation.

When selecting a prediction equation to use, it is important to note the population from which the equations was derived from. Dezenberg et al.³ indicated that the use of an equation developed for adults to predict %BF may result in overestimation in children. Furthermore, there are few available skinfold prediction equations valid across populations. The Slaughter equation¹ has been used in different ethnic groups producing high correlation coefficients (BMI, $r = 0.78$; $p < 0.001$) with BIA in a Mexican study (mean age 9.47 ± 1.55 years)⁷². Additionally, the results revealed a good correlation between BIA and the Slaughter¹ skinfold equation ($r = 0.74$; $p < 0.001$)⁷². Similarly, in a study conducted in Pakistan among children 9 to 19 years, the Slaughter skinfold prediction equation¹ accurately predicted %BF at 98.4% accuracy compared to DXA measurement⁵⁷ followed by the Dezenberg³ equation at 90.3%. The skinfold prediction equations selected for the current study were developed for use in children are Slaughter et al.¹, for 8 to 29 years old African and Caucasian children and young adults; Dezenberg³, 4 to 10.9- year-old Caucasian and African American children and Wickramasinghe², for 5 to 15-year old Asian children. In our study, a moderate ($r = 0.61$) positive correlation between predicted %BF using BIA and calculated by the Slaughter equation¹ was observed.

The current study had a homogenous population of African children from the same community. Contrary to the Slaughter¹ and Dezenberg³ studies, whereby 90% of the children in the Dezenberg equation³ study were Caucasian, the Wickramasinghe study² only focused on an Asian population, while the Slaughter study included white and black children. The Slaughter study does not stipulate the proportion of black vs. white children in the study¹. In our study, all the prediction equations under predicted %BF in Black South African children. Even though the age groups used in the three selected prediction equations are within the age range of children in the current study, the ethnicity and environment of the study population may have influenced the observed underestimation and prediction in %BF. Previous studies have reported higher fat percentages among Asian children^{73,74}; thus the Wickramasinghe equation was developed for Asian children and may influence %BF in a different ethnic group. The differences in %BF were previously reported even within the different Asian groups⁷⁵.

In the current study, girls had higher fat mass as measured by skinfold and BIA. Similar results were reported in other studies⁷⁶⁻⁷⁸, with similar age ranges: 4-20 years; 6-9 years and 7-9 years,

respectively. A recent publication from the BC–IT study reported a high prevalence of excessive FM as determined by stable isotopes in the same population⁶⁸. The Freedman et al.⁷⁹ study also showed higher % fat among girls even though the values were only based on skinfold measurement. Physiological reasoning behind the high body fat in girls is on the basis that fat deposition aggregates around the hips^{80–82} consistent with a wider pelvic structure and breasts, with physical alterations triggered by changing levels of oestrogen, testosterone⁸³, and growth hormone, along with an increase in the number and size of adipocytes⁸⁴.

In our study, the Wickramasinghe et al.² prediction showed the most applicable prediction of %BF in the combined group of boys and girls, compared to the BIA equation. Measurements of %BF by both skinfold thickness and BIA have been inconsistent in different studies. A study by Otte and colleagues⁸⁵, that compared % BF by BIA and skinfold found a significant difference of (18.50±6.50 and 16.81±6.74) respectively. Contrary, Lubis et al⁸⁶ reflected no difference in %BF between BIA and Skinfold thickness at a population level and between males and females with a (p=0.20 for both).

Similarly, in the Hastuti et al.,⁸⁷ study, the skinfold equations by Durnin and Rahaman⁸⁸ and that of Wickramasinghe² significantly over-estimated %BF (p<0.001) in boys and girls, while the equation of Watanabe et al.⁸⁹ underestimated the %BF (p<0.001). The equation of Slaughter et al.¹ gave the lowest bias, but it slightly overestimated %BF in boys (-2.02±5.22% difference; p<0.001) and underestimated %BF in girls (0.78±4.48% difference; p<0.001). However, in the Hastuti et al.,⁸⁷ study, all equations showed a high correlation with %BF obtained from BIA (r = 0.85-0.87; p<0.001) and acceptable range of limits of agreement (LOA; 2.6-5.1%), with the lowest being from Slaughter et al.,¹. These results differ from our study, where the Wickramasinge prediction equation² had the smallest differences in %BF compared to BIA. Similarly, all predicted equations used in our study underestimated % BF in this South African sample. The observed underestimation by Slaughter¹ and Dezenberg³ skinfold prediction equations was previously recorded by Cameron et al.,⁹⁰ in a South African study conducted on 9-year-olds aimed at validating the Slaughter¹ and Dezenberg³ prediction equations using DXA as a reference method. Ethnic and age differences in populations studied could be the contributory factor to the observed discrepancies.

The underestimation of the Slaughter equation¹ has previously been reported in other populations. For example, in a study by Kehoe et al.⁷⁷, conducted among 6-to 9-year-old Indian children, the Slaughter equation¹ underestimated %BF only in children with the lowest adiposity. Similarly, in a Chilean study by Aguirre et al.⁷⁸, conducted among children 7-to 9-years, the Slaughter equation¹ underestimated BF% by -12 and - 9% in girls and boys, respectively. In the Aguirre et al.⁷⁸ study, the Slaughter equation¹ showed the greatest underestimation of % BF compared to the Ramirez-Zea⁵¹ equations. Similarly, in our study, an underestimation of %BF

using the Slaughter equation¹ was -12.65 for girls and -11.92 for boys. Aguirre et al.⁷⁸ attributed the under-prediction by Slaughter¹ to the fact that the equation was developed in the 80's when obesity was not as prevalent among children as today. The underestimation is observed in children of the same age groups but different ethnic groups.

In the Kehoe et al.⁷⁷ study, the Dezenberg³ equation underpredicted %BF in all the groups, while the Wickramasinghe² and Shaikh⁹¹ equations underpredicted in boys and over predicted in girls. These differences are observed even though our study and their study used the same procedures: the same BIA (50 kHz) frequency, inbuilt manufacturer's equation, the sum of the subscapular and triceps skinfold thicknesses measurements and the same age range. The effect of ethnicity and environmental factors may account for the differences observed in the two studies.

In the Hastuti et al.⁸⁷ study, conducted in Indonesia among 12-15-year-old adolescents, the Slaughter equation¹ slightly overestimated %BF in boys by $-2.02 \pm 5.22\%$ and underestimated %BF in girls by $0.78 \pm 4.48\%$ difference $p < 0.001$. In the Hastuti et al.⁸⁷ study, the Durnin and Rahaman and the Wickramasinghe equations significantly ($p < 0.001$) overestimated %BF in both sexes, while the Watanabe et al.⁸⁹ equations underestimated %BF in both sexes ($p < 0.001$). The observed differences may be due to the methods used. The Hastuti et al.⁸⁷ study compared the sum of four sites with BIA measurements⁸⁸, while our study used the sum of two skinfold thickness, and the age groups in the study differ.

The skinfold prediction equations by Wickramasinghe³ and Slaughter¹ showed a moderate to strong positive correlation with BIA (r^2 ranges between 0.63-0.79) in the Hastuti et al.⁸⁸ study. Similarly, in our study, the Slaughter equation¹ also demonstrated a moderately positive correlation with BIA ($r = 0.61$, $p < 0.001$) and Wickramasinghe equation² ($r = 0.59$, $p < 0.001$), a low correlation was observed with Dezenberg equation³ ($r = 0.47$, $p < 0.001$). Moderate to high correlation ranging from $r = 0.51$ to $r = 0.90$ was also observed by Goss et al.⁹⁸ in adolescent boys

The strength of this study is that it was able to compare %BF derived from skinfold thickness with BIA in a relatively large and homogenous sample of young African children. The non-conclusive results presented in different studies indicate a need for pretesting prediction equations in populations and age groups before adoption. Of importance for this study is that it provided a platform for developing validation studies in this sample. Future studies that explore the validation of TBW for the determination of %BF and FFM are needed. The limitations of this study were that it did not include different racial groups of South African children. However, the children were selected from one of the major ethnic groups in South Africa. Further, the age range was narrow, only limited to 6-8-year olds. This study had a homogenous population with similar economic status.

Future research should consider the inclusion and comparison of different ethnic groups in the same age group, and the age range also needs to be broadened. Given that South Africa is an unequal and multidimensional society, future studies should consider socio-economic differences. All these studies reflect the importance of categorising age in the analysis.

CONCLUSION

All three equations demonstrated a smaller %BF than the %BF measured by BIA, but the difference is smallest with the Wickramasinghe² equation. Development of field base age-specific %BF equations for South African children are urgently needed. Based on these findings, it is apparent that validated equations of %BF against TBW for South African are urgently needed.

Conflict of interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: Lynn T. Moeng-Mahlangu, Makama A. Monyeki, Herculina S. Kruger.

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Review and editing: Lynn T. Moeng-Mahlangu, Makama A. Monyeki, John J. Reilly, Herculina S. Kruger

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Ethical Approval

The study was approved by the Health Research Ethics Committee of the Faculty of Health Sciences (HREC) of the North-West University (ethic no: NWU-00025-17-A1), of which ethical approval and consent to participate statements can be made available.

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CHAPTER 5: ARTICLE 3: DEVELOPMENT AND VALIDATION OF BIOELECTRICAL IMPEDANCE ANALYSIS IN PREDICTING TOTAL BODY WATER AND ADIPOSITY IN 6- TO 8-YEAR-OLD SOUTH AFRICAN SCHOOL CHILDREN

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ABSTRACT

Background: Limited information is available on studies validating prediction equations against criterion methods, determining total body water (TBW) and fat-free mass (FFM) in children from South Africa. Equations available for predicting body composition are predominantly from high-income countries, and equations inclusive of low- to middle-income countries are needed.

Objective: The objective of this study was to develop and validate bioelectrical impedance analysis (BIA) to predict TBW and FFM in 6- to 8-year-old South African children.

Design: A cross-sectional study with 299 (149 development and 150 validation groups) 6- to 8-year children (mean age of 7.63 ± 0.84) was used. TBW and FFM were determined with Deuterium Oxide Dilution, the reference method and predicted using BIA (Bodystat 1500) with 50 kHz frequency. Paired t-tests compared the mean differences between BIA and Deuterium Dilution using the Statistical Package for Social Sciences (SPSS V₂₆®). The new BIA equation was determined by multiple regression, and the accuracy of prediction equations for both TBW and FFM was assessed using Bland Altman plots. The level of significance was set at $p \leq 0.05$.

Results: Published TBW and FFM equations for BIA validated in the study demonstrated under- or over-estimations. The newly developed South African equation had higher accuracy for both TBW and FFM prediction. Significant associations ($p < 0.001$) were found between TBW and impedance index for both development group ($R^2 = 74.3\%$) and cross-validation group ($R^2 = 82.6\%$). The newly developed BIA prediction equation for the estimation of TBW was $= 1.472 + 0.257*(Ht^2/R) + 0.225*(weight) - 0.769*(sex) + 0.333*(age)$ with a regression coefficient $R = 0.935$ and $SEE = 0.801$; and $FFM = 1.466 + 0.333 (Ht^2/R) + 0.292*(weight) - 0.997*(sex) + 0.491* (age)$, with a regression coefficient $R = 0.934$ and $SEE = 1.055$. No significant differences ($p > 0.05$) were found between measured and predicted values for the validation group. Bland Altman plots showed a good level of agreement between TBW and FFM predicted by the new equations against reference measures of TBW and FFM. The bias expressed as the mean difference in TBW or FFM measured by Deuterium Dilution of stable isotope techniques and BIA was 0.0 ± 0.69 l [95% CI: -1.352; 1.352] for TBW and 0.00 ± 0.90 kg FFM [95% CI: -1.764; 1.764]. The limits of agreement were respectively; -1.352; 1.352 and -1.764; 1.764 kg for TBW and FFM. The concordance correlation coefficients for the criterion values against the newly developed predicted BIA equations for TBW and FFM respectively were high (both $r = 0.91$).

Conclusion: It was evident that the published equations used for comparison in the current study are invalid to predict TBW and FFM in South African children. The newly developed equation demonstrates the potential for practical and accurate estimation of TBW and FFM for use in comparable groups of South African children aged 6-to 8- years.

Keywords: deuterium dilution technique, bioelectrical impedance analysis, body composition, obesity, fat-free mass, fat mass, South African children

Abstract word count: 448

Strengths and limitations

- One of the limitations of the study is the limited age range of 6- to 8-year-old and the fact that only a homogenous sample in terms of age and ethnicity was included.
- A newly developed equation for predicting TBW and FFM, and in its turn, to determine fat mass, which can help to identify children at risk of obesity.
- No previous studies explored the body composition of South African children through the reference method of Isotope dilution / DDM.

INTRODUCTION

Measuring body composition is an important component in assessing health and nutritional status, including measuring changes in fat mass[1,2], an indicator of overweight and obesity. Overweight and obesity are defined as excessive accumulation of fat[3]; however, in many studies on obesity prevalence and trends, body mass index (BMI) is used as a proxy for body fat[4-6]. Notably, despite the risks associated with obesity, a lot is still unknown about the condition, including techniques that may accurately predict body fat, especially in children[7]. Despite the recognized importance of measuring body composition in children, there are still limited methods used in both clinical and field settings[8]. Accurate measures of body composition should be grounded on reliable methods validated through reliable models with analysis based on appropriate statistical procedures [8,9]. It is essential to know that all measurements are imperfect and always with some measurement error[10]. Even when BMI alone is used as a proxy for body fatness, references or cut-off points may differ. Such differences in BMI cut-off points are observed in the applications of Child Growth Foundation (CGF), International Obesity Task Force (IOTF) and World Health Organization (WHO) cut-off points[11].

Increasing childhood obesity has precipitated the need for accurate but straight forward methods for determining adiposity[8]. Some of the available body composition techniques require costly equipment, require specialized expertise and are mainly used in laboratory settings and research[12,13]. Despite the recognized importance of measuring body composition in children, there are still limited methods that can confidently be used in both clinical and field settings[8]. For this reason, the accuracy of commonly used field methods needs to be validated against criterion methods[14], which may not always be accessible.

Bioelectrical impedance analysis (BIA), which is a commonly used field tool, is practical for assessing fat mass (FM) and FFM in field studies[15]. The need for accurate techniques to

evaluate body composition is critical in assessing nutritional status and metabolic risks. A change in body composition, especially during pre-adolescent growth spurt years around the ages of 6 to 8 years[16], can pose a threat to health[17]. The BIA is, however, limited by the fact that it is not known if the TBW and FFM equations used are population-specific and need to be validated against criterion methods such as Deuterium Dilution Method (DDM) and DXA. The majority of BIA studies performed in children use guidelines that are based on adults' standards while some aspects of the standards may not be relevant for children[18], and this is compounded by the fact that BIA accuracy depends on the type of device used[19].

Various bioelectrical impedance equations have been developed to predict TBW[20,21]. However, the development of age-specific equations is suggested due to the age variances in electrolyte concentration in the extracellular space relative to the intracellular space[22,23]. Selected equations that have been validated in age groups similar to this study will be validated in the present study. These include, among others: the Kushner equation for estimating TBW developed for Peruvian children, inclusive of 6-10-year old children[24]; Leman 5-18-year-olds in West Africa; Rush in 5-14-year-olds in Europe, 5-17 years old in the UK[25]. While all these equations were developed for children, their validity for other ethnic and economic groups still needs to be validated[26], including South African children. No South African studies were found that compared TBW and FFM measured by BIA against DDM for children. Against this background, the current study aim was twofold; to determine the accuracy of existing prediction equations and develop and validate new prediction equations in 6- to 8-year old South African children.

Material and methods

Participants

A total of 299 African children 6- to 8-year-olds of predominantly Sotho-Tswana ethnic group, who are part of a larger BC–IT study, were selected from four urban township primary schools in North West Province, South Africa. More details of the BC–IT study are published elsewhere[27]. A sample was randomly selected from children within the 6 - 8-year age range between 2017 and 2018; whereby every third healthy (i.e., not on chronic medications, no form of physical disorders, no heart and lung disorders) child had an opportunity to participate in the study. Permission was received from the Department of Education, school principals and parents of the participating schools. The parents and their children gave consent and assent to participate in the study. The study was granted permission (Ethic no: NWU-00025-17-S1) by the Health Research Ethics Committee of the Faculty of Health Sciences (HREC) of the North-West University.

Measurements

Anthropometric measurement

Height (cm) and weight (kg), waist circumference (cm), biceps, subscapular and suprailiac skinfolds (mm) were measured according to standard procedures[28]. Subsequently, BMI was calculated as weight divided by height squared (mass in kg/height in metres), and BMI z-scores were calculated relative to WHO reference data[29]. The WHO BMI z-score classification for underweight: -2 SD; normal weight: -2 SD to $+1$ SD; overweight: $> +1$ to $+2$ SD; and obesity: $> +2$ SD[29].

Bioelectrical impedance analysis (BIA)

The BIA procedures used are published previously[27]. In brief, body composition was assessed using BIA Bodystat® with a measurement frequency of 50 kHz. Measures of height, sex and age were entered manually, while body mass was recorded automatically using 0.5kg to adjust for clothing weight in all subjects. The Bodystat1500® software inbuilt prediction equations were used to produce an output specifying TBW, FFM (using a hydration factor of 0.73, independent of age), BF and %BF, as well as impedance readings.

Deuterium Oxide Dilution

The Deuterium Oxide Dilution protocol used in the present study followed the guidance provided by the International atomic Energy agency (IAEA)[31], and more detail of this process are published elsewhere[27]. Saliva samples of 0.4 ml were equilibrated for six hours at 25°C with hydrogen gas in the presence of a platinum (Pt) catalyst. The isotope of the gas was determined using stable isotope of Fourier-transform infrared spectroscopy (FTIR)[31]. Then, TBW was calculated using the measurement of the abundance of isotope (deuterium) after complete equilibration in accordance with acceptable standards[31,33]. Fat free mass was calculated using age-specific and sex-specific Lohman hydration factors in children from TBW[33]. Fat mass was calculated by the difference between body mass and FFM: $FM\ (kg) = \text{body mass}\ (kg) - FFM\ (kg)$ [34]. Results are expressed as % body mass: $FM\ (\%) = FM\ (kg) / \text{body mass}\ (kg) \times 100$. The McCarthy et al.[35], classification of fatness was applied (underfat=2nd, overfat=85th, and obese=95th percentiles), which was developed on Caucasian children. Classifications of body status in this sample are described in detail in our previous publication[27].

Table 1: Published bioelectrical impedance analysis (BIA) based prediction equations used in the article

Reference	Prediction equation	Age (years)	Countries	RMSE (SEE)	r ²
TBW equations					
Davies and Gregory [36]	TBW= 0.60 Ht ² /R + 0.50	5-17	UK	0.94	1.69
Danford <i>et al.</i> [37]	TBW (kg) = 1.84 + 0.45 (Ht ² /R) + 0.11(weight)	5-9	Illinois	0.62kg	0.98
Kushner[38]	TBW (l)a = 0.593(Ht ² / R) + 0.065(BW) +0.04	6 - 10	Chicago and Peru	1.24kg	0.99
Leman <i>et al.</i> [39]	TBW = 1.67 + 0.35 (Ht ² / R) + 0.24 (Weight) – 0.74 (Sex)	5 – 18	South-Western Nigeria	0.7 l	0.987
Wickramasinghe <i>et al.</i> [3]	TBW= 0.41 impedance index+0.17 weight +1.1 sex code-0.44	5-15	Sri Lanka	2.1	0.86
FFM equations					
Rush <i>et al.</i> [40]	FFM = 0.622 (Ht ² / R) + 0.234 (weight) + 1.166	5 – 14	European, Maori and Pacific Island	2.44 kg	0.96
Wickramasinghe <i>et al.</i> [3]	FFM= 0.56 impedance index + 0.22 weight + 1.6 sex code – 0.22	5-15	Sri Lanka	2.7	0,87

BW = body weight; Ht = height; Ht² / R =height²/resistance; TBW=Total Body Water; RMSE=Root mean square error; SEE Standard Error of estimate; FFM= Fat free mass; UK=United Kingdom.

Statistical analysis

The validity of the prediction equation for estimating body fat for South African children comparing BIA and Deuterium Dilution was analysed statistically using the Statistical Package for Social Sciences (SPSS® V26). The level of significance was set at $p \leq 0.05$.

The selected BIA prediction within the age limits of the current study found in the literature was cross-validated on our sample, as presented in Table 1. The sample was sorted by sex and randomly split using the SPSS into two sub-samples and assigned to the prediction equation development group (n = 149) and a cross-validation group (n = 150) as recommended in body composition validation studies[41,1]. Paired-sample t-tests were used to determine the significance of the differences measured. Chi-square was used to determine the significant differences for categorical variables.

We used Pearson correlation coefficients to determine the relationships between DDM measured TBW, FFM and FM and predictive variables from BIA. Variables with a $p > 0.05$ were not considered for regression analyses. Multiple regression analyses were computed for DDM, FFM (kg), FM (kg) as dependent variables while independent variables were values predicted from BIA (FFM, FM, %BF, impedance, impedance index), and anthropometric variables (weight, height, BMI, WC, Weight to Hip Ratio (WtHR) as well as sex (0= boys; and 1= girls) and age. Additionally, we computed the multicollinearity to evaluate the variance inflation factor (VIF). A VIF of < 10 and

a condition number (CN) of <30 were considered appropriate. Best prediction equations were those that had the largest R^2 with minimum root means-squared error (RMSE)[3]. When backward regression was performed, the results were the same. Bland and Altman[42] plots were used to compare the TBW difference (bias = measured-predicted values) and the means of TBW (measured+predicted values) from the criterion DDM as the criterion method and BIA. The limits of agreement (bias $\pm$ expressed as 2 SD; above and low) were analysed and paired t-tests demonstrated a significant absolute bias with proportional bias evaluated by multiple regression investigated the relationship between the variables used to calculate the new predictions.

The absolute bias (measured-predicted values), its error (i.e standard deviation of the absolute mean bias (SD)) and significance, and the limits of agreement (absolute bias $\pm$ 2 sd) were provided for each equation. Paired t-tests was used to assess the significance of the absolute bias. The proportional bias was evaluated by Pitman's test (1939 as cited in Lee[43]).

Results

General Characteristics

The results of the anthropometry and body composition from 299 (i.e., mean age of 7.6 $\pm$ 0.8) participants (development (n=149) and validation (n=150) groups) are presented in Table 2. No significant differences were found in age, weight and body composition between the development vs validation groups. Significant variations between the development and the validation groups in the number of children who were classified as underweight, normal weight, overweight, and obesity are evident when BMI Z-scores were found.

Table 2: General characteristics and body composition of the participants

	Total (n=299)		Development group	Validation Group	P-value for the Group differences
	Boys: n; %	Girls: n; %	(n=149; %)	(n=150; %)	
	Mean $\pm$ SD	Range	Mean $\pm$ SD	Mean $\pm$ SD	
Anthropometry					
Sex (n; %)	Boys (125; 42); Girls (174; 58)		Boys (65; 44); Girls (84; 56)	Boys (60; 40); Girls (90; 60)	0.95
Age (year)	7.6 $\pm$ 0.8	6.0–8.9	7.6 $\pm$ 0.8	7.6 $\pm$ 0.8	0.76
Height (cm)	121.2 $\pm$ 7.0	103.5–139.9	121.1 $\pm$ 6.8	121.3 $\pm$ 7.2	0.75
Weight(kg)	23.4 $\pm$ 5.1	14.8–48.7	23.39 $\pm$ 5.2	23.5 $\pm$ 5.0	0.86
BMI Z-score	-0.1 $\pm$ 1.1	-2.9–3.6	-0.10 $\pm$ 1.1	-0.1 $\pm$ 1.1	0.84
HAZ	-0.6 $\pm$ 1.0	-3.1 – 3.1	-0.66 $\pm$ 1.0	-0.6 $\pm$ 0.9	0.54
Body composition					
D ₂ O					
TBW (l)	13.2 $\pm$ 2.2	8.30–20.9	13.1 $\pm$ 2.2	13.2 $\pm$ 2.3	0.82
FFM (kg)	17.1 $\pm$ 2.9	10.7–27.1	17.0 $\pm$ 2.9	17.1 $\pm$ 3.0	0.82
FM (kg)	6.3 $\pm$ 2.8	2.2–22.8	6.3 $\pm$ 3.0	6.3 $\pm$ 2.71	0.93

% FM (BIA)	26.3±6.5	12.0–46.8	26.2±6.8	26.4±6.2	0.76
BIA					
Impedance (Ω)	756.8±80.2	543.0–966.0	758.8±81.17	754.8±79.5	0.66
Reactance	66.1±8.9	20.9–101.20	65.8±9.48	66.3±2.3	0.67
Resistance	752.5±80.4	540.0–953.0	755.0±80.9	749.9±80.1	0.58
H ² /Z ₅₀ (cm ² /Ω)	19.8±3.6	11.6–30.2	19.6±3.3	19.9±3.8	0.46
BMI Z-score categories	n (%)		n (%)	n (%)	
Normal	217(73)	-	108(72.5)	109(72.6)	
Grade 1 thinness	37(12)	-	18(12.1)	19(12.7)	
Grade 2 thinness	10(3.3)	-	6(4)	4(2.7)	<0.0001
Overweight	28(9.4)	-	14(9.4)	14(9.3)	
Obese	7(2.3)	-	3(2)	4(2.7)	
D ₂ O FM categories					
Normal	190(63)	-	91(61)	99(66)	
Underweight	29(10)	-	15(11)	12(8)	
Overweight	45(15)	-	22(15)	23(15)	<0.0001
Obese	35(12)	-	19(13)	16(11)	

FM(kg)= expressed as median and interquartile; TBW= total body water; kg=kilogram; l =liter; Overweight/obese²= %BF >25% in boys and >30% in girls; ±=plus minus; n=sample size; sd=standard deviation; cm=centimeters; ±=plus-minus; D₂O= heavy water (deuterium oxide) ; FFM =fat free mass; FM=fat mass; %FM=percentantage fat mass; BIA=Bioelectrical impedance analysis; H²/Z₅₀= height squared devide by impedance index

Comparison of body composition by BIA and the DDM

Table 3 compares mean values obtained by DDM and those obtained by BIA using the Bodystat1500®. Limits of agreement obtained by BIA and the DDM, mean differences/bias, r^2 , paired sample correlations, and SEE for the total sample and sex are also presented. No significant differences were found between TBW and FFM, respectively, of the total group, boys or girls. High correlations between TBW and FFM, respectively, measured by DDM and BIA Adjusted R square values ranged from 0.65 to 0.86 SEE values for FFM reflect low prediction errors according to the guidelines of Lohman[44] of 2.0 to 2.5 kg for males and 1.5 to 1.8 kg for females.

Table 3: Comparison of body composition data obtained by BIA (Bodystat) and the DDM measurements in 6-to 8- year old children

Overall (n=299)	Measured Mean (D ₂ O)	Predicted Mean (BIA)	Mean differences (paired value D ₂ O-BIA)/ Bias	P-value	Paired correlations	Adjusted r ²	LOA, Upper; Lower	SEE
TBW (l)	13.1±2.3	13.2±2.5	.01±1.4	.85	.83	.69	2.844;-2.644	1.4
FFM kg	17.1±2.9	17.1±3.1	.00±1.2	.41	.92	.85	1.752;-2.952	1.2
BOYS (n=125)								
TBW (l)	13.6±2.1	13.5±2.2	.1±1.3	.54	.81	.65	2.618;-2.478	1.3
FFM kg	17.7±2.7	17.7±2.7	.0±1.2	.72	.90	.81	2.322;-2.382	1.2
GIRLS (n=174)								
TBW (l)	12.8±2.3	12.8±2.7	.0±1.4	.81	.84	.70	2.724;-2.764	1.5
FFM kg	16.6±3.0	16.7±3.3	-.1±1.2	.43	.93	.86	2.252;-1.152	1.2

D₂O= deuterium oxide dilution; l=liter; BIA = bioelectrical impedance; TBW = total body water; FFM =fat free mass; LOA=Level of agreement; n=sample size; SEE- Standard error of estimate

Cross-validation of the new and the published equations in the validation sample

Table 4 below presents the means, r², bias, error, limits of agreement and SEE/pure error for cross-validation of equations from the literature in the sample. The results indicate that the Davies and Gregory equation[36] demonstrated a significantly large mean difference and underestimated TBW compared to DDM measurements in both boys and girls. Narrow and insignificant margins were observed between TBW measurement by Kushner et al.[49], Leman et al.[44], Danford et al.[37], and Wickramasinghe et al.[3] equations and DDM. The Rush et al.[45] and Wickramasinghe et al.[3] prediction equations overestimated FFM with large margins among boys and girls. In both sexes, the bias for FMM when using the new equation is very close to zero, but the limits of agreement are wide at around plus or minus 5kg. Therefore, it was apparent from the results that some of the available BIA prediction equations for TBW and FFM are not accurate for our sample.

Table 4 Cross-validation of the new and the published equations in the validation sample for boys and girls

Equation						
Boys (n=60)	Mean	R ²	Absolute bias ^b (p-value)	Error ^c	Limits of agreement ^d	SEE/ Pure error ^e
TBW_D2O measurement	13.6±2.10					
New TBW equation	13.7±1.91	0.88	-0.1(0.249)	1.00	-3.84; 3.64	0.72
Bodystat	13.6±2.17	0.84	0.0(0.750)	0.84	-4.22; 4.02	0.82
KushnerA_TBW[38]	13.9±2.28	0.82	-0.4(0.025)	1.09	-4.87; 4.07	0.87
Davies and_GregoryA_TBW[36]	11.9±1.90	0.82	1.6(0.000)	2.00	-2.12; 5.32	0.87
Leman_TBW[39]	13.6±2.11	0.88	-0.1(0.026)	2.10	-4.23; 4.03	0.71
Wickramasinghe_TBW[3]	13.8±1.01	0.87	-0.2(0.007)	0.78	-2.18; 1.78	0.73
Danford_TBW[37]	13.5±1.89	0.86	0.0(0.002)	1.00	-3.70; 3.70	0.76
FFM (kg)D2O	17.6±2.80					
New FFM equation	17.7±2.50	0.88	-0.1(0.249)	1.32	-5.00; 4.84	0.93
Bodystat	17.7±2.80	0.85	-0.1(0.581)	1.08	-5.59; 5.39	1.06
Rush_FFM	19.2±2.96	0.88	-1.6(0.000)	1.44	-7.40; 4.20	0.96
Wickramasinghe_FFM[3]	18.3±2.70	0.88	-0.7(0.000)	1.37	-5.69; 4.49	0.96
Girls (n=90)						
TBW_D2O	12.9±2.39					
New_TBW equation	12.8±2.67	0.77	0.1(0.091)	1.26	-5.13; 5.23	1.14
Bodystat	13.0±2.83	0.92	-0.1(0.675)	1.35	-5.44; 5.64	0.67
KushnerA_TBW[38]	13.4±2.66	0.82	-0.5(0.000)	1.26	-5.40; 4.71	1.00
Davies_GregoryA_TBW[36]	11.5±2.21	0.82	1.4(0.000)	1.15	-2.931; 5.73	1.00
Leman_TBW[39]	12.7±2.50	0.90	0.2(0.017)	1.22	-4.70; 5.10	0.72
Wickramasinghe_TBW[3]	14.7±2.36	0.90	-1.8(0.000)	1.19	-6.42; 2.82	0.74
Danford_TBW[37]	13.2±2.22	0.89	-0.3(0.000)	1.15	-4.65; 4.05	0.79
FFM (kg)D2O	16.8±2.39					
New FFM equation	16.6±2.98	0.92	0.2(0.076)	1.34	-5.64; 5.84	0.87
Bodystat	16.9±3.57	0.84	-0.1(0.618)	1.43	-7.10; 6.90	1.25
Rush_FFM[40]	18.9±3.46	0.90	-2.1(0.000)	1.46	-8.88; 4.68	0.99
Wickramasinghe_FFM[3]	19.6±3.16	0.90	-2.8(0.000)	1.39	-8.99; 3.39	0.98

^a Significant at p< 0.001

^b negative value of the bias reflects an overestimation by the prediction equation (significant at 0.05)

^c SEE/Pure error, prediction error of 0.0 – 1.8 kg is considered ideal (Houtkooper *et al.*, 1996)

^d the smaller the 95% limits of agreement, the greater the agreement of the equation with the deuterium dilution technique

Development of new prediction equations

Though no significant differences were found between FFM and TBW measured by the Bodystat 1500®MDD and DDM in this sample, the inconsistencies with other BIA prediction equations (Table 4) and DDM for measuring TBW and FFM warranted the development of an equation that we can be confidently used for this sample of children. Stepwise multiple regression and the regression statistics were computed for the prediction equations (Table 5). In the regression models, the FFM (kg) and TBW (l) were used as dependent variables whilst BIA index (Ht^2/R), age, sex (dichotomized as 0 = boys and 1= girls), waist circumference (cm), and mass were used as independent variables. As a result, BIA index (Ht^2/R), age, sex (dichotomized as 0 = boys and 1= girls) and weight were significant predictors variables included in the equations. It was apparent that waist circumference showed a condition index (CI) or condition number (CN) of 29 for TBW and FFM, which meant no association between independent variables. Waist

circumference was not selected in the final equation since it did not meet collinearity with a CN conditions, reflecting no significant association between independent variables, and it was excluded in the final equation.

In the development group, we used BIA index (Ht^2/R), age, sex (dichotomized as 0 = boys and 1= girls) and weight to develop the following equations, which presented the highest adjusted R and the lowest SEE value, are used as in Table 5:

TBW = $1.472 + 0.257*(Ht^2/R) + 0.225*(weight) + -0.769*(sex) + 0.333*(age)$ with a regression coefficient $R = 0.935$ and $SEE = 0.801$.

FFM = $1.466 + 0.333 (Ht^2/R) + 0.292*(weight) - 0.997*(sex) + 0.491* (age)$, with a regression coefficient $R = 0.934$ and $SEE = 1.401$.

Where TBW in litre (l); FFM in kg; Ht^2/R in cm^2/Ω ; age in year; sex: male = 0 and female = 1 and weight in kg.

Table 5: Regression models for the prediction of TBW and FFM

Variables included in the model	Regression coefficient (SE)	SEE			Collinearity Statistics	
		R		P	CN	VIF
TBW						
Constant	1.472			<.0001		
Weight	0.225	0.923	0.867	<.0001	.326	3.067
SEX	0.769	0.935	0.801	<.0001	.845	1.183
H ² /R	0.257	0.880	1.068	<.0001	.309	3.238
Age	0.333	0.942	0.833	<.0001	.790	1.266
FFM						
Constant	1.466			<.0001		
Weight	0.292	0.923	1.138	<.0001	.309	3.238
SEX	-0.997	0.934	1.055	<.0001	.845	1.183
H ²	0.333	0.879	1.401	<.0001	.309	3.338
Age	0.491	0.943	0.991	<.0001	.790	1.266

TBW: Total body water (l); FFM: fat-free mass (kg); SEE: standard error of the estimate (kg or l); Sex: male=0, female=1; CN=condition number; VIF: variance inflation factor

Validation of the new prediction equations on a validation group

Table 6 presents a comparison between TBW and FFM validating the new equations with the validation group. The use of paired-sample t-test results shows no significant differences ($p=0.68$) between TBW measured by DDM (13.2 ± 2.3 l) and estimated using the newly developed BIA equation (13.2 ± 2.6 l) in the validation group. Similarly, insignificant bias (0.028) difference ($t=0.582$, $p=0.87$) was found between the new BIA equation and for FFM determined by DDM (17.1 ± 3.0 kg), a bias of 0.00 ± 0.90 , $t=0.582$ and predicted with the newly developed BIA equation (17.1 ± 2.8 kg).

Table 6: One sample t-test for comparison between TBW and FFM measured by DDM and the newly developed BIA equation in the validation group

	Mean±SD	R ²	Absolute bias	LOA	t	SEE
TBW by DDM	13.2±2.3	0.91	0.00±0.69	-1.352; 1.352	0.473	0.70
TBW new BIA equation	13.2±2.2					
FFM by DDM	17.1±3.0	0.91	0.00±0.90	-1.764; 1.764	0.582	0.90
FFM new BIA equation	17.1±2.8					

TBW = total body water (l); FFM = fat free mass (kg); SD = standard deviation; t = one-sample t statistic

The Bland-Altman[42] plots show no significant mean difference between newly developed BIA predicted TBW plotted against the mean of the criterion and newly developed predicted BIA TBW (Figure 1a). Furthermore, no significant difference was found between the criterion method and predicted BIA FFM plotted against the mean of the criterion and predicted BIA FFM as illustrated (Figure 1b) The bias expressed as the mean difference in TBW or FFM measured by DDM and BIA was 0.00±0.69 l for TBW and 0.00±0.90 kg FFM [95% CI: -1.764; 1.764] with a 95% confidence interval in cross-validation. Respectively, the limits of agreement were for TBW and FFM were -1.352 to 1.352 and -1.764; 1.7642.4kg. The concordance correlation coefficients for the criterion values against the newly developed predicted BIA equations for TBW and FFM were significantly high (both $r=0.91$). The new equations demonstrated small bias, strong correlations and small pure error compared to the criterion method for TBW and FFM.

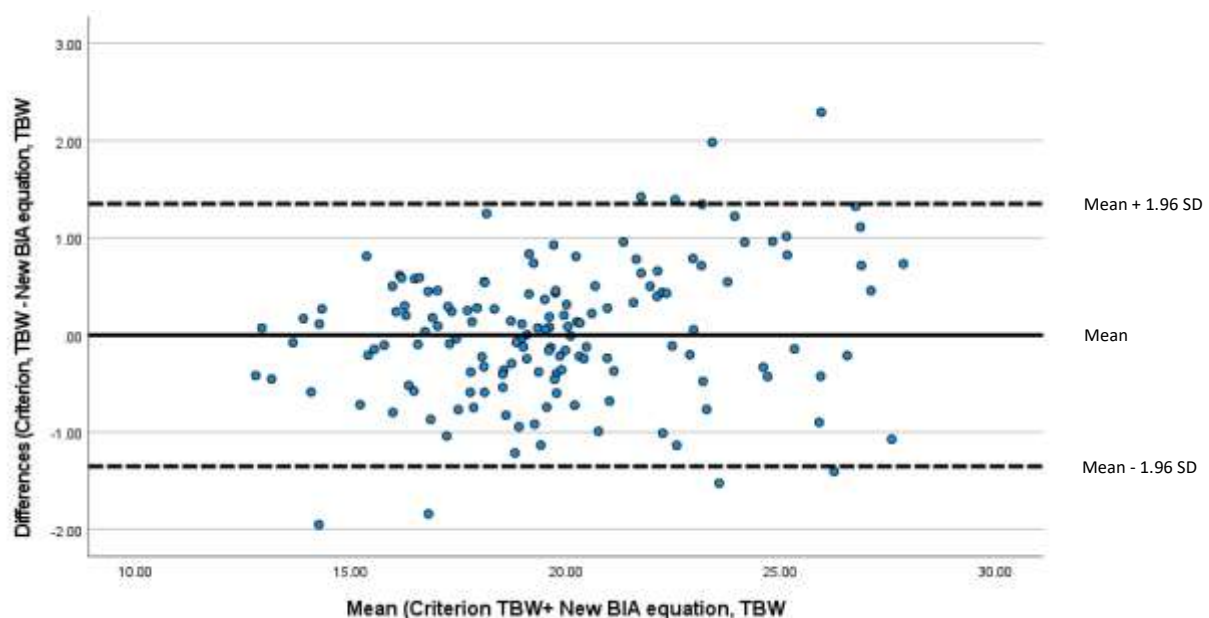


Figure 1a

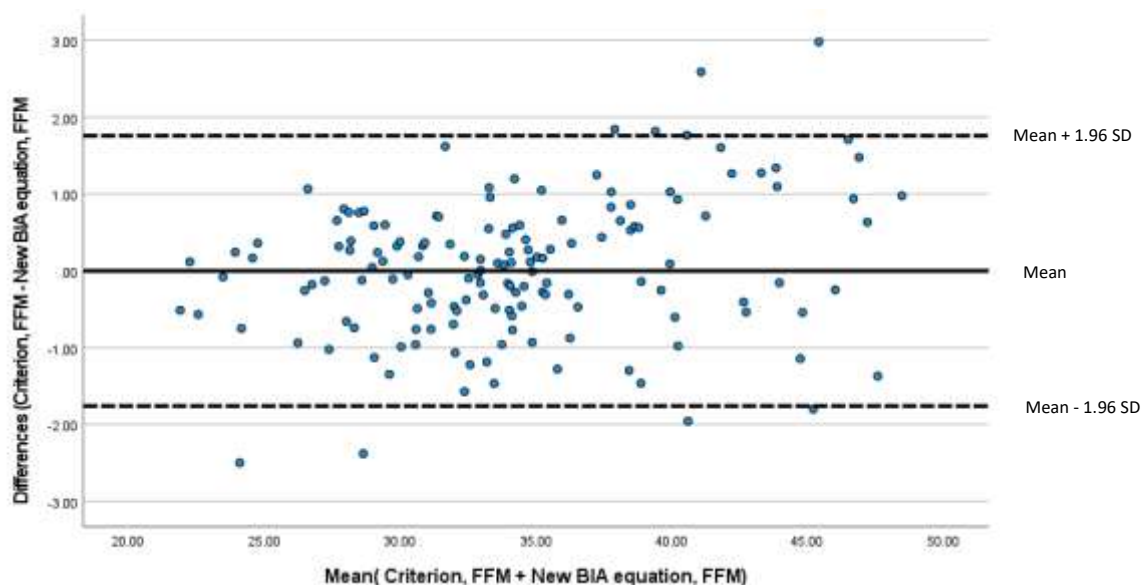


Figure 1b

Figures 1 (1a & 1b): Bland-Altman[43] plots for the newly developed BIA equation and measure of deuterium dilution for differences and means for TBW (1) and FFM (2)

DISCUSSION

The purpose of our study was two-fold: to determine the accuracy of existing BIA[30] prediction equations for TBW and FFM and to develop and validate the newly developed BIA equations for TBW and FFM in the 6-8-year-old South African children. To the best of our knowledge, there are no BIA prediction equations for South African that were developed or cross-validated to estimate TBW and FFM against measures of TBW and FFM by deuterium dilution methods for 6- to 8-old children. The study's main findings are that most BIA published TBW and FFM equations demonstrated unacceptable discrepancies of under- and over-estimations in our sample. Additionally, when the newly developed and validated BIA prediction equations were plotted on Bland-Altman plots, the results showed a good level of agreement between TBW and FFM against the criterion technique of Deuterium Dilution, with negligible bias and narrow limits of agreement, especially for FFM.

Comparison of body composition by BIA and the DDM

In the current study, no significant mean differences were found between TBW and FFM measured by BIA (Bodystat 1500®MDD, MultiScan 5000, Body Stat, and DDM of the total sample, and among boys and girls. Additionally, the two methods were highly and significantly correlated. Similarly, studies on the Caucasian population especially in high-income countries, reported that BIA strongly agrees with the reference methods [22,44,45]. Bioimpedance analysis has been regarded as reliable and accurate in estimating body composition [18,46]. However, other studies had reported significant bias between the original Tanita equation and DDM, namely in Tunisia [47], Bangladesh [48] and Cameroon [49]. Fuller et al. [55] indicated that variability in

the results is attributable to the differences in methods; other factors including physical body size and racial differences. Ben Jemaa and co-workers [47] noted that if the factors named above are not considered in Tanita's BIA equations, the accuracy of prediction equations may be compromised.

Comparison of TBW and FFM from D₂O as the reference method and BIA-based published prediction equations

In our study, the Davies and Gregory equation showed a significantly large mean difference and underestimated TBW compared to D₂O measurements in both boys and girls. While the Rush et al [40] prediction equations overestimated FFM among boys and girls, and the Wickramasinghe et al. BIA prediction equation overestimated TBW and FFM among girls. Our findings are somewhat comparable with studies from Brazil [51] conducted on adolescents aged 10-19 years, in which BIA was found to overestimate FFM and TBW and underestimated FM compared to DDM. In another Brazilian study by Bila et al. [52] conducted among 6- 9-year-old children, significant differences were observed between TBW obtained by DDM and BIA (ANOVA; $p=0.05$). The differences were observed in the total group (44.9% vs 47.6%) and males (45.4% vs 49.3%) but not females. Similar inconsistencies between TBW measured by various BIA equations compared to DDM measurements were observed in a study conducted among pre-school children in Cameroon by Essa'a et al. [49]. The prediction equations that were validated in our study had different variables, which may have affected the performance of these equations in our sample. The Rush [40] and Davies and Gregory [36] equations that did not include age and sex had the highest bias in this sample, indicating that the development of a prediction equation that includes gender and age is necessary.

Development and validation of new prediction equations

When developing the new BIA equations for TBW and FFM prediction, the development group ($n=149$) was used. However, the new equation was validated using the validation group ($n=150$). In the development of prediction equations, stepwise regression was used to test the effect of various variables in producing the lowest and acceptable SEE.

In the current study, sex alone did not improve the R^2 and RMSE/SEE. However, sex combined with other independent variables used to predict TBW and FFM had a high R^2 and low RME/SEE but did not significantly differ. Previous studies have highlighted that variables used to determine the accuracy of the equation and contribute positively or negatively to the accuracy of the prediction equation. The results of our study highlighted differences in TBW and FFM between boys and girls. In addition, including sex improved accuracy of the prediction equations ($R=0.935$ and SEE 0.801 for TBW and $R=0.934$ and SEE=1.055 for FFM). Our study further demonstrated the importance of other variables such as weight, age and BIA impedance index (Ht^2/R) in the development of BIA-based prediction equation. As such, our findings are comparable with

findings from Tunisian children by Ben Jemaa et al. [47]), Jackson et al. [9], on African women by Dioum et al. [53], and North African adults by Aglago et al. [54]. The BIA developed equation in our study used models concurrent with other similar validation studies where new equations are developed and cross-validated [39,55].

In previous studies, Houtkooper et al. [56], Ht^2/R was the best predictor of body composition accuracy and improved prediction accuracy when age and sex codes were included. Kushner [38], in his review studies on the validation of prediction equations of FFM and TBW compared to BIA, found that the majority of the studies reported that impedance index (Ht^2/R) produces less bias, while few other studies, including Jackson and Pollock [56], identified Ht/Ht^2 and weight to show the least bias. Kushner [38] further highlighted that though the studies included broad age ranges and body sizes, the inclusion of sex and age assisted in improving the level of agreement, irrespective of the body size. In the study by Liu et al. [58] all the variances applied (weight, height, age, sex, and ethnicity) to predict TBW and FFM were found to equally contribute to the accuracy of the prediction equation.

When the newly developed equation in the present was cross-validated with the validation group, no significant difference and a narrow bias between measured and predicted values were found, TBW ($p = 0.47$ bias of 0.00 ± 0.69) and FFM ($p=0.60$ bias of 0.0 ± 0.90). Testing the newly developed equation in a different group was necessary since it is reported that the accuracy of an equation may be reduced when applied to other samples [58].

The Bland-Altman plots for validation in our study demonstrated a good level of agreement between the predicted and measured TBW and FFM. The pure error in our predicted equation was 1.4l for TBW and 1.2kg for FFM. This was in line with the one reported by Ben Jemaa et al. [47] in Tunisian children (aged 8 to 11 years) of 1.26 l TBW and 1.646kg FFM and Hastuti et al. [59] on Indonesian men of 1.34l and 1.84 kg for FFM [57,60]. The regression analysis showed an assumption of proportional bias. These results are not exclusive; since the current findings are comparable to a study by Khan et al. [48].

Variances in the different validation studies are also influenced by the measurement equipment used by Wickramasinghe et al. [3]. It was highlighted that BIA does not directly measure TBW but relies on mathematically derived prediction equations that are mainly derived from high-income countries among Caucasian populations [3,61]. The method used to analyze the enrichment of the deuterium in the saliva sample may play a role in the accuracy of the analysis [32]. In our study, Fourier Transform Infrared spectroscopy (FTIR) was used to analyze the saliva sample. In the Houtkooper et al. [56] study, analysis of the DDM was done using isotope ratio mass spectrometry (IRMS) or Infrared Spectroscopy and produced low SEE and high R^2 compared to an analysis by tritium and liquid-scintillation counting. Therefore, it may not be surprising that the

variable results found in DDM validation studies may be due to the analytical method used to analyze the saliva sample.

Limitations and recommendations

One of the limitations of the study is the limited age range of 6- to 8-year-olds and the fact that only a homogenous sample in terms of age and ethnicity was included. Bioelectrical impedance analysis equations were found not to be sufficiently generalizable and population specific. Therefore, BIA equations are not sufficiently generalizable to be useful, and so, most or all populations need the development and validation of new equations specific to their population. Additionally, the new equation developed will need to be validated in other ethnic groups within the same age groups in South Africa. The study's strength is that it was able to evaluate a large sample of 299 African children attending primary school to develop and validate a BIA prediction suitable for 6-to 8-year old South African children from predominantly Tswana and Sotho ethnic groups. Also, we were unable to find any published BIA vs DDM studies in South African children in the literature, thus making comparisons difficult. Further studies which could confirm or refute our findings are recommended.

CONCLUSION

It was evident that most of the published equations used for comparison in the current study are invalid to predict TBW and FFM in South African children. The newly developed equations demonstrate potential for practical and accurate estimation in predicting TBW and FFM for use in South African children aged 6-to 8-year old from a predominantly Tswana and Sotho ethnic group. However, predictions equations probably need to be population-specific.

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Competing interests

The authors declare no competing interests.

Authors' contributions

TSMM wrote the manuscript, and interpreted the data, and proofread the final manuscript. MAM (as principal investigator in the BC–IT study) designed (project conception, development of overall research plan, and study oversight), conducted (hands-on conduct of the experiments and data collection), contributed to the writing of the paper, funding acquisition of the BC–IT study, supervision, project administration, analysis, and interpretation of data, and critical reviewing of the manuscript. JJR advised on the study design, review and commented on the manuscript. JX commented on the manuscript, and HSK contributed to the study's design and provided comments on the manuscript. All authors read and approved the final manuscript.

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CHAPTER 6: SUMMARY, CONCLUSION, LIMITATIONS AND RECOMMENDATIONS

6.1 Summary

The main aim of the research presented in this thesis was to validate the level of agreement between commonly used measures, namely Bioelectrical Impedance Analysis (BIA) and skinfold thickness and a subjective (Body Image perception) measure of body fatness using DDM as the reference method. The chapter summarises the interpretation of the main research findings, and conclusions. Further, the chapter ends with the study limitations and recommendations for future studies. Chapters 1, 2 and 5 of the thesis are written in compliance with NWU guidelines (NWU, 2017:29), except chapter 3, 4 and 5, that have been written in compliance with the journal where they will be published. Clause 5.10.5 states that a thesis written in article format must be presented for examination purposes as an integrated unit, inclusive of chapter 1, which outlines an introduction, the problem statement, and a synoptic conclusion as prescribed by faculty rules (NWU, 2017:29).

Chapters 1 and 2 are written using the North-West University (NWU) referencing guidelines (2017) and present the background, problem statement, study objectives, proposed hypotheses of the study, and the reviewed literature related to the body composition measurements and the validation equations for assessing or determining body composition. The reviewed literature identified gaps in previous validation studies, especially those conducted in low and middle-income countries. There was paucity of literature on validation studies from Africa.

The literature review highlighted that to date, studies on body composition have produced equivocal results on the accurate measures of body composition. Given the gender, ethnic and age differences in various populations, scientists in various parts of the world continue to search for methods that would accurately predict, be accessible to, and be population-specific to assess body composition. The search is complicated by the advances in technology, which also need to be validated in different population groups.

The review also highlighted that there is no single accurate method to measure *in vivo* humans, and previous scholars acknowledge that the only method to measure body composition accurately is by dissecting a human cadaver. However, dissecting cadavers can only be achieved after demise, and the person to be dissected may have died of a disease that may alter the body composition.

This reality necessitates the need to continuously validate existing commonly used or developing new methods that can accurately predict body composition. The review underlined limitations of various methods used to measure weight and body composition, including the commonly used

body mass index (BMI), that most studies use to estimate body fatness. Hence, body composition prediction has been the subject of intense debate within the scientific community and in clinical settings for decades. Various scholars recommend that existing prediction equations should be validated to assess their suitability in different population groups. Of interest was the role that body image perception can play in the success of obesity management programmes.

Further, while there are some methods regarded as criterion or reference methods for measuring body composition, comparison of the results from each of the methods have lead to ambiguous results. Several authors have recommended that prediction equations developed by earlier scholars need validation in different populations (Leal, 2019:11). Validation of previous predication equations developed by earlier scholars is necessitated by advancements in technology, the changing diets and changing obesity trends that alter body composition. Furthermore, different measurement tools are validated using different criterion methods creates an additional dilemma in identifying the most suitable method.

The literature further revealed that ethnicity, gender, age, and the level of fatness are central parameters in the measurement of obesity. Demonstrating that a method that could accurately predict fatness in one population may not be suitable for use in another population. The population for the current study was a homogenous group in terms of ethnicity and socio-economic status, and the age range was narrow (6- to 8-year old children). Therefore, replication of the current study may be different if used in a different ethnic group or heterogeneous group of South African children.

As outlined in the introductory chapter of this thesis, a response is required to the research questions, which refers to the validation of commonly used subjective measures against deuterium dilution techniques of stable Isotope as the reference method. A cross-sectional study design was used; Anthropometric measurements and body composition measures are described in chapter one. Further, in chapters 3-5, each article outlines different methods used to achieve the objective. The articles can be read independently; each article is written using the guidelines prescribed by the peer-reviewed journals selected. The methods compared in the study included; Body Image Perception vs BMI measurement and DDM, BIA vs skinfold equations, lastly comparison of Fat-Free Mass (FFM) and Total Body Water (TBW) by BIA and DDM.

6.2 Main findings of this thesis:

The results reflected significant differences and poor levels of agreement between the various methods used in the population of 6-8-year-old children. The study results were in line with previous studies that reported inconsistencies in levels of agreement for the assessing fatness between various body weight or fatness measurements. However, no comparable studies could be found to compare body image perception vs stable isotopes dilution.

The objective of Chapter 3 (Article 1) was to assess the level of agreement between objectively determined body composition versus perceived body image outcomes in 6-to 8-year old South African children. The article was unique in that it compared body image perception of boys and girls with DDM as a criterion method. According to the knowledge of the author of this thesis, no previous study was found that made such a comparison. The study also revealed the gross underestimation of fatness by the 6- to 8-year-old children based on a choice of silhouettes followed by underestimating BMI z-scores.

The objective of Chapter 4 (Article 2) was to assess the level of agreement between skinfold prediction equations and BIA for estimating body fat in South African children. The three skinfold prediction equations tested showed varying levels of agreements with the BIA. All three equations underestimated percentage body fat (%BF) compared to %BF measured by BIA. The difference was smallest with the Wickramasinghe *et al*, (2008) equation, though all three predicted equations showed a poor SEE against measured %BF using BIA. Age-specific %BF equation incorporating optimum methods using DDM are desperately needed.

The last objective, presented in Chapter 5 (Article 3), presented as objective 3, focused on developing and validating BIA in predicting TBW and adiposity in 6-to 8-year-old South African schoolchildren. The published TBW and FFM equations for BIA validated in the study demonstrated under- or over-estimations, though narrow in other studies. The newly developed South African equation had higher accuracy for both TBW and FFM prediction. This article is unique in the sense that it validates and develops TBW and FFM equations for the first time in children aged 6-8-years old in South Africa using stable isotope techniques of FTIR.

6.3 Conclusion

The three articles are interrelated, and together with the comprehensive thesis, they aim to respond to the research aim: to validate the level of agreement between commonly used body composition measures, namely BIA and, Skinfold Thickness and a subjective (Body Image perception) measure of body fatness using Isotope as the reference method. The conclusions drawn are based on the hypotheses posed in Chapter 1.

Hypothesis 1: Variations between perception of body image and objectively determined body composition will indicate a poor level of agreement in 6- to 8-year-old children exist.

The results indicate discrepancies between fatness based on body image perception compared to the BMI z-scores classification and DDM measurement. From a sample of 202 children, (32.2%, 55.1%, 8.8%, and 2.4%) perceived their body size as underweight, normal, overweight, and obese, respectively. Based on the BMI z-score, (18.8%, 72.8%, 6.9%, and 1.5%) were classified as underweight, normal, overweight, and obese, respectively. Body fatness

measurement showed that (2.5%, 48.0%, 21.8%, and 29.7%) were underweight, normal weight, overweight, and obese, respectively. The application of silhouettes and BMI z-scores resulted in either overestimation or underestimation of own body size. Overall, the levels of agreements (kappa, κ) between body size perception, body fatness, and BMI for age, respectively, were small ($\kappa=0.083$, $p=0.053$ and $\kappa=0.154$, $p<0.001$). The level of agreement between body size perception, body fatness, and BMI z-score was poor. The hypothesis was therefore accepted.

Hypothesis 2: Agreement between %BF obtained by skinfold prediction equations and that obtained by BIA in 6-8-year-old South African boys and girls will be low.

The levels of agreement differed between the BIA measurement and the three prediction equations. Significant mean differences ($p<0.001$) were found in the predicted %BF means determined by BIA ($25.12\pm6.42\%$), Dezenberg *et al*, (1999) ($17.84\pm5.36\%$), Slaughter *et al*, (1988) ($12.76\pm4.89\%$), Wickramasinghe *et al*, (2008) ($23.19\pm7.42\%$) and for the total group and by gender. Additionally, significant paired mean differences were found for BIA and Slaughter *et al*, (1988) ($t_{201} = 33.896$, $p<0.001$), Wickramasinghe ($t_{201} = -4.217$, $p < 0.001$), and Dezenberg *et al*, (1999) ($t_{201} = -16.910$, $p<0.001$). BIA moderately and positively correlated with the Slaughter equations ($r=0.61$, $p < 0.001$), and with the Wickramasinghe *et al*, (2008) equation ($r=0.54$, $p<0.001$), but a low correlation was observed with the Dezenberg *et al*, (1999) equation ($r = 0.47$, $p < 0.001$). For all the equations, the standards for evaluating prediction errors (Standard Error of the Estimate (SEE)) were above 5. The magnitude of the regression lines varied from low to moderate ($r^2 = 0.46-0.61$). The Bland-Altman regression plots showed relatively large positive and negative deviations from the mean differences lines, and trends of systematic under-and over-estimation of %BF across the %BF spectrum, in particularly when the Slaughter equation is applied. The hypothesis is partially accepted.

Hypothesis 3: Total body water calculated from published equations will under-/overestimate TBW calculated from a newly developed equation.

The published TBW and FFM equations for BIA validated in the study demonstrated under- or over-estimations. The newly developed South African equation had a higher accuracy for both TBW and FFM prediction. Significant associations ($p< 0.001$) were found between TBW and impedance index (Ht^2/R) for both development group ($R^2 = 74.3\%$) and cross-validation group ($R^2= 82.6\%$). The newly developed BIA prediction equation for the estimation of TBW was $= 1.472 + 0.257*(Ht^2/R) + 0.225*(weight) - 0.769*(sex) + 0.333*(age)$ with a regression coefficient $R = 0.935$ and $SEE = 0.801$. The BIA prediction equation to estimate FFM was $= 1.466 + 0.333 (Ht^2/R) + 0.292*(weight) - 0.997*(sex) + 0.491* (age)$, with a regression coefficient $R = 0.934$ and $SEE = 1.055$. No significant differences ($p>0.05$) were found between measured and predicted values for the validation group. Bland-Altman plots showed a good level of agreement between TBW and FFM predicted by the new equations against reference measures of TBW and FFM. The bias

expressed as the mean difference in TBW or FFM measured by DDM and BIA was 0.0 ± 0.69 l [95% CI: -1.352; 1.352] for TBW and 0.00 ± 0.90 kg FFM [95% CI: -1.764; 1.764]. The limits of agreement were respectively; -1.352kg; 1.352kg and -1.764kg; 1.764kg for TBW and FFM. The concordance correlation coefficients for the criterion values against the newly developed predicted BIA equations for TBW and FFM, respectively, were high (both $r=0.91$). When the newly developed and validated BIA prediction equations were plotted on Bland-Altman plots, the results showed a good level of agreement between TBW and FFM against the criterion technique of Deuterium Dilution, with negligible bias and narrow limits of agreement, especially for FFM. The hypothesis is, therefore, accepted.

To this end, the three articles in the thesis lined to each other in the sense that available prediction equations of body composition were validated, and levels of agreements determined as well as the new equations for TBW and FFM for children attending primary schools in the North-West Province of South Africa were developed.

6.4 Contribution of the study

Notwithstanding its limitations, the study has contributed to the scientific body of knowledge due to the methods used and results that have a public health impact. The comparison of the relationship between body image perception and objective measurements by DDM in the thesis is unique – no single study was found that had made such a comparison. Also, it is evident that South Africa may have a much more significant obesity problem than what is reported in BMI based studies. This study also contributed by developing a new equation to predict TBW and FFM for 6- to 8-year-old South African children. The available published BIA equations are unacceptable in estimating TBW and FFM for 6-to 8-year-old South African children. The newly developed prediction equations for TBW and FFM provide a good level of agreement with the DDM in the sample.

An important message of the study is that even though South Africa has a high overweight and obesity prevalence, the reality is that the currently relied-on tools are grossly underestimating overweight and obesity. It should be noted that, fatness is a major risk for non-communicable diseases and may be undiagnosed in individuals with a normal BMI who might be 'thin' but possess excess fat; therefore, physical activity and healthy eating should be advocated, irrespective of weight status. As such, these findings might be useful to policymakers to focus on fatness measured with body composition methods, and not only body weight, to be able to timely intervene to prevent risks factors of health associated with fatness. The results also highlight the need to strengthen school programmes to promote healthy eating and physical activity. Participating schools will be able to understand the magnitude of the obesity problem, and together with the parents, use the study's recommendations to develop intervention programmes.

The importance of body image perception in influencing behaviour change may need to be incorporated in behaviour change strategies and programmes.

6.5 Limitations of the study

- Firstly, the results of the study are not representative of the whole South African population; the study is limited to predominantly Tswana and Sotho ethnic groups within a confined socio-economic group. Secondly, factors that may contribute to the obesity rates were not assessed. Thirdly the age range was narrow; therefore, further studies are needed to expand the study to include other age groups.
- The study population was from North-West Province, South Africa only, and thus the results cannot be generalised to the entire South African population.
- The impact of parental preferences and environmental factors on body perception was not assessed.
- Further, the study did not assess how the current perceived weight may impact behaviour change.

6.6 Recommendations and future research

The following recommendations are proposed to influence future research:

- A heterogeneous sample inclusive of other ethnic groups is needed to validate BIA and skinfold equations used in the study.
- The current study used in-built BIA equations. It is uncertain whether the use of equations developed by other authors compared with skinfold thickness would have produced a different level of agreement.
- The study sample had a narrow age range, limited to 6- to 8-year-old children. The influence of age in the assessment of body perception could not be determined, so future studies should include a broader age range and have different age categories that can be compared.
- Future studies are needed to assess external factors that may influence the body size preferences of children (such as the role of parents, peers and media).
- In the study, children were classified within the normal weight range based on BMI measurement, who had a higher %BF. Attention should be given to assess the influence of diet and physical inactivity among these children.

- Given the reviewed literature, it is my perception that the impact of cultural ideals, poverty and community perceptions in the rising obesity in South Africa may be underestimated. Therefore, future studies need to focus on factors contributing to the preference, e.g., culture, big bodies as a norm and environmental factors and the role of poverty in making healthy choices in this community and South Africa in general.

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APPENDIX A: ETHICS DOCUMENTATION



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Research Ethics Regulatory Committee

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ETHICS APPROVAL CERTIFICATE OF STUDY

Based on approval by Health Research Ethics Committee (HREC) on 22/02/2018, the North-West University Research Ethics Regulatory Committee (NWU-RERC) hereby approves your study as indicated below. This implies that the NWU-RERC grants its permission that provided the special conditions specified below are met and pending any other authorisation that may be necessary, the study may be initiated, using the ethics number below.

Study title: Body composition by isotope techniques and objectively assessed physical activity in 6–8 year-old children: BC-IT study

Study Leader: Prof MA Monyeke

Ethics number:

N W U - 0 0 0 2 5 - 1 7 - A 1

Submission Study Number Year Status: S = Submission, R = Re-Submission, P = Provisional Authorisation, A = Authorisation

Application Type: Larger study

Commencement date: 19/04/2017

Risk:

2

Approval of the study is initially provided for a year, after which continuation of the study is dependent on receipt of the annual (or as otherwise stipulated) monitoring report and the concomitant issuing of a letter of continuation.

Special conditions of the approval (if applicable):

- Please provide the HREC with permission letters from the school governing bodies and the principals of the schools still outstanding, for approval before recruitment can begin.

General conditions:

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, please note the following:

- The study leader (principal investigator) must report in the prescribed format to the NWU-RERC via HREC:
 - annually (or as otherwise requested) on the monitoring of the study, and upon completion of the study
 - without any delay in case of any adverse event or incident (or any matter that interrupts sound ethical principles) during the course of the study.
- Annually a number of studies may be randomly selected for an external audit.
- The approval applies strictly to the proposal as stipulated in the application form. Should any changes to the proposal be deemed necessary during the course of the study, the study leader must apply for approval of these amendments at the HREC, prior to implementation. Should there be any deviations from the study proposal without the necessary approval of such amendments, the ethics approval is immediately and automatically forfeited.
- The date of approval indicates the first date that the study may be started.
- In the interest of ethical responsibility the NWU-RERC and HREC retains the right to:
 - request access to any information or data at any time during the course or after completion of the study;
 - to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process.
 - withdraw or postpone approval if:
 - any unethical principles or practices of the study are revealed or suspected,
 - it becomes apparent that any relevant information was withheld from the HREC or that information has been false or misrepresented,
 - the required amendments, annual (or otherwise stipulated) report and reporting of adverse events or incidents was not done in a timely manner and accurately,
 - new institutional rules, national legislation or international conventions deem it necessary.
- HREC can be contacted for further information or any report templates via Ethics-HREC@nwu.ac.za or 018 299 1206.

The RERC would like to remain at your service as scientist and researcher, and wishes you well with your study. Please do not hesitate to contact the RERC or HREC for any further enquiries or requests for assistance.

Yours sincerely

Prof. Refilwe Phaswana-Mafuya

Chair NWU Research Ethics Regulatory Committee (RERC)

District Education Department Research Approval



Education and Sport Development
Department of Education and Sport Development
Departement van Onderwys en Sport Ontwikkeling
Lefapha la Thuto le Tlhaboloiso ya Metsihamako
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DR KENNETH KAUNDA DISTRICT OFFICE OF THE DISTRICT DIRECTOR

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Contact 082 317 4656

27 June 2017

To: **University of the North West
Faculty of Health & Science
Physical Activity Sport & Recreation**

Attention: **Prof. M. Monyeke (Principal Investigator)
Prof. H. Moss (Director – PhASRec)**

From: **Mr. H. Motara
District Director**

PERMISSION TO CONDUCT RESEARCH:

"Body Composition by Isotope Techniques and Objectively Assessed Physical Activity in 6 – 8 Year old Children: BC-IT Study."

We refer to your e-mailed correspondence regarding your request for permission to conduct research under the above-stated topic in primary schools around Dr. Kenneth Kaunda District.

Accordingly, permission is hereby granted for you to conduct the research as per your request, subject to the following provisions.

- That the onus to contact your target schools about your request and this subsequent letter of permission rests with your good self. Your Target Schools are: Dan Tlome, Pudulogo, Tshupane, Lesego, Potch and Keotshepile Primary Schools
- A parent-consent seeking process must be undertaken via the School Governing Bodies of affected schools, for learners identified to participate in the research.
- Considering that the research work will involve learners, it is requested that you ensure that the general functionality of affected schools is not compromised by the research project, or alternatively, a recovery plan be developed to recoup lost teaching time.
- Your research findings must be made available to the Department of Education & Sport Development in Dr. Kenneth Kaunda District upon request.
- The principle of confidentiality will be observed in its strictest terms in relation to information sourced from the research work conducted.

Thanking you

**Mr. H. Motara
District Director
Dr. Kenneth Kaunda District**



"Towards Excellence in Education and Sport Development"

APPENDIX B: RECRUITMENT FLIER, LETTER TO THE SCHOOLS AND DEPARTMENT OF EDUCATION, AND INFORMED AND ASSENT CONSENT FORMS

RECRUITMENT FLYER



RECRUITMENT FLYER

North-West University

TITLE OF THE STUDY: Body composition by isotope techniques and objectively assessing physical activity in 6-to 8-year-old children: BC-IT study

Your child is invited to participate in a study aimed at examining birth weight, body composition by BIA and body composition by stable isotope techniques at 6-to 8- years South African children: its relationship to physical activity by Prof. M.A. Monyeki at the North-West University, (Potchefstroom Campus), Physical Activity, Recreation and Sport Science Focus Area within the Faculty of Health Sciences. The study involves 4 hour visit on collection of saliva for stable Isotope, questionnaires (i.e. demographic, diet, physical activity) fitness tests, objective measure of physical activity by wearing Actigraph, body composition by BIA at the Physical Activity, Sports and Recreation (PHASRec) laboratory.

No compensation will be given, except that incentives will be provided during data collection. If your child is at least 6 to 8 years old, healthy and not on medical drugs, and would like more information about participating, contact:

Principal investigator professor M.A. Monyeki email: andries.monyeki@nwu.ac.za or tel: 018 2991790 or 018 299 1807

LETTERS TO THE SCHOOLS AND DEPARTMENT OF EDUCATION



Faculty of Health Sciences
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<http://www.nwu.ac.za>

Date:

The School Principal and SGB

REQUEST TO RECRUIT PARTICIPANTS FROM YOUR SCHOOL FOR A RESEARCH PROJECT

Dear Sir,

We the researcher from the Physical Activity, Sport and Recreation Focus Area (PHASRec) at the Faculty of Health Sciences, North-West University are hereby making a request to recruit participants from your school for a research project.

Background of the study:

Globally, obesity affects 500 million persons, and is predicted to increase to one billion people by 2030. Obesity occurs due to number of circumstances such as recovery from undernutrition or low birth weight and excessive fatness as well as physical inactivity. In this area especially in adolescents overweight and obesity prevalence is reported to be 13.7%. The observed percentages warrant studies to be done earlier in life to determine the actual causes for effective strategic intervention given the fact that overweight or obese child are likely to become obese adults. It should be noted that over fatness is detrimental for health. The long term consequences of having excessive fatness in the body is linked with the development of non-communicable lifestyle diseases such as high blood pressure and high glucose levels. Many people also report very little regular physical activity, which contribute to a lack of performance at work. These combinations increase the burden of disease and result in low capital performance. The information we currently have to manage these health issues, have been determined by means of people self-reporting their physical activity and

using proxy measurements for over-fatness. Since over-fatness at an early age is known to carry into adulthood, early interventions are now the focus of research. Therefore, it is necessary to apply new technologies that have been developed recently to make accurate measurements. These new techniques include stable isotopes as well as objective measures such as, bioelectrical impedance (BIA) and accelerometers (Acti-Graph). The aims of this study is **to examine birth weight, body composition by BIA and body composition by stable isotope techniques in 6–8 years South African children (Potchefstroom areas), and its relationship to physical activity.**

The outcome of this project is to try to come up with the strategic interventions plans which are scientifically based on how to reduce childhood obesity.

We are requesting you a permission to do the project with learners in grades 1 to 3. The project is planned to take place according to the schedule to be given to you upon agreement to take part in the project. The project will have a minimal effect on the school time table. No blood will be drawn from the children, but a saliva sample will be collected with cotton wool.

As the investigator in the project I will be pleased to come to your school and give a briefing about the project.

A token of appreciation will also be given to a learner upon completion of the tests. Measurements are provided in under 20 seconds. The measurements to be done are the following:

1. Demographic information (personal information and family; education of the parents, employment, type of dwelling, household amenities and marital status).
2. Anthropometric measurements (i.e. body height; weight; skinfolds thickness (triceps, subscapular and calf skinfolds), and waist.
3. Cardiovascular risk factor measurements by the use of Finometer.
4. Physical activity questionnaire (PAQ-C) for only 8 years old learners
5. ActiGraph for physical activity.
6. Health-related physical fitness (i.e. 20m shuttle run, standing broad jump, sit-and-reach, bent arm hang, sit-ups).
7. Dietary questionnaire, health habits and knowledge of food groups.
8. Body image.
9. Birth weight from the hospital/ clinic card.
10. Use of stable isotope to measure percentage body fatness (learners will be subjected to the ingestion of deuterium water and thereafter a saliva will be collected to measure

total body water and subsequently percentage body fat). The ingestion of deuterium water is safe as been proven by the International Atomic Energy Agency.

11. Use of bioelectrical impedance analysis (BIA) to measure percentage body fatness. With the bioelectrical impedance a child will be attached with the electrodes which will be connected to Bioimpedance Analyzer. Depending on the monitor, body composition.

Data to be collected on these children will be used solely for research purposes, and it will be treated as anonymous in any form of a report.

Your cooperation in this regard will be highly appreciated.

Yours sincerely,
Thank you,

Prof. M. Andries. Monyeki
(Principal Investigator, NWU-Potchefstroom)

Prof. Hanlie Moss
Director: Physical Activity, Sports and
Recreation, NWU-Potchefstroom

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Date:

The District Operational Director

Department of Education
North-West Province
Potchefstroom

**REQUEST TO RECRUIT PARTICIPANTS FROM SCHOOL FOR A RESEARCH
PROJECT
WITHIN YOUR DISTRICT**

Dear Sir,

We the researcher from the Physical Activity, Sport and Recreation Focus Area (PHASRec) at the Faculty of Health Sciences, North-West University are hereby making a request to recruit participants from school for a research project within your district.

Background of the study:

Globally, obesity affects 500 million persons, and is predicted to increase to one billion people by 2030. Obesity occurs due to number of circumstances such as recovery from undernutrition or low birth weight and excessive fatness as well as physical inactivity. In this area especially in adolescent's overweight and obesity prevalence is reported to be 13.7%. The observed percentages warrant studies to be done earlier in life to determine the actual causes for effective strategic intervention given the fact that overweight or obese child are likely to become obese adults. It should be noted that over fatness is detrimental for health. The long-term consequences of having excessive fatness in the body is linked with the development of non-communicable lifestyle diseases such as high blood pressure and high glucose levels. Many people also report very little regular physical activity, which contribute to a lack of performance at work. These combinations increase the burden of disease and result in low capital performance. The information we currently have to manage these health issues, have been determined by means of people self-reporting their physical activity and using proxy measurements for over-fatness. Since over-fatness at an early age is known to carry into adulthood, early interventions are now the focus of research. Therefore, it is necessary to apply new technologies that have been developed recently to make accurate measurements. These new techniques include stable isotopes as well as objective measures such as, bioelectrical impedance (BIA) and accelerometers (Acti-Graph). The aims of this study is **to examine birth weight, body composition by BIA and body composition by**

stable isotope techniques in 6-8 years South African children (Potchefstroom areas), and its relationship to physical activity.

The outcome of this project is to try to come up with the strategic interventions plans which are scientifically based on how to reduce childhood obesity.

We are requesting you a permission to do the project in learners in grades 1 to 3. The project is planned to take place according to the schedule to be given to you upon agreement to take part in the project. The project will have a minimal effect on the school timetable.

No blood will be drawn from the children.

As the investigator in the project I will be pleased to come to your school and give a briefing about the project.

A token of appreciation will also be given to a learner upon completion of the tests.

The measurements to be done are the following:

- Demographic information (personal information and family; education of the parents, employment, type of dwelling, household amenities and marital status)
- Anthropometric measurements (i.e. body height; weight; skinfolds thickness (triceps, subscapular and calf skinfolds), and waist.
- Cardiovascular risk factor measurements by the use of Finometer 4. Physical activity questionnaire (PAQ-C) for only 8 years old learners
- ActiGraph for physical activity
- Health-related physical fitness (i.e. 20m shuttle run, standing broad jump, sit-and-reach, bent arm hang, sit-ups)
- Dietary questionnaire, health habits and knowledge of food groups
- Body image
- Birth weight from the hospital/ clinic card
- Use of stable isotope to measure percentage body fatness (learners will be subjected to the ingestion of deuterium water and thereafter a saliva will be collected to measure total body water and subsequently percentage body fat). The ingestion of deuterium water is safe as been proven by the International Atomic Energy Agency.
- Use of bioelectrical impedance analysis (BIA) to measure percentage body fatness. With the bioelectrical impedance a child will be attached with the electrodes which will be connected to Bioimpedance Analyzer. Depending on the monitor, body composition measurements are provided in under 20 seconds.

5. The schedule of the project will be as follow (Specific dates for selected schools will be finalised per arrangement with the principals concerned):

Month	Duration
April/May 2017	3 hours per child in a selected school
April/May 2017	3 hours per child in a selected school

Identified children will be transported to the North-West University laboratory for the measurements per grade on specific days. Transport will be paid by the university.

Due to the fact that participants will be asked to fast 10 hours without eating breakfast in the morning, therefore sandwiches and drink provision will be made available upon completion of the measurements. The outcomes of this project will benefit the children and the schools with the information regarding the physical activity status and the determinants of health for future.

Data to be collected on these children will be used solely for research purposes, and it will be treated as anonymous in any form of a report.

Hoping for a positive response.

Yours sincerely,

Thank you,

Prof. M. Andries. Monyeki
(Principal Investigator, NWU-Potchefstroom)

Prof. Hanlie Moss
(Director: Physical Activity, Sports and Recreation, NWU-Potchefstroom)

**WRITTEN INFORMED CONSENT AND ASSENT FORMS
WRITTEN INFORMED CONSENT AND ASSENT FORMS; PARENT CONSENT
FORM**

INFORMED CONSENT LETTER



HREC Stamp

PARTICIPANT INFORMATION LEAFLET AND ASSENT FORM FOR CHILDREN



**TITLE OF THE RESEARCH PROJECT: BODY COMPOSITION BY ISOTOPE TECHNIQUES
AND OBJECTIVELY ASSESSING PHYSICAL ACTIVITY IN 6–8 YEAR-OLD CHILDREN: BC-
IT STUDY**

REFERENCE NUMBERS:

PRINCIPAL INVESTIGATOR: Prof. M.A. Monyeki

**ADDRESS: Physical activity, Sport and Recreation (PhASRec), Faculty of
Health Sciences, North-West University, Private Bag X6001, Potchefstroom, 2520**

CONTACT NUMBER: 018 2991790

You are being invited to take part in a research project which seeks to investigate body composition by isotope techniques and objectively assessing physical activity in 6 to 8 year-old children. Please take some time to read the information presented here, which will explain the details of this project. Please ask the researcher any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you could be involved. Also, your participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part.

This study has been approved by the **Health Research Ethics Committee of the Faculty of Health Sciences of the North-West University (NWU.....)** and will be conducted according

to the ethical guidelines and principles of the international Declaration of Helsinki and the ethical guidelines of the National Health Research Ethics Council. It might be necessary for the research ethics committee members or relevant authorities to inspect the research records.

What is this research study all about?

- This study will be conducted in primary schools within the Tlokwe local municipality and will involve about 300 children aged between 6 to 8 years of age. Data with regards to demographic information, birth weight, physical activity, and cardiovascular function will be collected. Body composition by means of anthropometric measurements of height, body mass, skinfolds, and circumferences will be measured. Water mixed with a deuterium dose will be given to children and afterwards saliva will be collected to determine body fat and muscle mass by means of stable isotope. Body composition will also be determined by means of bioelectric impedance analysis for comparison purposes. Data collection will be conducted by experienced health researchers trained in Human Movement Science.

The objectives of this research are:

- i. determine birth weight (retrospectively), body composition by BIA and body composition by isotope techniques, and its relationship to physical activity in 6 to 8 years of age in South African children.
- ii. investigate a longitudinal development of body composition with birth weight (retrospectively) and physical activity in South African children.
- iii. determine the associations between anthropometry, body composition, physical activity level and metabolic health in normal-weight, overweight and obese children using stable isotope techniques and establish improved predictors of future health risk.

Why have you been invited to participate?

- You have been invited to participate because you are between 6 to 8 years old and in a primary school from the area selected for recruitment.
 - You have also complied with the following inclusion criteria: healthy aged 6 to 8 years attending primary schools within the Tlokwe Local Municipality
 - You will be excluded if: you are not healthy and, less than 6 years and above 8 years old, and attending primary schools outside Tlokwe Local Municipality. Unable to perform any of the health-related physical activity tests.

What will your responsibilities be?

- You will be expected to answer a number of questionnaires (a sociodemographic information, including data on personal information and family - i.e. education of the parents, employment, type of dwelling, household amenities and marital status) personal image, physical activity, healthy habits, lifestyle and health, knowledge on food choice, will be collected by means of a IAEA questionnaire. The questionnaire will take about 15 minutes to complete under the supervision of teachers, and consists of 51 questions; Take part in the EUROFIT test battery which will last for 10 minutes, and anthropometric measurements of height, body mass, waist circumference, mid arm circumference, triceps, biceps, supra-iliac and subscapular skinfolds which will last for 10 minutes; and give saliva for stable isotope (at different time zones, at arrival and after 2 hours of the dose of deuterium) and do assessment of body composition by bioelectric impedance analysis for approximately 4–5 minutes. Lastly, an accelerometer will be attached to determine habitual physical activity and be placed on your waist with a belt for 24 hours for seven days.

Will you benefit from taking part in this research?

- The direct benefits for you as a participant will be to recognize the importance of keeping a healthy body composition and the benefits associated with regular participation in physical activity. It may increase the children's awareness of a regular participation in physical activity in reduction of obesity and keeping a healthy lifestyle. You will be able to gain access to their results. Their data will also be used to explain to teachers orally how to improve their healthy lifestyle.
- The indirect benefit will be an expansion of existing expert knowledge in the field of Human Movement Science and related fields which can be transferred to the wider community. This knowledge can be used for further studies addressing applicability of our findings.

Are there risks involved in your taking part in this research?

- There are no known risks associated with participating in this research project, but the anticipated risks in this study are minimal. This may be due to the fact that you might get tired during the ± 10 minutes of physical fitness testing. To minimised fatigue the researchers will aim to maximise potential benefits that are likely to have a valuable impact on the children either directly or indirectly. No risk for using isotope deuterium when collecting saliva, and use of accelerometry (ActiGraph) for physical fitness tests and BIA in children are known. These instruments are safe to use in field research in children participants. No severe physical, psychological, social stress or other negative consequences will be encountered in this study. An experienced researcher will take the children through the process of all the test items. A medical doctor (Dr. Venter) will be on standby during the total duration of the project, should any injuries occur. In case of illness or injury, children will immediately be withdrawn from the project and will be allowed to leave at any time if they feel so

Are there risks involved in your child taking part in the project and producing us with his/her data? If so what will be done as precautionary measures?

Risks	Precautionary measures
Loss of anonymity	All participants will be allocated a number to whom their data will be linked to protect their anonymity.
Data management and loss of original data sets	Data will be kept safe and secure in the service provider's office and electronic data (Statistica for Windows and SmartaBase) will be password protected
Unethical future dissemination of data sets	Data will be stored for a period of 20 years after which it will be destroyed in the safest way possible.
Loss of class time	No additional class time will be lost.
Fatigue and injury	All necessary measures are put in place during programmes offered to avoid and if necessary manage such risks.

The benefits outweigh the risk

The benefits in this study outweigh the risks. This research is not designed to help you personally, however you will receive a free medical examination and receive your own results informing you whether you have high blood pressure, high body fat. The findings of this research can be used to help prevent, treat and manage non-communicable diseases (NCDs) of lifestyle associated with overweight and obesity in South African people.

What will happen in the unlikely event of some harm/form of discomfort occurring as a direct result of your taking part in this research study?

- Should you have the need for further discussions during/after the participation in this project; an opportunity will be arranged for you to speak directly with the researcher so that we can help you.

Who will have access to the data?

- Anonymity will be only partial because you will be doing the post-test measurements and wearing ActiGraph with your classmates. We as researchers will do everything to make sure that the answers you gave on the questionnaire and sample collected will be kept secret and we will not disclose your name to anyone. Reporting of findings will be anonymous and the data of the groups and not on an individual level and by means of only using your research number that will be given to you and we will not disclose your name to anyone. Only the researchers will know which data belongs to you.

What will happen with the data/samples?

- Data/samples will be kept safe and secure by locking hard copies in locked cupboards in the researcher's office and for electronic data it will be password protected. Data will be stored for 7 years. Data will be analysed in South Africa and reported in various forms. Participants' permission will be requested for data sharing with other collaborators for research reports.

Will you be paid to take part in this study and are there any costs involved?

- You will not receive any payment except that, incentives will be provided to you during data collection.
- There will thus be no costs involved for you, if you do take part.

Can I withdraw from the study?

- Your participation in this research is completely voluntary. You may choose not to take part at all. If you decide to participate in this research, you may stop participating at any time. If you decide not to participate in this study or if you stop participating at any time, you will not be penalized or lose any benefits such as school marks to which you otherwise qualify.

Is there anything else that you should know or do?

- You can contact Professor M.A. Monyeki at 018 299 1790 if you have any further queries or encounter any problems.
- You can contact the Health Research Ethics Committee via Mrs Carolien van Zyl at 018 299 2089; carolien.vanzyl@nwu.ac.za if you have any concerns or complaints that have not been adequately addressed by the researcher. If you would like to take part in the study, please read and sign the consent form.
- You will receive a copy of this information and consent form for your own records.

How will you know about the findings?

- The findings of the research will be shared with you by giving you a summary of your results.

Declaration by participant

By signing below, I agree to take part in a research study entitled: Determining body composition by isotope techniques and objectively assessing physical activity in 6 to 8 year-old children.

I declare that:

- I have read this information and consent form and it is written in a language with which I am fluent and comfortable.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is **voluntary** and I have not been pressurised to take part.
- I may choose to leave the study at any time and will not be penalised or prejudiced in any way.
- I may be asked to leave the study before it has finished, if the researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to.

Signed at (*place*) on (*date*) 20....

.....
Signature of participant

.....
Signature of witness

Declaration by investigator

I (*name*) declare that:

- I explained the information in this document.
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above
- I did/did not use an interpreter.

Signed at (*place*) on (*date*) 20....

.....
Signature of investigator

.....
Signature of witness

CONSENT

Declaration by parent (Informed Consent: 0-13 years)	
Declaration by the participant (Written Assent: 7-13 years)	
Declaration by the participant (Verbal Assent: 0-7 years)	

Declaration by parent (Parental informed Consent: 0-13 years)

By signing below, I give consent that my child may take part in the project and that his information may be used for research purposes.

I declare that:

I have read this information and consent form and it is written in a language with which I am fluent and comfortable. I have had a chance to ask questions to both the person obtaining consent, as well as the service provider and all my questions have been adequately answered, if needed. I understand that my child taking part in this project is **voluntary** and I have not been pressurised to give consent. I may choose to withdraw my consent at any time and my child will not be penalised or prejudiced by this in any way. I understand that the data collected during this project may be used for possible future research purposes (as indicated by the objectives) as long as anonymity is ensured. I give consent that data collected may be used for research purposes.

YES	NO
-----	----

Signed at (place) on (date) 20....

.....
Signature of participant

.....
Signature of witness

Declaration by the participant (Written Assent: 7-13 years)

By signing below, I agree to take part in this research project

I declare that:

I have understood instructions given to me verbally. Information and permission was given in a language with which I am fluent and comfortable. I have had a chance to ask questions to my parent and the person obtaining consent, as well as the researcher and all my questions have been adequately answered. I understand that taking part in this study is voluntary (my own decision) and that nobody forced me to do so. I may choose to withdraw my assent at any time and I will not be penalised or discriminate against in any way. I may be asked to leave the study before it is finished, if the researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to. I am also told that the information or data collected on me will solely be used for research purpose until it is fully analysed.

Signed at (place) on (date) 20....

.....
Signature of child

.....
Signature of witness

VERBAL ASSENT (0-7 years)

Do you want to participate in the activities today? Point with your finger to the face that shows how you feel about taking part in today's activities.

YES



NO



Child's choice:

Declaration by person obtaining consent

I (name) declare that:

I explained the information in this document to the teacher, parent (if possible) and the child themselves. I encouraged all parties to ask questions and took adequate time to answer them. I am satisfied that all parties adequately understand all aspects of the project, as discussed above. I did not use any interpreter.

Signed at (place) on (date) 20....

.....
Signature of person obtaining consent

.....
Signature of witness

Problems and queries to:

1. Professor Andries Monyeki: Tel: 018 2991790 or email: andries.monyeki2nwu.ac.za
2. Health Research Ethics Committee (Mrs Carolien van Zyl): 018 299 2089; carolien.vanzyl@nwu.ac.za

SETSWANA VERSION OF LEKWALO LA GO DUMELA GOITSISIWE LEKWALO LA GO DUMELA GOITSISIWE



Setempe sa HREC

PAMPITSHANA YA TSHEDIMOSETSO YA MOTSAKAROLO LE FOROMO YA BANA YA TUMELO



**SETLHOGO SA YA PATLISISO YA POROJEKE: POPO YA MMELE KA MOKGWA WA
SETEGENIKI SA ELEMENTE YA DIKHEMIKALE LE GO SEKA-SEKA KA MAITLHOMO A
TIRISO YA MMELE MO BANENG BA DINGWAGA DI MAGARENG GA 6-8: DITHUTO BC-IT**

DINOMORO PAPISO:

TLHOGO YA BOTLHOTLHOMISI: Mop. M.A. Monyeke

**ATERESE: Tiriso ya mmele, Motshameko le Boitapoloso (PhASRec), Legopo la Disaense
le Boitekanelo,
Yunibesithi ya Bokone Bophirima, Poso ya sephiri X6001, Potchefstroom, 2520**

NOMORO YA KOPANO: 018 2991790

O lalediwa go tsaya karolo mo porojekeng ya patlisiso e e batlang go tlhotlhomisa popego ya mmele ka mokgwa wa setegeniki wa dielemente tsa dikhemikale le ka go seka-seka maitlhommo a tiriso ya mmele mo baneng ba bogolo bo ka nnang 6 go ya go 8ka dingwaga. Tsweetswee tsaya nako go bala tshedimosetso e e filweng fano, e e tla tlhalosang ka ga porojeke e. Tsweetswee botsa mmatlisisi dipotso dingwe le dingwe ka ga karolo nngwe le nngwe ya porojeke en aka seo o sa setlhaloganyeng ka botlalo. Go botlhokwa thata gore o kgotsofale ka botlalo le gore o tlhaloganya sentle se patlisiso e e se kenyeletsang le gore o ka amega jang. Ebile, go tsayakarolo ga gago ke ka **boithaopi ka gotlhe** mme o letleletswe go ikgolola mo go tseyengkarolo. Fa o re nnyaa, se se ka se go ame ka boitatolo le ka tsela nngwe le nngwe. Gape o gololesegile go ikogela kwa morago mo thutong ka ntlha nngwe le nngwe, le fa ele gore o dumela go tsayakarolo.

Thuto e letleletswe ke ba **Komiti ya Boitshwari ya Patlisiso ya Boitekanelo ya Legopo la Disaense tsa Boitekanelo tsa Yunibesithi ya Bokone-Bophirima(YBB.....)** mme e tla tsamaiswa go ya ka melao ya motheo le taolo ya Polelo ya Boditshabatsaba ya ga Helsinki le melao ya motheo ya Khansele ya Boitshwari ya Patlisiso ya Bosetshaba ya Boitekanelo. Go ka

tlhokagala gore maloko a komiti ya boitshwaro ya patlisiso kgotsa balaodi ba ba maleba go tlhatlhoba bosupi jwa patlisiso.

Thuto e ya Patlisiso ke ka ga eng?

- Thuto e e tla tsamaisiwa mo dikolong tse di potlana mogare ga motse selegae wa masepale wa Tlokwe mme e tla akaretsa bana ba ba kannang 300 ba le dingwaga di le magareng ga 6 go ya go dile 8. Tshedimosetso e e kgobokantsweng mabapi le setlhopha se se riling, bokete jwa matsalo, tiriso ya mmele, le kokoannyo ya tiriso ya tsamaiso ya madi. Popego ya mmele ka go dirisa selekanyetsi sa boleele sa anteropometiriki, bokete jwa mmele, menoganoletlalo, le go dikologa go tla lekanyetswa. Bana ba tla neelwa selekanyetso sa elemente ya khemikhale gabedi sa metsi a a kopantsweng mme morago ga moo mathe a tla kgobokangwa go ikaelela mafura a mmele letshobokano ya mesifa ka mokgwa wa elemente ya khemikhale e e tlhomameng. Popego ya mmele e ka itlhome gape ka mokgwa wa seka-seka sekgoreletsi sa motlakase ka maitlhome a papiso. Kokoano ya tshedimosetsoe tla dirwa ke babatlisisi ba boitekanelo ba ba katisitsweng mo Disaenseng tsa Tsikinyego ya Botho.
- Maitlhome a patlisiso e ke ana:
 - i. ikaelela boima jwa matsalo (kakanyo e fetileng), popego ya mmele ka BIA le popego ya mmele kaelemente ya khemikhale ya setegeniki, le kamano ya tiriso ya mmele mo dingwageng dile 6 go ya go dile 8 mo baneng ba Aforikaborwa.
 - ii. Tlhotlhomisa tsweletso ya tlhabololo ya nako e erileng ya popego ya mmeleka bokete jwa mmele (kakanyo e fetileng), le tiriso ya mmele mo baneng ba Aforikaborwa.
 - iii. Itlhomele ditokololo magareng ga anteropometiriki, popego ya mmele, tepatepanyo ya tiriso ya mmele, le boitekanelo jwa taolo ya dikhemikhake ya mmele mo boketeng jo bo tlwaelegile, bana ba digompo leba banonneng go dirisiwa mokgwa wa elemente ya khemikhale ya setegeniki le go tlagisa tebelelo pele e e ntshwafaditsweng ya matshosetsi a boitekanelo a bokamoso.

Goreng o laleditswe go tsayakarolo?

- O laleditswe go tsayakarolo ka gonne o mo magareng a dingwaga dile 6 go ya go dile 8 mo sekolong sa poraemare go tswa mo tikologong e e tlhophilweng go baithaopi. O dumelane gape le mokgwa wa go tsenyeletsa: dingwaga dile 6 go ya go dile 8 tsa itekanelo ba tsena kwa diklong tsa poraemare mogare ga Masepale wa Selegae wa Tlokwe. O tla tswalelwa kwantle fa fela: ga wa itekanela mme, ole ka fat lase ga dingwaga dile 6 le go feta dile 8 o tsena kwa dikolong tsa poraemare mogare Masepale wa Selegae wa Tlokwe. O sa kgone go diragatsa nngwe ya ditiro tsa mmele tse di ikamanyang le boitekanelo.

Ke eng se e tla nnang maikarabelo a gago?

- O tla be o lebeleletse go araba palo ya dipotso (tshedimosetso ya loago lo lo mabapi, go tsenyeletswa tshedimosetso ya gago le ba lelapa – eleng thuto ya batsadi, tiro, mofuta wa bonno, dilwana tsa legae le maemo a lenyalo) setshwano sa botho, tiriso ya mmele, ditlwaello tsa itekanelo, boitekanelo le mokgwa wa go tshela, kitso ya go tlhopha dijo, di tla kgobokanngwa ka mokgwa wa dipotso tsa IAEA. Dipotso di tla tsaya metsotso ele 15 go e fetsa ka fa tlase ga tebelelo ya barutabana, mme e na le dipotso dile 51; Tsayakarolo mo tekong ya legala ya EUROFIT e e tla tsayang metsotso ele 10, le selekanyetsi sa elemente ya dikhemikhale ya setegeniki ya boleele, bokete jwa mmele, bokgolokwe jwa letheke, bogare jwa letsogo, mesifamegolo ya fa morago mogodimo ga letsogo, mesifamegolo ya letsogo mogodimo kwa pele, menagano ya legetla le go feteletsa go go tla tsayang metsotso ele 10; mme a neelana ka mathe go elemente ya khemikhale ya setegeniki e e tsepameng (ka mofuta e e farologaneng ya dinako fa ba goroga le morago ga diura dile 2 tsa nno ya mofuta wa haedrojini) le go dira tsheka-tsheko ya popego ya mmele ka sekeleki sa mokgwa wa setegeniki go lekanyetsa motlakase go emisa tsamao ya motlakase metsotso e e ka nnang ele 4-5. Lwa bofelo, sediriswa sa sepotlakisi se a

tlhongwa go lebelela ditlwaelo tsa tiriso ya mmele le go bewa mo lethekeeng la gago ka lebanta diura di le 24 malatsi ale supa.

A go sengwe se o tla se boelwang ka go tsayakarolo mo patlisisong e?

- Dipoelo tse di lebaneng le wena jaaka motsayakarolo e tla nna go lemoga botlhokwa jwa go tshola popego ya mmele o o itekanetseng le dipoelo tse di gokaganang le go tsayakarolo ka gale mo tirisong ya mmele. E tla oketsa le goitse ga bana ga go tsayakarolo ga ka gale mo tirisong ya mmele mo go fokotseng bokima le go tshwarelela bophelo jo bo itekanetseng. O tla kgona go bona katamelo go dipholong tsa tsona. Tshedimisetso e e kokoantsweng e tla dirisiwa gape go tshalosetsa barutabana ka molomo gore bophelo jwa bone jwa boitekanelo bo ka tokafala jang.
- Dipoelo tse di sa lebanang e tla nna phuthologo ya kitso ya botswerere e e leng teng mo dithutong tsa Disaense tsa Tsamaiso ya Botho le dithuto tse di itsalanyang tse di ka fetisetwang kwa baaging ka bophara. Kitso e e kanna ya dirisiwa go tseweletsa dithuto tse pele go bueng ka ga itiriso ya diphithlelelo tsa rona.

A na go nale kotsi e eleng teng go tsaya karolo mo patlisisong e?

- Ga gona kotsi e e itsagalang e e gokaganang le go tsayakarolo mo porojekeng e ya patlisiso, fela dikotsi tse di solofetsweng mo thutong e di dinnye. Se e kanna ka lebaka la gore o kanna wa lapa ka nako ya teko ya tirisommele e nonofileng ya metsotso ele +- 10. Go fokotsa golapa babatlisisi bat la ikaelela go godisa dipoelo tsa bokgoni tse di nang le gone gore di kanna le seabe se se matla se se boleng mo baneng e ka nna e e lebaneng kgotsa e sa lebanang. Ga gona kotsi go dirisa mokgwa wa khemikhale wa setegeniki fa o kgobokanya mathe, le go dirisa sediriswa sa sepotlakisi (ActiGraph) go diteko tsa tirisommele e nonofileng le BIA mo baneng ya itsagala. Di diriswa tse di bolokeselele go ka diriswa thutong ya patlisiso mo go tsayakarolong ga bana. Ga gona tirisommele, saekholoji, kgathelelo loago kgotsa dingwe tse di botlhoko le ditlamorago tse di go tla rakanwang le tsona mo thutong ena. Mmatlisisi yo o boiphithlelo o tla tsaya bana go ya ka tsamaiso ya teko ya dithulaganyo. Ngaka ya ditshipi (Dr,Venter) o tla nna a le mo tebellong ka nakoya tsamaiso ya porojeke, gore fa go ka nna le kgobalo e e diragalang. Fa go ka diragala gore gonne le kotsi ya tshoganyetso kgotsa golwala, bana ba tla gogelwa kwa morago ka gangwe go tswa mo porojekeng mme ba letlelelwa tlogela le go tsamaisa nako enngwe le enngwe fa ba ikutlwa jaalo.

A go na le kotsi e e tlamang ngwana go tsaya karolo mo porojekeng le go re tshagisetse tshedimisetso ya gagwe? Fa gontse jalo ke eng se se tla dirwang jaaka dilekanyo tse di tlamelang?

Dikotsi	Dilekanyo tse di tlamelang
Go latlhegelwa o sa itsagale	Batsayakarolo botlhe batla neelwa di nomore tseo tshedimisetso ya bona e tla lomaganngwa go sireletsa go sa itsagale ga bona.
Tsamaiso ya tshedimisetso le tatlhegelo ya disete tsa motheo wa tshedimisetso	Tshedimisetso e tla beiwa ka tlhokomelo le tshireletso mo di ofising tsa ba neela ditirelo le tshedimisetso ya eleketeroniki (Matlhabaphefo a Dipalopalo le motheo wa eleketeroniki) e tla sireletswa ka lefoko la sephiri
Go anamisa disete tsa tshedimisetso ka Bokamoso jo bo senang boitswharo	Tshedimisetso e tla bolokiwa lobaka la dingwaga dile 20 morago ga moo e be e sentswe ka tsela e e bolokosegileng e sa retele
Go latlhegelwa ke nako ya mophato	Ga gona nako ya mophato e e okeditsweng e e tla latlhegang.
Golapa le go gobala	Ditlhokego tsotlhe tsa dilekanyetso di dirisiwa ka nako ya go neelana ka mananeo go thibelela fa go tlhokega go tsamaisa kotsi e entseng jalo.

- Di poelo dibotlhokwa go na le kotsi
- Di poelo mo thutong e di dbotlokwa go na le dikotsi. Patlisiso e ga ya ikaelelwa go gothusa ka bo wena, le fa gotse jalo o tla amogela tlhatlhobo ya bongaka ya mahala mme o amogele dipholo tsa gago di gi itsise fa ele gore o na le kgatelelo ya madie e kwa godim, mafura a a kwa godimo a mmele.
- Di phitlhelelo tsa patlisiso e di ka dirisiwa go thusa go thibela, direla le go laola malwetse a a sa tshelaneng(NCDs) a mokgwa wa go tshela o o gokaganang le mahura le bokima mo bathong ba Aforikaborwa.

Go tla diragala eng fa go ka nna le tirafalo ya go tlhorisa go go diragalang ele ditswamorago tsa go tsaya karolo mo thutong e ya patlisiso?

- Fa o ka nna le tlhoko go tsweletsa dipuisanyo mo tsamaong/morago ga gotsayakarolo mo porojekeng; tshono e tla rulaganngwa gore wena o bueka tolamo le mmatlisisi gore re kgone go thusa.

Ke mang yo o tla nnang le katamelo go tshedimosetso e e kgobokantsweng?

- Go sa itsagale e tla nna fela ka kgethologo ka gonne o tla be o dira teko ya kwa morago ga dilekanyetsao mme o rwele sediriswa sa (ActiGraph) le ditsala ya dithuto. Rona jaaka ba batlisisi re tla dira sengwe le sengwe go dira bo nnete jwa gore dikarabo tse o re neelang tsona mo dipotsong le disampole tse di kokoantsweng di tla dula ele sephiri mme di ka se tlhagise le leina la gago go mongwe le mongwe. Pego ya diphitlhelelo e ka se itsagale mme tshedimosetso ya ditlhopho mme eseng ya phaphathi ka bongwe le ka mokgwa wa go dirisa fela nomoro ya patlisiso ya gago e o tla e neelwang mme e ka se tlhagisi leina la mongwe le mongwe. Ke ba batlisisi fela bat la itseng ke tshedimosetso efe e eleng ya gago.

Go ile go diragalang ka tshedimosetso/disampole?

- Tshedimosetso e e kgobokantsweng/disampole di tla dula di bolokesegile di sireletsegile ka go lotlelela dikhopi tsa pampiri mo dirakeng mo ofising ya mmatlisisi mme go tshedimosetso ya elekenotiriki e tla sireletswa ka leinasephiri. Tshedimosetso e tla bolokwa dingwaga dile 7. Tshedimosetso e tla seksekwa mo Aforikaborwa mme e begwe ka dipopego dingwe le dingwe. Tsetlelelo ya batsayakarolo e tla lopiwa ka kabelano ya tshedimosetso le baitshwaraganyi ba bangwe go dipegelo tsa patlisiso.

A na o tlele go duelwa go tsaya karolo mo thutong mme a go na le ditshenyegelo dingwe tse di amegang?

- Ga o kitla o amogela tuelo nngwe ntle le gore, go na le dikgothatso tse o tla di neelwang ka nako ya go kgobokanya tshedimosetso. Ga go kitla go nna jaana le ditshenyegelo tse di amegang go wena, fa gona o tsaya karolo.

A mme nka ikgaoganya go tswa mo thutong?

- Go tsayakarolo gag ago mo patlisisong e key a boithaopo ka gotlhe. O na le gone go ka tlhopho go se tseye karolo gotlhelele. Fa o tsaya tshwetso ya go tsayakarolo mo patlisisong e, o ka emisa go tsayakarolo nako nngwe le nngwe. Fa o o tsaya tshwetso ya go sa tseyekarolo mo thutong e kgotsa fa o emisa go tsayakarolo nako nngwe le nngwe, o ka seke wa duedisiwa kgotsa wa latlhegelwa ke dipolelo dinngwe jaaka matshwao a sekolo ao le gale letleletsweng go a bona.

A go na le sengwe se o ka ratang go se itse?

- O ka kopana le moporofesara M.A. Monyeke mo 018 299 1790 fa o na le dipotsiso dingwe kgotsa o itemogela mathata.
- O ka kopana le Komiti ya Boitshwaro Ya Patlisiso ya Boitekanelo go ya ka Mme Carolien van Zyl mo 018 299 2089; carolie.vanzyl@ac.za fa o na ngongorego kgotsa ditlhobaello dingwe tse di sa buelang ka go lekanyetsa ke mmatlisisi. Fa o tla rata go tsaya karolo mo thutong, tsweetswee buisa mme o saene foromo ya tumelano.
- O tla amogela khopi ya tshedimosetso e mme foromo ya tumelo go dipegelo tsa gago.

O tla itse jang ka ga diphithhelelo?

- Diphithhelelo tsa patlisiso di tla abelanwa le wena ka go go neela tshosobanyo ya dipholo tsa gago.

KITSISO KA MOTSAYAKAROLO

Ka gosaena kwa tlase, Nna ke dumela go tsaya karolo mo thutong ya patlisiso e e bidiwang: **Boikaelelo jwa popego ya mmele ka mokgwa wa tiriso khemikhale ya setegeniki le go sekaseka ka maitlhommo tiriso ya mmele mo baneng ba bogolo jwa dingwaga dile 6 go ya go le di 8.**

Ke itsise gore:

- Ke badile tshedimosetso e le foromo ya tumelo mme e kwadilwe ka puo e ke leng boleta le kgomotso ka yona.
- Ke nnile le sebaka sa go botsa dipotso mme dipotso tsotlhe di ne di arabegile ka selekanyetsa.
- Ke tlhaloganya gore go tsaya karolo mo thutong e ke boithaopi mme ga ke a ka gatelelwa go tsaya karolo.
- Nkanna ka tlhopha go tlogela thuto nako nngwe le nngwe mme nka se duedisiwe kgotsa kgethololo ka tsela nngwe.
- Nkanna ka tlhopha go tlogela thuto pele e fela, fa mmatlisisi a ikutlwela gore ke a dikgatlhegelong tsa tse dikgolo, kgotsa fa ele gore ga ke latele maano a thuto, jaaka go dumalanwe.

E saenilwe kwa (tulo) Ka (letlha) 20....

.....
Tshaeno ya motsayakarolo

.....
Tshaeno ya paki

KITSISO KA MOTLHOTLHOMISI

Nna (Leina) Ke itsise gore:

- Ke tlhalositse tshedimosetso mo lokwalong le go
- Ke rotloeeditse ena go botsa dipotso le go tsaya nako e e lekaneng go di araba.
- Ke kgotsofetse gore o tlhalogantse ka selekanyo dikarolo tsotlhe tsa patlisiso, jaaka go rerisanwe fa godimo
- Ke dirisitse/ga ke a dirisa mofetoledi.

E saenilwe kwa (tulo) ka (letlha) 20....

.....
Tshaeno ya motlhotlhomisi

.....
Tshaeno ya paki

DUMELA

Boipolelo ka motsadi (Tumelo e itsisitsweng: 0-13 dingwaga)	
Boipolelo ka motsayakarolo (Tumelo e e Kwetsweng: 7-13 dingwaga)	
Boipolelo ka motsayakarolo (Tumelo ka goblela: 0-7 dingwaga)	

Boipolelo ka motsadi (Tumelo e e itsisitsweng: 0-13 dingwaga)

Ka go saena kwa tlase, Nna Ke neelana ka tumelo gore ngwana wa me a ka tsaya karolo mo porojekeng le gore tshedimose tso ya gagwe e ka nna ya dirisiwa ka ga maikaeleo a patlisiso.

Ke dumela gore:

Ke badile tshedimose tso le foromo ya tumelo mme e kwetswe ka puo e ke leng boleta le thokgama mo go yona. Ke nnile le lekgetlo la go botsa dipotso go ka bobedi motho yo o neng a bona tumelo, ga mmogo le motlamedi wa tirelo mme le dipotso tsa ka tsotlhe di arabilwe ka tekanyo, fa go le botlhokwa. Ke a tlhaloganya gore ngwana waka go tseyeng karolo mo porojekeng e ke **boithaopo** mme ga ke a gatelelelwa go neelana ka tumelo. Nkanna ka tlhopha go goga tumelo ya ka nako nngwe le nngwe mme ngwana wa me ga a kake a duela kgotsa go kgethololwa ka se ka tsela efe nngwe. Ke a tlhaloganya gore tshedimose tso e e kokoantsweng mo tsewetsong ya porojeke e e ka nna ya dirisiwa ka ga go bona maikaeleo a patlisiso ya isago (jaaka go supilwe ka maitlhomong) fa fela go netefaditswe gore ga a tlhagisiwe ka leina. Ke neelana ka tumelo gore kokoanyo ya tshedimose tso e ka nna ya dirisiwa ka maikaeleo a patlisiso.

E saenilwe kwa (lefelo) ka (letlha) 20....

EYA

NNYAA

.....
Tshaeno ya motsayakarolo

.....
Tshaeno ya mopaki

APPENDIX C: DEMOGRAPHIC INFORMATION QUESTIONNAIRE

Country _____ Country code _____
Name of school _____ School Number _____
Name of interviewer _____ Date _____ (dd/mm/yyyy)
Questionnaire 4-digit ID _____ Class/Grade _____

Section A: Tell us about you and your family

We would like to learn more about you and your family. Please answer all questions.

Remember that there are no right or wrong answers, and that every person is different. We will not share any of your personal information with anyone else, and all of your answers will remain private.

1. Participant: _____
(Write your name in full, do not use abbreviations)
2. Are you a boy or a girl? (tick one) ☐ Boy ☐ Girl
3. How old are you? _____ Years
4. Including yourself, how many people currently live in your home? _____
5. 5. How many rooms are there in your house? _____ rooms
(Including rooms like the kitchen, lounge/sitting room, bedrooms and any outside structures; do not include bathrooms)
6. In your home, what is the main source of water? (tick one)
☐ Indoor tap water
☐ Outside tap water
☐ Other water source
7. What kind of toilet do you usually use at home?
☐ Flush toilet inside the house
☐ Flush toilet outside the house
☐ Pit latrine/bucket
☐ Other type, Specify _____

8. Tick all items that you have in your home now?

Household items	☐ if Yes
Electricity	
Television	
Radio	
Car/motorbike	
Bicycle	
Fridge/refrigerator	
Washing machine	
Telephone/Cell phone	
Computer	
Satellite dish/cable	
Microwave oven	

9. What is used for cooking in your home? **You can circle more than one.**

- ☐ Gas
- ☐ Electricity
- ☐ Wood
- ☐ Charcoal
- ☐ Paraffin stove
- ☐ Other, specify _____

10. Does your mother/guardian work? ☐ Yes ☐ No

If yes, what type of work does she do? _____

11. Does your father/guardian work? ☐ Yes ☐ No

If yes, what type of work does he do? _____

APPENDIX D: ANTHROPOMETRIC MEASUREMENTS DATA FORM

Subject number (ID):

Name:

Sport/ Activity:.....

Surname

Date of Birth:			
	Day	Month	Year

first names

Test Date:			
	Day	Month	Year

Height:

Gender: M ☐ F ☐

	<i>ID</i>	<i>Site</i>	<i>Trail 1</i>	<i>Trail 2</i>	<i>Mean/ Median</i>
Basic	1	Body mass			
	2	Stature			

Skinfolds (SF) (mm)	3a	Triceps : L			
	3b	Subscapular : L			
	3c	Biceps : L			
	3d	Supraspinale : L			

Girths GR (cm)	4a	Arm (relaxed) : L			
	4b	Arm (flexed & tensed) : L			
	4c	Waist (minimum)			

APPENDIX E: BIA DATA FORM

Subject number (ID):

Name: Sport:

Surname

first names.....

Date of Birth:
Day Month Year

Test Date:
Day Month Year

Height:

Gender: M ☐ F ☐

Step 1 Assess hydration level of a child
(Note: specific gravity must be <1.025)

Pass **Fail**

Step 2 – Assess height

Step 3 – Bioelectrical Impedance
Assessment (Note: Approved Equipment:
Tanita TBF – 300W (Standard Mode)
Record mass at initial assessment

Kg

STABLE ASSEMENT PRINT –OUT HERE



Calculated Body Fat (Fat% on Tanita print-out)

APPENDIX F: STABLE ISOTOPE DATA FORM

SAMPLE DATA SHEET FOR TBW ESTIMATION BY DEUTERIUM OXIDE DILUTION

Person performing the test: _____ Date: ____/____/____

Day Month Year

1. Participant

Name: _____ Code/ID: _____

Mass: ____ . ____ kg Stature: ____ . ____ cm BMI ____ kg/m²

Date of birth: ____/____/____ Age: ____ years Gender: M F

Healthy: | YES | NO

Notes (health): _____

2. Dose

Dose bottle number: _____

Dose weight: ____ . ____ g

Did the participant fast overnight? | YES | NO

If not, how long was the fast before the dose? _____

Was the container opened just before the dosage? | YES | NO

Was the dose consumed correctly? | YES | NO

If not, what was the weight of the dose not consumed. ____ . ____ g

The container was rinsed with 2 × 50 mL water. | YES | NO

The same straw was used. | YES | NO

Notes: _____

3. Specimen times

Time of baseline saliva sample: ____:____

Time dose was taken: ____:____

Pre-dose saliva samples: _____

Post-dose saliva samples:

2 hours: ____:____

APPENDIX G: BC-IT: BODY IMAGE SILHOUETTES

ID Number: _____

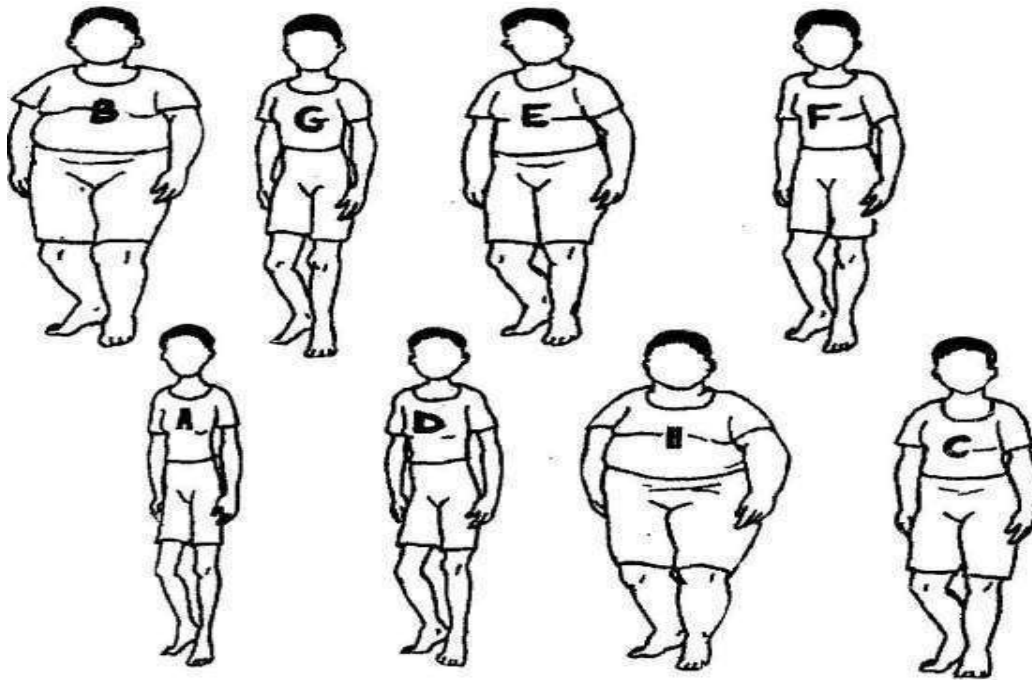
Girls only



1. When you look at these pictures, which one do you think you look like? _____
2. Which one would you like to look like? _____

ID Number: _____

Boys only



1. When you look at these pictures, which one do you think you look like? _____
2. Which one would you like to look like? _____

APPENDIX H: PROOF OF LANGUAGE EDITING



Embed Educational Services

6 Andover Road
Westdene
Johannesburg
2092
11th March 2021

Certificate of Language Editing

Validation of body fatness equations using selected anthropometric measurements, BIA and body image, compared to deuterium dilution in a group of 6-8 years old black South African: BC-IT study.

TL Moeng-Mahlangu

Carol A Macdonald
MA Wits MA Reading Ph D Edinburgh (PEG)

APPENDIX I: GUIDELINES FOR AUTHORS

FOR ARTICLE 1

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Do not use non-standard abbreviations unless they appear at least three times in the text.
Keep abbreviations to a minimum.

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<i>Species names</i>	Write in italics (e.g., <i>Homo sapiens</i>). Write out in full the genus and species, both in the title of the manuscript and at the first mention of an organism in a paper. After first mention, the first letter of the genus name followed by the full species name may be used (e.g., <i>H. sapiens</i>).
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Short title 100 characters State the topic of the study

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Cigarette smoke exposure and innate immunity
SODIS and childhood diarrhoea

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Abstract

The Abstract comes after the title page in the manuscript file. The abstract text is also entered in a separate field in the submission system.

The Abstract should:

- Describe the main objective(s) of the study
- Explain how the study was done, including any model organisms used, without methodological detail
- Summarize the most important results and their significance
- Not exceed 300 words

-
- Abstracts should not include:
- Citations
 - Abbreviations, if possible

Introduction

The introduction should:

- Provide background that puts the manuscript into context and allows readers outside the field to understand the purpose and significance of the study
- Define the problem addressed and why it is important
- Include a brief review of the key literature
- Note any relevant controversies or disagreements in the field
- Conclude with a brief statement of the overall aim of the work and a comment about whether that aim was achieved.

Materials and Methods

The Materials and Methods section should provide enough detail to allow suitably skilled investigators to fully replicate your study. Specific information and/or protocols for new methods should be included in detail. If materials, methods, and protocols are well established, authors may cite articles where those protocols are described in detail, but the submission should include sufficient information to be understood independent of these references.

Supporting reproducibility with protocols

To enhance the reproducibility of your results, we recommend and encourage you to make your protocols public.

There are several options:

Protocols associated with Research Articles

Protocol documents may be uploaded as Supporting Information or linked from the Methods section of the article. For laboratory protocols, we recommend protocols.io. Include the DOI link in the Methods section of your manuscript using the following format: [http://dx.doi.org/10.17504/protocols.io.\[PROTOCOL DOI\]](http://dx.doi.org/10.17504/protocols.io.[PROTOCOL DOI]). This allows editors and reviewers to consult the detailed step-by-step protocol when evaluating your manuscript. You can choose to keep the protocol private on the protocols.io platform until your article is published—at which time it will be published automatically.

Protocols published in their own right

PLoS ONE offers two options for publishing stand-alone protocol articles: Lab Protocols that describe verified methodologies and Study Protocols that describe detailed plans and proposals for research projects. Specific guidelines apply to the submission of [Lab Protocol](#) and [Study Protocol](#) manuscripts. Read the detailed instructions for submitting [Lab Protocols](#) and [Study Protocols](#).

Results, Discussion, Conclusions

These sections may all be separate, or may be combined to create a mixed Results/Discussion section (commonly labelled “Results and Discussion”) or a mixed Discussion/Conclusions section (commonly labelled “Discussion”). These sections may be further divided into subsections, each with a concise subheading, as appropriate. These sections have no word limit, but the language should be clear and concise.

Together, these sections should describe the results of the experiments, the interpretation of these results, and the conclusions that can be drawn.

Authors should explain how the results relate to the hypothesis presented as the basis of the study and provide a succinct explanation of the implications of the findings, particularly in relation to previous related studies and potential future directions for research.

PLOS ONE editorial decisions do not rely on perceived significance or impact, so authors should avoid overstating their conclusions. See the *PLOS ONE* [Criteria for Publication](#) for more information.

Acknowledgments

Those who contributed to the work but do not meet our authorship criteria should be listed in the Acknowledgments with a description of the contribution.

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Do not include funding sources in the Acknowledgments or anywhere else in the manuscript file. Funding information should only be entered in the financial disclosure section of the submission system.

References

Any and all available works can be cited in the reference list. Acceptable sources include:

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- Manuscripts on preprint servers, providing the manuscript has a citable DOI or arXiv URL.

Do not cite the following sources in the reference list:

- Unavailable and unpublished work, including manuscripts that have been submitted but not yet accepted (e.g., “unpublished work,” “data not shown”). Instead, include those data as supplementary material or deposit the data in a publicly available database.
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References are listed at the end of the manuscript and numbered in the order that they appear in the text. In the text, cite the reference number in square brackets (e.g., “We used the techniques developed by our colleagues [19] to analyze the data”). PLOS uses the numbered citation (citation-sequence) method and first six authors, et al.

Do not include citations in abstracts.

Make sure the parts of the manuscript are in the correct order *before* ordering the citations.

Formatting references

Because all references will be linked electronically as much as possible to the papers they cite, proper formatting of the references is crucial.

PLOS uses the reference style outlined by the International Committee of Medical Journal Editors (ICMJE), also referred to as the “Vancouver” style. Example formats are listed below. Additional examples are in the [ICMJE sample references](#).

A reference management tool, EndNote, offers a current [style file](#) that can assist you with the formatting of your references. If you have problems with any reference management program, please contact the source company's technical support.

Journal name abbreviations should be those found in the [National Center for Biotechnology Information \(NCBI\) databases](#).

Source	Format
Published articles	Hou WR, Hou YL, Wu GF, Song Y, Su XL, Sun B, et al. cDNA, genomic sequence cloning and overexpression of ribosomal protein gene L9 (rpL9) of the giant panda (<i>Ailuropoda melanoleuca</i>). <i>Genet Mol Res</i> . 2011;10: 1576-1588. Devaraju P, Gulati R, Antony PT, Mithun CB, Negi VS. Susceptibility to SLE in South Indian Tamils may be influenced by genetic selection pressure on TLR2 and TLR9 genes. <i>Mol Immunol</i> . 2014 Nov 22. pii: S0161-5890(14)00313-7. doi: 10.1016/j.molimm.2014.11.005. Note: A DOI number for the full-text article is acceptable as an alternative to or in addition to traditional volume and page numbers. When providing a DOI, adhere to the format in the example above with both the label and full DOI included at the end of the reference (doi: 10.1016/j.molimm.2014.11.005). Do not provide a shortened DOI or the URL.
Accepted, unpublished articles	Same as published articles, but substitute “Forthcoming” for page numbers or DOI.
Online articles	Huynen MMTE, Martens P, Hilderlink HBM. The health impacts of globalisation: a conceptual framework. <i>Global Health</i> . 2005;1: 14. Available from: http://www.globalizationandhealth.com/content/1/1/14
Books	Bates B. <i>Bargaining for life: A social history of tuberculosis</i> . 1st ed. Philadelphia: University of Pennsylvania Press; 1992.
Book chapters	Hansen B. New York City epidemics and history for the public. In: Harden VA, Risse GB, editors. <i>AIDS and the historian</i> . Bethesda: National Institutes of Health; 1991. pp. 21-28.

Source	Format
Deposited articles (preprints, e-prints, or arXiv)	Krick T, Shub DA, Verstraete N, Ferreira DU, Alonso LG, Shub M, et al. Amino acid metabolism conflicts with protein diversity. arXiv:1403.3301v1 [Preprint]. 2014 [cited 2014 March 17]. Available from: https://128.84.21.199/abs/1403.3301v1 Kording KP, Mensh B. Ten simple rules for structuring papers. BioRxiv [Preprint]. 2016 bioRxiv 088278 [posted 2016 Nov 28; revised 2016 Dec 14; revised 2016 Dec 15; cited 2017 Feb 9]: [12 p.]. Available from: https://www.biorxiv.org/content/10.1101/088278v5 doi: 10.1101/088278
Published media (print or online newspapers and magazine articles)	Fountain H. For Already Vulnerable Penguins, Study Finds Climate Change Is Another Danger. The New York Times. 2014 Jan 29 [Cited 2014 March 17]. Available from: http://www.nytimes.com/2014/01/30/science/earth/climate-change-taking-toll-on-penguins-study-finds.html
New media (blogs, web sites, or other written works)	Allen L. Announcing PLOS Blogs. 2010 Sep 1 [cited 17 March 2014]. In: PLOS Blogs [Internet]. San Francisco: PLOS 2006 - . [about 2 screens]. Available from: http://blogs.plos.org/plos/2010/09/announcing-plos-blogs/ .
Masters' theses or doctoral dissertations	Wells A. Exploring the development of the independent, electronic, scholarly journal. M.Sc. Thesis, The University of Sheffield. 1999. Available from: http://cumincad.scix.net/cgi-bin/works/Show?2e09
Databases and repositories (Figshare, arXiv)	Roberts SB. QPX Genome Browser Feature Tracks; 2013 [cited 2013 Oct 5]. Database: figshare [Internet]. Available from: http://figshare.com/articles/QPX_Genome_Browser_Feature_Tracks/701214
Multimedia (videos, movies, or TV shows)	Hitchcock A, producer and director. Rear Window [Film]; 1954. Los Angeles: MGM.

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Authors can submit essential supporting files and multimedia files along with their manuscripts. All supporting information will be subject to peer review. All file types can be submitted, but files must be smaller than 20 MB in size.

Authors may use almost any description as the item name for a supporting information file as long as it contains an "S" and number. For example, "S1 Appendix" and "S2 Appendix," "S1 Table" and "S2 Table," and so forth.

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Example caption

S1 Text. Title is strongly recommended. Legend is optional.

In-text citations

We recommend that you cite supporting information in the manuscript text, but this is not a requirement. If you cite supporting information in the text, citations do not need to be in numerical order.

Read the [supporting information guidelines](#) for more details about submitting supporting information and multimedia files.

Figures and tables

Figures

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Cite figures in ascending numeric order at first appearance in the manuscript file.

[Read the guidelines for figures](#) and [requirements for reporting blot and gel results](#).

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Figure captions must be inserted in the text of the manuscript, immediately following the paragraph in which the figure is first cited (read order). Do not include captions as part of the figure files themselves or submit them in a separate document.

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The caption may also include a legend as needed.

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Manuscripts submitted to *PLoS ONE* are expected to report statistical methods in sufficient detail for others to replicate the analysis performed. Ensure that results are rigorously reported in accordance with community standards and that the statistical methods employed are appropriate for the study design.

Consult the following resources for additional guidance:

- [SAMPL guidelines](#), for general guidance on statistical reporting
- [PLoS ONE guidelines](#), for clinical trials requirements
- [PLoS ONE guidelines](#), for systematic review and meta-analysis requirements
- [EQUATOR](#), for specific reporting guidelines for a range of other study types

Reporting of statistical methods

In the methods, include a section on statistical analysis that reports a detailed description of the statistical methods. In this section:

- List the name and version of any software package used, alongside any relevant references
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- Provide the repository identifier for any code used in the analysis (See our [code-sharing policy](#).)

Statistical reporting guidelines:

- Identify research design and independent variables as being between- or within-subjects
- For pre-processed data:
 - Describe any analysis carried out to confirm the data meets the assumptions of the analysis performed (e.g. linearity, co-linearity, normality of the distribution).
 - If data were transformed include this information, with a reason for doing so and a description of the transformation performed
- Provide details of how outliers were treated and your analysis, both with the full dataset and with the outliers removed
- If relevant, describe how missing/excluded data were handled
- Define the threshold for significance (alpha)
- If appropriate, provide sample sizes, along with a description of how they were determined. If a sample size calculation was performed, specify the inputs for power, effect size and alpha. Where relevant, report the number of independent replications for each experiment.
- For analyses of variance (ANOVAs), detail any post hoc tests that were performed
- Include details of any corrections applied to account for multiple comparisons. If corrections were not applied, include a justification for not doing so
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 - Report the alpha level used
 - Discuss whether the variables were assessed for collinearity and interaction
 - Describe the variable selection process by which the final model was developed (e.g., forward-stepwise; best subset). [See SAMPL guidelines](#).
- For Bayesian analysis explain the choice of prior trial probabilities and how they were selected. Markov chain Monte Carlo settings should be reported.

Reporting of statistical results

Results must be rigorously and appropriately reported, in keeping with community standards.

- **Units of measurement.** Clearly define measurement units in all tables and figures.
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- **Regression analyses.** Include the full results of any regression analysis performed as a supplementary file. Include all estimated regression coefficients, their standard error, p-values, and confidence intervals, as well as the measures of goodness of fit.
- **Reporting parameters.** Test statistics (F/t/r) and associated degrees of freedom should be provided. Effect sizes and confidence intervals should be reported where appropriate. If percentages are provided, the numerator and denominator should also be given.
- **P-values.** Report exact p-values for all values greater than or equal to 0.001. P-values less than 0.001 may be expressed as $p < 0.001$, or as exponentials in studies of genetic associations.
- **Displaying data in plots.** Format plots so that they accurately depict the sample distribution. 3D effects in plots can bias and hinder interpretation of values, so avoid them in cases where regular plots are sufficient to display the data.
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Data reporting

All data and related metadata underlying the findings reported in a submitted manuscript should be deposited in an appropriate public repository, unless already provided as part of the submitted article. See [instructions on providing underlying data to support blot and gel results](#)

[Read our policy on data availability](#).

Repositories may be either subject-specific (where these exist) and accept specific types of structured data, or generalist repositories that accept multiple data types. We recommend that authors select repositories appropriate to their field. Repositories may be subject-specific (e.g., GenBank for sequences and PDB for structures), general, or institutional, as long as DOIs or accession numbers are provided and the data are at least as open as CC BY. Authors are encouraged to select repositories that meet accepted criteria as trustworthy digital repositories, such as criteria of the Centre for Research Libraries or Data Seal of Approval. Large, international databases are more likely to persist than small, local ones. [See our list of recommended repositories](#).

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- Deposit data in the integrated repository of choice.
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All appropriate data sets, images, and information should be deposited in an appropriate public repository. [See our list of recommended repositories](#).

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This information should describe sources of funding that have supported the work. It is important to gather these details prior to submission because your financial disclosure statement cannot be changed after initial submission without journal approval. If your manuscript is published, your statement will appear in the Funding section of the article.

Enter this statement in the Financial Disclosure section of the submission form. Do not include it in your manuscript file.

The statement should include:

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This information should not be in your manuscript file; you will provide it via our submission system.

All potential competing interests must be declared in full. If the submission is related to any patents, patent applications, or products in development or for market, these details, including patent numbers and titles, must be disclosed in full.

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For manuscripts disputing previously published work, it is *PLOS ONE* policy to invite a signed review by the disputed author during the peer review process. This procedure is aimed at ensuring a thorough, transparent, and productive review process.

If the disputed author chooses to submit a review, it must be returned in a timely fashion and contain a full declaration of all competing interests. The Academic Editor will consider any such reviews in light of the competing interest.

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Upon submission, authors must confirm that the manuscript, or any related manuscript, is not currently under consideration or accepted elsewhere. If related work has been submitted to *PLOS ONE* or elsewhere, authors must include a copy with the submitted article. Reviewers will be asked to comment on the overlap between related submissions.

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Registered Reports

Submission and format requirements for [Registered Report Protocols and Registered Reports](#) are similar to those for a regular submission and may be specific to your study type. For instance, if your Registered Report Protocol submission is about a Clinical Trial or a Systematic Review, follow the appropriate guidelines.

For Registered Report Protocols:

- Provide enough methodological detail to make the study reproducible and replicable
- Confirm that data will be made available upon study completion in keeping with the [PLoS Data policy](#)
- Include ethical approval or waivers, if applicable
- Preliminary or pilot data may be included, but only if necessary to support the feasibility of the study or as a proof of principle
- For meta-analyses or Clinical Trials, use the protocol-specific reporting guidelines [PRISMA-P](#) or [SPIRIT](#) respectively

For more guidance on format and presentation of a protocol, consult the [sample template hosted by the Open Science Framework](#). [Discipline-specific and study-specific templates](#) are also available.

If data need to be collected, modified or processed specifically for your study, or if participants need to be recruited specifically for your study, then it should occur only after your Registered Report Protocol is accepted for publication.

For Registered Report Research Articles:

- Report the results of all planned analyses and, if relevant, detail and justify all deviations from the protocol.
- The manuscript may also contain exploratory, unplanned analyses.

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All research involving human participants must have been approved by the authors' Institutional Review Board (IRB) or by equivalent ethics committee(s), and must have been conducted according to the principles expressed in the [Declaration of Helsinki](#). Authors should be able to submit, upon request, a statement from the IRB or ethics committee indicating approval of the research. We reserve the right to reject work that we believe has not been conducted to a high ethical standard, even when formal approval has been obtained.

Subjects must have been properly instructed and have indicated that they consent to participate by signing the appropriate informed consent paperwork. Authors may be asked to submit a blank, sample copy of a subject consent form. If consent was verbal instead of written, or if consent could not be obtained, the authors must explain the reason in the manuscript, and the use of verbal consent or the lack of consent must have been approved by the IRB or ethics committee.

All efforts should be made to protect patient privacy and anonymity. Identifying information, including photos, should not be included in the manuscript unless the information is crucial and the individual has provided written consent by completing the [Consent Form for Publication in a PLOS Journal \(PDF\)](#).

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- Studies of diagnostic accuracy: [STARD](#)
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- Microarray experiments: [MIAME](#)
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Methods sections of papers on research using human subjects or samples must include ethics statements that specify:

- **The name of the approving institutional review board or equivalent committee(s).** If approval was not obtained, the authors must provide a detailed statement explaining why it was not needed
- **Whether informed consent was written or oral.** If informed consent was oral, it must be stated in the manuscript:
 - Why written consent could not be obtained
 - That the Institutional Review Board (IRB) approved use of oral consent
 - How oral consent was documented

For studies involving humans categorized by race/ethnicity, age, disease/disabilities, religion, sex/gender, sexual orientation, or other socially constructed groupings, authors should:

- Explicitly describe their methods of categorizing human populations
- Define categories in as much detail as the study protocol allows
- Justify their choices of definitions and categories, including for example whether any rules of human categorization were required by their funding agency
- Explain whether (and if so, how) they controlled for confounding variables such as socioeconomic status, nutrition, environmental exposures, or similar factors in their analysis

In addition, outmoded terms and potentially stigmatizing labels should be changed to more current, acceptable terminology. Examples: “Caucasian” should be changed to “white” or “of [Western] European descent” (as appropriate); “cancer victims” should be changed to “patients with cancer.”

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The individual in this manuscript has given written informed consent (as outlined in PLoS consent form) to publish these case details.

For more information about *PLoS ONE* policies regarding human subjects research, see the [Publication Criteria](#) and [Editorial Policies](#).

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Clinical trials are subject to all [policies regarding human research](#). *PLoS ONE* follows the [World Health Organization's \(WHO\) definition of a clinical trial](#):

A clinical trial is any research study that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects on health outcomes [...] Interventions include but are not restricted to drugs, cells and other biological products, surgical procedures, radiologic procedures, devices, behavioural treatments, process-of-care changes, preventive care, etc.

All clinical trials must be registered in one of the publicly-accessible registries approved by the [WHO](#) or [ICMJE](#) (International Committee of Medical Journal Editors). Authors must provide the trial registration number. Prior disclosure of results on a clinical trial registry site will not affect consideration for publication. We reserve the right to inform authors' institutions or ethics committees, and to reject the manuscript, if we become aware of unregistered trials.

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- Explain in the Methods the reason for failing to register before participant recruitment

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[and guide](#). Submissions must also include the study protocol as supporting information, which will be published with the manuscript if accepted.

Authors of manuscripts describing the results of clinical trials must adhere to the [CONSORT](#) reporting guidelines appropriate to their trial design, available on the [CONSORT Statement web site](#). Before the paper can enter peer review, authors must:

- The name of the registry and the registration number must be included in the Abstract.
- Provide a copy of the trial protocol as approved by the ethics committee and a completed [CONSORT checklist](#) as supporting information (which will be published alongside the paper, if accepted). This should be named S1 CONSORT Checklist.
- Include the [CONSORT flow diagram](#) as the manuscript's "Fig 1"

Any deviation from the trial protocol must be explained in the paper. Authors must explicitly discuss informed consent in their paper, and we reserve the right to ask for a copy of the patient consent form.

The name of the registry and the registry number must be provided in the Abstract. If the trial is registered in more than one location, please provide all relevant registry names and numbers.

Lab Protocols

[Lab Protocols](#) consist of two interlinked components: a protocol hosted on the [protocols.io](#) platform and a peer-reviewed article on *PLoS ONE* that contextualises the protocol.

[protocols.io](#) is a secure open access platform that specializes in laboratory protocols. It allows scientists to share, discover and reuse up-to-date protocol knowledge. The platform provides specialist tools and guidance on how to add each element of the protocol, including the title, abstract, steps, files, links, reagents, measurements, formulae, videos, charts and more.

The *PLoS ONE* article component must comply with the general submission guidelines (detailed above in this article).

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- Link, in the Introduction section, to at least one supporting peer-reviewed publication in which the protocol was applied to generate data.
- Link, in the Materials and Methods section, to the [protocol.io](#) component, using the digital object identifier (DOI) and format provided by protocols.io, for example [https://dx.doi.org/10.17504/protocols.io\[....\]](https://dx.doi.org/10.17504/protocols.io[....]).
- Describe the appropriate controls, sample sizes and replication needed to ensure that the data are robust and reproducible.
- Provide the protocol as a [supporting information](#) (S1) file for printing purposes. You can download a PDF from [protocols.io](#) for this purpose.
- Optionally, provide minimal new data relevant to the development of the protocol e.g., for additional benchmarking, validation or troubleshooting purposes.

[Download a sample Lab Protocol template](#)

Lab Protocols describing routine methods, or extensions or modifications of routine methods, add little or no value to the published literature and will not be considered for publication.

Manuscripts that report new methods should be submitted as [research articles](#), not as Lab Protocols

Lab Protocols are subject to the same [editorial and peer review process](#) as all other articles, except that the peer review process may be expedited and carried out by one internal Academic Editor and one external reviewer.

Lab Protocols are eligible for both [signed and published peer review](#).

We encourage you to post your protocol to the protocols.io platform before submitting your manuscript to *PLOS ONE*, or at the latest, before the editorial and peer review process. This approach is optional, but beneficial, because:

- Your DOI is assigned on the protocols.io platform. You need this identifier to link out from the Material and Methods section of your manuscript.
- You can [keep your protocol private](#) on the protocols.io platform (until you are satisfied that it is ready for publication), but still assign a DOI.
- The protocol will be accessible to editors and reviewers during the editorial and peer review process.

If you prefer to submit your manuscript to *PLoS ONE* before uploading your protocol to protocols.io, please provide your protocol as a [supporting information](#) (S1) file. You can use protocols.io's [editorial service](#) at no cost: they will check and publish your protocol for you. As part of *PLoS ONE*'s partnership with protocols.io, your waiver code for this purpose will be provided in the first decision letter.

Preprint posting is not available for Lab Protocols and [bioRxiv](#) does not accept them.

Study Protocols

[Study Protocols](#) describe plans for conducting research projects and consist of a single article on *PLoS ONE*.

Study Protocols must comply with the *PLoS ONE* general submission guidelines (detailed above in this article) and any guidelines specific to the related research study type. In addition, the protocol must:

- Relate to a research study that has not yet generated results.
- Be submitted before recruitment of participants or collection of data for the study is complete.
- Meet the same standards for [ethics of experimentation and research integrity](#) as the research study. If it involves [human](#) or [animal](#) subjects, [cell lines](#) or [field sampling](#), or has [potential biosafety implications](#), prior approval from the relevant ethics body must be obtained prior to submission. Please contact us if you have a valid reason for not obtaining approval.

Additional prerequisites apply for these study types:

- [Clinical trials](#):
 - The trial must be registered prior to submission of your protocol in one of the publicly accessible registries approved by the WHO or ICMJE (International Committee of Medical Journal Editors).
 - The name of the registry and the trial or study registration number must be included in the Abstract.
 - A copy of the protocol that was approved by the ethics committee must be submitted as a supplementary information file. Please provide an additional English translation if the original document is not in English.
 - A SPIRIT [schedule of enrollment, interventions, and assessments](#) must be included as the manuscript's Figure 1, and a completed [SPIRIT checklist](#) must be uploaded as Supporting Information file S1.
- [Systematic reviews and meta-analyses](#):
 - A completed [PRISMA-P checklist](#) must be provided as a supporting information (SI) file. See [PRISMA-P Explanation and Elaboration](#) for more information on completing your checklist.

Study Protocols must also comply with general *PLoS ONE* [criteria for publication](#) and in addition you should:

- include the word "Protocol" in your Title.
- include a detailed description of the planned study in the Materials and Methods section. This should provide sufficient methodological detail for the protocol to be reproducible and replicable. Your description should cover all relevant and applicable facts and hypothesis, including:
 - the aim, design, and setting
 - the sample size calculation
 - how data saturation will be determined (for qualitative studies)
 - the characteristics of participants e.g., inclusion and exclusion criteria, sample selection criteria, variables to be measured, randomization and blinding criteria (where applicable), and how informed consent will be obtained
 - how materials will be selected and used e.g., where and how they will be sourced, the processes, interventions, or comparisons to be used, the outcomes to be measured, and when and how they will be measured
 - the data management plan
 - safety considerations
 - the type of data and statistical analyses to be used
 - the status and timeline of the study, including whether participant recruitment or data collection has begun
 - where and when the data will be made available. See our [Data Availability policy](#) for more.
- include an analysis of preliminary or pilot data, only if it is necessary to support the feasibility of the study or as a proof of principle. This is optional.
- we encourage authors you to register with [OSF](#) and provide the your registration number in the Materials and Methods section. This is optional.

- optionally add any other SI files, figures or tables that elaborate or authenticate the protocol: e.g., any reporting checklists applicable to your study type.

Read the [supporting information guidelines](#) for more details about adding SI files.

Download [our sample Study Protocol template](#) or an OSF [discipline or study-specific template](#).

Study Protocols are subject to the same [editorial](#) and [peer review](#) process as all other articles, and are eligible for both [signed and published peer review](#).

You can expedite the review process by providing:

- proof of external funding. This is typically your funding approval letter and a list of the names and credentials of the funders who conducted the external peer review of the protocol. Include an English translation if needed.
- proof of ethics approval (if required). This is typically the approval or waiver letter from the relevant ethics body and a copy of the protocol approved by this body.

These documents are used for internal purposes and do not form part of the published Study Protocol.

Expedited review is conducted by an internal Staff Editor only and bypasses the external review process.

If the Study Protocol describes a replication study or involves re-analysis of published work, we will invite the author of the initial or replicated study to provide a signed review.

We encourage you to share your Study Protocol with other researchers, either before or after submission. You can publish it on your website or [protocols.io](#), or submit it for posting on [medRxiv](#) or another preprint server.

Animal research

All research involving vertebrates or cephalopods must have approval from the authors' Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee(s), and must have been conducted according to applicable national and international guidelines. Approval must be received prior to beginning research.

Manuscripts reporting animal research must state in the Methods section:

- The full name of the relevant ethics committee that approved the work, and the associated permit number(s).
- Where ethical approval is not required, the manuscript should include a clear statement of this and the reason why. Provide any relevant regulations under which the study is exempt from the requirement for approval.
- Relevant details of steps taken to ameliorate animal suffering.

Example ethics statement

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the University of Minnesota (Protocol Number: 27-2956). All surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

Authors should always state the organism(s) studied in the Abstract. Where the study may be confused as pertaining to clinical research, authors should also state the animal model in the title.

To maximize reproducibility and potential for re-use of data, we encourage authors to follow the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines for all submissions describing laboratory-based animal research and to upload a completed [ARRIVE Guidelines Checklist](#) to be published as supporting information.

Non-human primates

Manuscripts describing research involving non-human primates must report details of husbandry and animal welfare in accordance with the recommendations of the Weatherall report, [The use of non-human primates in research](#), including:

- Information about housing, feeding, and environmental enrichment.
- Steps taken to minimize suffering, including use of anesthesia and method of sacrifice, if appropriate.

Random source animals

Manuscripts describing studies that use random source (e.g. Class B dealer-sourced in the USA), shelter, or stray animals will be subject to additional scrutiny and may be rejected if sufficient ethical and scientific justification for the study design is lacking.

Unacceptable euthanasia methods and anesthetic agents

Manuscripts reporting use of a euthanasia method(s) classified as unacceptable by the [American Veterinary Medical Association](#) or use of an anesthesia method(s) that is widely prohibited (e.g., chloral hydrate, ether, chloroform) must include at the time of initial submission, scientific justification for use in the specific study design, as well as confirmation of approval for specific use from their animal research ethics committee. These manuscripts may be subject to additional ethics considerations prior to publication.

Humane endpoints

Manuscripts reporting studies in which death of a regulated animal (vertebrate, cephalopod) is a likely outcome or a planned experimental endpoint, must comprehensively report details of study design, rationale for the approach, and methodology, including consideration of humane endpoints. This applies to research that involves, for instance, assessment of survival, toxicity, longevity, terminal disease, or high rates of incidental mortality.

Definition of a humane endpoint

A humane endpoint is a predefined experimental endpoint at which animals are euthanized when they display early markers associated with death or poor prognosis of quality of life, or specific signs of severe suffering or distress. Humane endpoints are used as an alternative to allowing such conditions to continue or progress to death following the experimental intervention (“death as an endpoint”), or only euthanizing animals at the end of an experiment. Before a study begins, researchers define the practical observations or measurements that will be used during the study to recognize a humane endpoint, based on anticipated clinical, physiological, and behavioural signs. [Please see the NC3Rs guidelines for more information](#). Additional discussion of humane endpoints can be found in this article: Nuno H. Franco, Margarida Correia-Neves, I. Anna S. Olsson (2012) How “Humane” Is Your Endpoint? — Refining the Science-Driven Approach for Termination of Animal Studies of Chronic Infection. PLoS Pathog 8(1): e1002399 doi.org/10.1371/journal.ppat.1002399.

Full details of humane endpoints use must be reported for a study to be reproducible and for the results to be accurately interpreted.

For studies in which death of an animal is an outcome or a planned experimental endpoint, authors should include the following information in the Methods section of the manuscript:

- The specific criteria (i.e. humane endpoints) used to determine when animals should be euthanized.
- The duration of the experiment.
- The numbers of animals used, euthanized, and found dead (if any); the cause of death for all animals.
- How frequently animal health and behaviour were monitored.
- All animal welfare considerations taken, including efforts to minimize suffering and distress, use of analgesics or anaesthetics, or special housing conditions.

If humane endpoints were not used, the manuscript should report:

- A scientific justification for the study design, including the reasons why humane endpoints could not be used, and discussion of alternatives that were considered.
- Whether the institutional animal ethics committee specifically reviewed and approved the anticipated mortality in the study design.

Observational and field studies

Methods sections for submissions reporting on any type of field study must include ethics statements that specify:

- Permits and approvals obtained for the work, including the full name of the authority that approved the study; if none were required, authors should explain why
- Whether the land accessed is privately owned or protected
- Whether any protected species were sampled
- Full details of animal husbandry, experimentation, and care/welfare, where relevant

Paleontology and archaeology research

Manuscripts reporting paleontology and archaeology research must include descriptions of methods and specimens in sufficient detail to allow the work to be reproduced. Data sets supporting statistical and phylogenetic analyses should be provided, preferably in a format that allows easy re-use. [Read the policy](#).

Specimen numbers and complete repository information, including museum name and geographic location, are required for publication. Locality information should be provided in the manuscript as legally allowable, or a statement should be included giving details of the availability of such information to qualified researchers.

If permits were required for any aspect of the work, details should be given of all permits that were obtained, including the full name of the issuing authority. This should be accompanied by the following statement:

All necessary permits were obtained for the described study, which complied with all relevant regulations.

If no permits were required, please include the following statement:

No permits were required for the described study, which complied with all relevant regulations.

Manuscripts describing paleontology and archaeology research are subject to the following policies:

- **Sharing of data and materials.** Any specimen that is erected as a new species, described, or figured must be deposited in an accessible, permanent repository (i.e., public museum or similar institution). If study conclusions depend on specimens that do not fit these criteria, the article will be rejected under *PLoS ONE*'s [data availability criterion](#).
- **Ethics.** *PLoS ONE* will not publish research on specimens that were obtained without necessary permission or were illegally exported.

Systematic reviews and meta-analyses

A systematic review paper, as defined by [The Cochrane Collaboration](#), is a review of a clearly formulated question that uses explicit, systematic methods to identify, select, and critically appraise relevant research, and to collect and analyze data from the studies that are included in the review. These reviews differ substantially from narrative-based reviews or synthesis articles. Statistical methods (meta-analysis) may or may not be used to analyze and summarize the results of the included studies.

Reports of systematic reviews and meta-analyses must include a completed [PRISMA \(Preferred Reporting Items for Systematic Reviews and Meta-Analyses\)](#) checklist and flow diagram to accompany the main text. Blank templates are available here:

- Checklist: [PDF](#) or [Word document](#)
- Flow diagram: [PDF](#) or [Word document](#)

Authors must also state in their “Methods” section whether a protocol exists for their systematic review, and if so, provide a copy of the protocol as supporting information and provide the registry number in the abstract.

If your article is a systematic review or a meta-analysis you should:

- State this in your cover letter
- Select “Research Article” as your article type when submitting
- Include the PRISMA flow diagram as Fig 1 (required where applicable)
- Include the PRISMA checklist as supporting information

Meta-analysis of genetic association studies

Manuscripts reporting a meta-analysis of genetic association studies must report results of value to the field and should be reported according to the guidelines presented in [Systematic Reviews of Genetic Association Studies](#) by Sagoo *et al.*

On submission, authors will be asked to justify the rationale for the meta-analysis and how it contributes to the base of scientific knowledge in the light of previously published results. Authors will also be asked to complete a [checklist \(DOCX\)](#) outlining information about the justification for the study and the methodology employed. Meta-analyses that replicate published studies will be rejected if the authors do not provide adequate justification.

Personal data from third-party sources

For all studies using personal data from internet-based and other third-party sources (e.g., social media, blogs, other internet sources, mobile phone companies), data must be collected and used according to company/website Terms and Conditions, with appropriate permissions. All data sources must be acknowledged clearly in the [Materials and Methods section](#).
[Read our policy on data availability.](#)

In the Ethics Statement, authors should declare any potential risks to individuals or individual privacy, or affirm that in their assessment, the study posed no such risks. In addition, the following Ethics and Data Protection requirements must be met.

For interventional studies, which impact participants’ experiences or data, the study design must have been prospectively approved by an Ethics Committee, and informed consent is required. The Ethics Committee may waive the requirement for approval and/or consent.

For observational studies in which personal experiences and accounts are not manipulated, consultation with an Ethics or Data Protection Committee is recommended. Additional requirements apply in the following circumstances:

- If information used could threaten personal privacy or damage the reputation of individuals whose data are used, an Ethics Committee should be consulted and informed consent obtained or specifically addressed.

- If authors accessed any personal identifying information, an Ethics or Data Protection Committee should oversee data anonymization. If data were anonymized and/or aggregated before access and analysis, informed consent is generally not required.

Note that Terms of Use contracts do not qualify as informed consent, even if they address the use of personal data for research.

[See our reporting guidelines for human subjects research.](#)

Cell lines

Authors reporting research using cell lines should state when and where they obtained the cells, giving the date and the name of the researcher, cell line repository, or commercial source (company) who provided the cells, as appropriate.

Authors must also include the following information for each cell line:

For de novo (new) cell lines, including those given to the researchers as a gift, authors must follow our policies for [human subjects research](#) or [animal research](#), as appropriate. The ethics statement must include:

- Details of institutional review board or ethics committee approval; AND
- For human cells, confirmation of written informed consent from the donor, guardian, or next of kin

For established cell lines, the Methods section should include:

- A reference to the published article that first described the cell line; AND/OR
- The cell line repository or company the cell line was obtained from, the catalogue number, and whether the cell line was obtained directly from the repository/company or from another laboratory

Authors should check established cell lines using the [ICLAC Database of Cross-contaminated or Misidentified Cell Lines](#) to confirm they are not misidentified or contaminated. Cell line authentication is recommended – e.g., by karyotyping, isozyme analysis, or short tandem repeats (STR) analysis – and may be required during peer review or after publication.

Blots and gels

Please review *PLoS ONE*'s requirements for [reporting blot and gel results and providing the underlying raw images](#).

Antibodies

Manuscripts reporting experiments using antibodies should include the following information:

- The name of each antibody, a description of whether it is monoclonal or polyclonal, and the host species.
- The commercial supplier or source laboratory.
- The catalogue or clone number and, if known, the batch number.
- The antigen(s) used to raise the antibody.
- For established antibodies, a stable public identifier from the [Antibody Registry](#).

The manuscript should also report the following experimental details:

- The final antibody concentration or dilution.
- A reference to the validation study if the antibody was previously validated. If not, provide details of how the authors validated the antibody for the applications and species used.

We encourage authors to consider adding information on new validations to a publicly available database such as [Antibodypedia](#) or [CiteAb](#).

Small and macromolecule crystal data

Manuscripts reporting new and unpublished three-dimensional structures must include sufficient supporting data and detailed descriptions of the methodologies used to allow the reproduction and validation of the structures. All novel structures must have been deposited in a community endorsed database prior to submission (please see our list of [recommended repositories](#)).

Small molecule single crystal data

Authors reporting X-Ray crystallographic structures of small organic, metal-organic, and inorganic molecules must deposit their data with the Cambridge Crystallographic Data Centre (CCDC), the Inorganic Crystal Structure Database (ICSD), or similar community databases providing a recognized validation functionality. Authors are also required to include the relevant structure reference numbers within the main text (e.g. the CCDC ID number), as well as the crystallographic information files (.cif format) as Supplementary Information, along with the checkCIF validation reports that can be obtained via the International Union of Crystallography (IUCr).

Macromolecular structures

Authors reporting novel macromolecular structures must have deposited their data prior to initial submission with the Worldwide Protein Data Bank (wwPDB), the Biological Magnetic Resonance Data Bank (BMRB), the Electron Microscopy Data Bank (EMDB), or other community databases providing a

recognized validation functionality. Authors must include the structure reference numbers within the main text and submit as Supplementary Information the official validation reports from these databases.

Methods, software, databases, and tools

PLoS ONE will consider submissions that present new methods, software, databases, or tools as the primary focus of the manuscript if they meet the following criteria:

Utility

The tool must be of use to the community and must present a proven advantage over existing alternatives, where applicable. Recapitulation of existing methods, software, or databases is not useful and will not be considered for publication. Combining data and/or functionalities from other sources may be acceptable, but simpler instances (i.e. presenting a subset of an already existing database) may not be considered. For software, databases, and online tools, the long-term utility should also be discussed, as relevant. This discussion may include maintenance, the potential for future growth, and the stability of the hosting, as applicable.

Validation

Submissions presenting methods, software, databases, or tools must demonstrate that the new tool achieves its intended purpose. If similar options already exist, the submitted manuscript must demonstrate that the new tool is an improvement over existing options in some way. This requirement may be met by including a proof-of-principle experiment or analysis; if this is not possible, a discussion of the possible applications and some preliminary analysis may be sufficient.

Availability

If the manuscript's primary purpose is the description of new software or a new software package, this software must be open source, deposited in an appropriate archive, and conform to the [Open Source Definition](#). If the manuscript mainly describes a database, this database must be open-access and hosted somewhere publicly accessible, and any software used to generate a database should also be open source. If relevant, databases should be open for appropriate deposition of additional data. Dependency on commercial software such as Mathematica and MATLAB does not preclude a paper from consideration, although complete open source solutions are preferred. In these cases, authors should provide a direct link to the deposited software or the database hosting site from within the paper. If the primary focus of a manuscript is the presentation of a new tool, such as a newly developed or modified questionnaire or scale, it should be openly available under a license no more restrictive than CC BY.

Software submissions

Manuscripts whose primary purpose is the description of new software must provide full details of the algorithms designed. Describe any dependencies on commercial products or operating system. Include details of the supplied test data and explain how to install and run the software. A brief description of enhancements made in the major releases of the software may also be given. Authors should provide a direct link to the deposited software from within the paper.

Database submissions

For descriptions of databases, provide details about how the data were curated, as well as plans for long-term database maintenance, growth, and stability. Authors should provide a direct link to the database hosting site from within the paper.

[Read the PLOS policy on sharing materials and software.](#)

New taxon names

Zoological names

When publishing papers that describe a new zoological taxon name, PLOS aims to comply with the requirements of the [International Commission on Zoological Nomenclature \(ICZN\)](#). Effective 1 January 2012, the ICZN considers an online-only publication to be legitimate if it meets the criteria of archiving and is registered in ZooBank, the ICZN's official registry.

For proper registration of a new zoological taxon, we require two specific statements to be included in your manuscript.

In the **Results** section, the globally unique identifier (GUID), currently in the form of a Life Science Identifier (LSID), should be listed under the new species name, for example:

***Anochetus boltoni* Fisher sp. nov.** urn:lsid:zoobank.org:act:B6C072CF-1CA6-40C7-8396-534E91EF7FBB

You will need to contact [Zoobank](#) to obtain a GUID (LSID). Please do this as early as possible to avoid delay of publication upon acceptance of your manuscript. It is your responsibility to provide us with this information so we can include it in the final published paper.

Please also insert the following text into the **Methods** section, in a sub-section to be called "Nomenclatural Acts":

The electronic edition of this article conforms to the requirements of the amended International Code of Zoological Nomenclature, and hence the new names contained herein are available under that Code from the electronic edition of this article. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix “<http://zoobank.org/>”. The LSID for this publication is: urn:lsid:zoobank.org:pub: XXXXXXX. The electronic edition of this work was published in a journal with an ISSN, and has been archived and is available from the following digital repositories: PubMed Central, LOCKSS [author to insert any additional repositories]. All PLOS articles are deposited in [PubMed Central](#) and [LOCKSS](#). If your institute, or those of your co-authors, has its own repository, we recommend that you also deposit the published online article there and include the name in your article.

Botanical names

When publishing papers that describe a new botanical taxon, PLOS aims to comply with the requirements of the International Code of Nomenclature for algae, fungi, and plants (ICN). The following guidelines for publication in an online-only journal have been agreed such that any scientific botanical name published by us is considered effectively published under the rules of the Code. Please note that these guidelines differ from those for zoological nomenclature, and apply only to seed plants, ferns, and lycophytes. Effective January 2012, the description or diagnosis of a new taxon can be in either Latin or English. This does not affect the requirements for scientific names, which are still to be Latin. Also effective January 2012, the electronic PDF represents a published work according to the ICN for algae, fungi, and plants. Therefore the new names contained in the electronic publication of PLOS article are effectively published under that Code from the electronic edition alone, so there is no longer any need to provide printed copies. Additional information describing recent changes to the Code can be found [here](#). For proper registration of the new taxon, we require two specific statements to be included in your manuscript.

In the **Results** section, the globally unique identifier (GUID), currently in the form of a Life Science Identifier (LSID), should be listed under the new species name, for example:

Solanum aspersum S.Knapp, sp. nov. [urn:lsid:ipni.org:names:77103633-1] Type: Colombia. Putumayo: vertiente oriental de la Cordillera, entre Sachamates y San Francisco de Sibundoy, 1600-1750 m, 30 Dec 1940, J. Cuatrecasas 11471 (holotype, COL; isotypes, F [F-1335119], US [US-1799731]).

Journal staff will contact IPNI to obtain the GUID (LSID) after your manuscript is accepted for publication, and this information will then be added to the manuscript during the production phase

In the **Methods** section, include a sub-section called “Nomenclature” using the following wording:

The electronic version of this article in Portable Document Format (PDF) in a work with an ISSN or ISBN will represent a published work according to the International Code of Nomenclature for algae, fungi, and plants, and hence the new names contained in the electronic publication of a PLOS article are effectively published under that Code from the electronic edition alone, so there is no longer any need to provide printed copies.

In addition, new names contained in this work have been submitted to IPNI, from where they will be made available to the Global Names Index. The IPNI LSIDs can be resolved and the associated information viewed through any standard web browser by appending the LSID contained in this publication to the prefix <http://ipni.org/>. The online version of this work is archived and available from the following digital repositories: [INSERT NAMES OF DIGITAL REPOSITORIES WHERE ACCEPTED MANUSCRIPT WILL BE SUBMITTED (PubMed Central, LOCKSS etc)].

All PLOS articles are deposited in [PubMed Central](#) and [LOCKSS](#). If your institute, or those of your co-authors, has its own repository, we recommend that you also deposit the published online article there and include the name in your article.

Fungal names

When publishing papers that describe a new botanical taxon, PLOS aims to comply with the requirements of the International Code of Nomenclature for algae, fungi, and plants (ICN). The following guidelines for publication in an online-only journal have been agreed such that any scientific botanical name published by us is considered effectively published under the rules of the Code. Please note that these guidelines differ from those for zoological nomenclature.

Effective January 2012, the description or diagnosis of a new taxon can be in either Latin or English. This does not affect the requirements for scientific names, which are still to be Latin.

Also effective January 2012, the electronic PDF represents a published work according to the ICN for algae, fungi, and plants. Therefore the new names contained in the electronic publication of PLOS article

are effectively published under that Code from the electronic edition alone, so there is no longer any need to provide printed copies.

Additional information describing recent changes to the Code can be found [here](#).

For proper registration of the new taxon, we require two specific statements to be included in your manuscript.

In the **Results** section, the globally unique identifier (GUID), currently in the form of a Life Science Identifier (LSID), should be listed under the new species name, for example:

Hymenogaster huthii. Stielow et al. 2010, sp. nov. [urn:lsid:indexfungorum.org:names:518624]

You will need to contact either [Mycobank](#) or [Index Fungorum](#) to obtain the GUID (LSID). Please do this as early as possible to avoid delay of publication upon acceptance of your manuscript. It is your responsibility to provide us with this information so we can include it in the final published paper. Effective January 2013, all papers describing new fungal species must reference the identifier issued by a recognized repository in the protologue in order to be considered effectively published.

In the **Methods** section, include a sub-section called “Nomenclature” using the following wording. Note that this example is for taxon names submitted to MycoBank; please substitute appropriately if you have submitted to Index Fungorum using the

prefix <http://www.indexfungorum.org/Names/NamesRecord.asp?RecordID=>.

The electronic version of this article in Portable Document Format (PDF) in a work with an ISSN or ISBN will represent a published work according to the International Code of Nomenclature for algae, fungi, and plants, and hence the new names contained in the electronic publication of a PLOS article are effectively published under that Code from the electronic edition alone, so there is no longer any need to provide printed copies.

In addition, new names contained in this work have been submitted to MycoBank from where they will be made available to the Global Names Index. The unique MycoBank number can be resolved and the associated information viewed through any standard web browser by appending the MycoBank number contained in this publication to the prefix <http://www.mycobank.org/MB/>. The online version of this work is archived and available from the following digital repositories: [INSERT NAMES OF DIGITAL REPOSITORIES WHERE ACCEPTED MANUSCRIPT WILL BE SUBMITTED (PubMed Central, LOCKSS etc)].

All PLOS articles are deposited in [PubMed Central](#) and [LOCKSS](#). If your institute, or those of your co-authors, has its own repository, we recommend that you also deposit the published online article there and include the name in your article.

Qualitative research

Qualitative research studies use non-quantitative methods to address a defined research question that may not be accessible by quantitative methods, such as people's interpretations, experiences, and perspectives. The analysis methods are explicit, systematic, and reproducible, but the results do not involve numerical values or use statistics. Examples of qualitative data sources include, but are not limited to, interviews, text documents, audio/video recordings, and free-form answers to questionnaires and surveys.

Qualitative research studies should be reported in accordance to the [Consolidated criteria for reporting qualitative research \(COREQ\) checklist](#) or [Standards for reporting qualitative research \(SRQR\) checklist](#). Further reporting guidelines can be found in the Equator Network's [Guidelines for reporting qualitative research](#).

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BMJ Open adheres to the highest standards concerning its editorial policies on publication ethics, scientific misconduct, consent and peer review criteria. To view all BMJ Journal policies please refer to the [BMJ Author Hub policies](#) page.

Copyright and authors' rights

As an open access journal, *BMJ Open* adheres to the Budapest Open Access Initiative definition of open access. Articles are published under an exclusive licence or non-exclusive licence for UK Crown employees or where BMJ has agreed CC BY applies. For US Federal Government officers or employees acting as part of their official duties, the terms are as stated in accordance with our licence terms. Authors or their employers retain copyright. Such open access articles can be reused under the terms of the relevant Creative Commons licence to facilitate reuse of the content. please refer to the *BMJ Open* [Author Licence](#). More information on [copyright and authors' rights](#). When publishing in *BMJ Open*, authors choose between two licence types – [CC-BY-NC](#) and [CC-BY](#) (Creative Commons open access licences require payment of an [article processing charge](#)). As an author you may wish to post your article in an institutional or subject repository, or on a scientific social sharing network. You may also link your published article to your preprint (if applicable). What you can do with your article, without seeking permission,

depends on the licence you have chosen and the version of your article. Please refer to the [BMJ author self archiving and permissions policies page](#) for more information.

- **Preprints**

Preprints foster openness, accessibility and collaboration by allowing authors to make their findings immediately available to the research community and receive feedback on an article before it is submitted to a journal for formal publication.

BMJ fully supports and encourages the archiving of preprints in any recognised, not-for-profit server such as [medRxiv](#). BMJ does not consider the posting of an article in a dedicated preprint repository to be prior publication. Preprints are reports of work that have not been peer-reviewed; Preprints should therefore not be used to guide clinical practice, health-related behaviour or health policy. For more information, please refer to our [Preprint policy page](#).

- **Article processing charges**

BMJ Open is an open access journal and levies an Article Processing Charge (APC) of 1,500 GBP (exclusive of VAT for UK and EU authors and GST for Australian and Indian authors) for all article types. There are no submission, colour or page charges.

Find out if you are eligible for institutional funding

A number of institutions have open access agreements with BMJ which can either cover the whole cost of open access publishing for authors at participating institutions or can allow authors to receive a discount of the Article Processing Charge (APC).

Visit [BMJ's open access agreements](#) page to find out whether your institution is a member and what discounts you may be entitled to.

BMJ also grants [waivers and discounts](#)* in specific circumstances, for more information on [publishing open access with BMJ](#) visit our Author Hub.

**Please note that applications for waivers or discounts should be made during initial submission and not after an article has been accepted. Editors are not involved in this process and the ability to pay has no bearing on editorial decisions. Payment will not be required unless your article is accepted. Accepted articles will not be published until payment has been received. BMJ does not refund APCs once paid.*

- **Peer review process**

All articles published in *BMJ Open* will have been sent for external, open peer review. Reviewers will not be asked to judge importance or breadth of appeal. Readers will be able to make these judgements for themselves. We recommend you use our [instructions for reviewers](#) as a checklist to ensure that your article is complete. Upon publication, all previous versions of the manuscript will also be made available, as will the reviewers' comments and authors' replies to those comments.

Post-publication peer review will also be possible via rapid responses.

BMJ Open will consider appeals on rejected manuscripts provided the authors can demonstrate that the decision on their manuscript was flawed or not in line with the journal's policies. For an appeal to be considered, authors must provide a detailed point-by-point response to all the concerns raised by the reviewers or editors involved with the manuscript. We ask that authors do not provide a revised manuscript during the appeal. All decisions made on appeals are final, and the decision process could take longer than with original submissions. Appeals must be made within 30 days of the reject decision.

Plagiarism is the appropriation of the language, ideas or thoughts of another without crediting their true source and representation of them as one's own original work. BMJ is a member of CrossCheck by CrossRef and iThenticate. iThenticate is a plagiarism screening service that verifies the originality of content submitted before publication. BMJ runs manuscripts through iThenticate during the peer review process. Authors, researchers and freelancers can also use iThenticate to screen their work before submission by visiting www.ithenticate.com.

- **Peer review of study protocols**

BMJ Open will consider publishing without peer review protocols that have formal ethical approval *and* funding from a recognised, open access advocating research-funding body (such as those listed by the [JULIET](#) project). Please provide proof that these criteria are met when uploading your protocol. Any protocols that do not meet both these criteria will be sent for open external peer review, with reviewer comments published online upon acceptance, as with research articles. Reviewers will be instructed to review for clarity and sufficient detail. The intention of peer review is not to alter the study design. Reviewers will be instructed to check that the study is scientifically credible and ethically sound in its scope and methods, and that there is sufficient detail to instil confidence that the study will be conducted and analysed properly.

As with research articles, protocols will be published under a Creative Commons licence.

- **ORCID**

Reviewers are now able to share their activity by connecting their review to their ORCID account. As *BMJ Open* is an open peer-reviewed Journal, information about the Journal, review, and article will be uploaded (title, DOI, URL, etc) into the reviewer's ORCID record. Records are uploaded once a final decision or revision has been made on the article. Please find more information about ORCID and BMJ's policy on our [Author Hub](#).

- [Reporting patient and public involvement in research](#)

BMJ encourages active patient and public involvement in clinical research as part of its patient and public partnership strategy. To support co-production of research we request that authors provide a Patient and Public Involvement statement in the methods section of their papers, under the subheading 'Patient and public involvement'.

We appreciate that patient and public involvement is relatively new and may not be feasible or appropriate for all papers. We therefore continue to consider papers where patients were not involved. Please note that this practice is only applicable for Research Articles, Study Protocols, and Cohort Profiles.

The [Patient and Public Involvement statement](#) should provide a brief response to the following questions, tailored as appropriate for the study design reported.

- At what stage in the research process were patients/the public first involved in the research and how?
- How were the research question(s) and outcome measures developed and informed by their priorities, experience, and preferences?
- How were patients/the public involved in the design of this study?
- How were they involved in the recruitment to and conduct of the study?
- Were they asked to assess the burden of the intervention and time required to participate in the research?
- How were (or will) they be involved in your plans to disseminate the study results to participants and relevant wider patient communities (e.g. by choosing what information/results to share, when, and in what format)?

If patients were not involved please state this.

In addition to considering the points above we advise authors to look at guidance for best reporting of patient and public involvement as set out in the [GRIPP2 reporting checklist](#).

If the Patient and Public Involvement statement is missing in the submitted manuscript we will request that authors provide it.

- [Data Sharing](#)

BMJ Open adheres to BMJ's Tier 2 data policy. We strongly *encourage* that data generated by your research that supports your article be made available as soon as possible, wherever legally and ethically possible. We also *require* data from clinical trials to be made available upon reasonable request.

To adhere to [ICMJE guidelines](#), we *require* that a data sharing plan must be included with trial registration for clinical trials that begin enrolling participants on or after 1st January 2019. Changes to the plan must be noted in the Data Availability Statement and updated in the registry record. All research articles must contain a Data Availability Statement. For more information and FAQs, please see BMJ's full [Data Sharing Policy](#) page.

- [Resources](#)

- [How can I maximise my chances of being published?](#)

BMJ Open will publish all submissions judged to be technically sound after peer review. Asking yourself these five questions will help maximise your chances of a successful submission.

1. [Does my research fall within BMJ Open's aims and scope?](#)
2. [Is the research question clear?](#)
3. [Is the study design appropriate?](#)
4. [Is the study valid?](#)
5. [Is the research presented correctly?](#)

These are intended to be aids to a successful submission and reflect the [type of judgement we ask reviewers to make](#). If you have any questions please [contact the editorial office](#).

- [Where can I find more general advice?](#)

The pages above all contain useful information for anyone preparing a research article for BMJ Open. The following links provide more detailed information on how to perform and report research. The links are not exhaustive and if you know of any good resources we have not listed, please let us know.

[General advice on designing, carrying out and reporting research](#)

[Authors' Submission Toolkit: A practical guide to getting your research published](#)

- [Resources for data management and sharing](#)

BMJ Open supports the idea that all appropriate datasets should be placed in open repositories. To assist, we have assembled some useful links and resources that cover best practice for data management plans through to sharing and citing complete datasets.

[Resources for data management and sharing](#)

- [ORCID](#)

BMJ has long supported the use of ORCID by integrating ORCID into our submission systems. BMJ Open mandates ORCID iDs for all submitting authors; this is published on the final article to promote discoverability and credit. Co-authors and reviewers are strongly encouraged to also connect their ScholarOne accounts to ORCID.

We are also working with ORCID to recognise the importance of the reviewer community. Reviewers are able to share their activity by connecting their review to their ORCID account to gain recognition for their contributions.

As BMJ Open is an open peer-reviewed Journal, information about the Journal, review, and article will be uploaded (title, DOI, URL, etc) into the reviewer's ORCID record. Records are uploaded once a final decision or revision has been made on the article. Please find more information about ORCID and BMJ's policy on our [Author Hub](#).

- [Submission guidelines](#)

Please review the below article type specifications including the required article lengths, illustrations, table limits and reference counts. The word count excludes the title page, abstract, tables, acknowledgements, contributions and references. Manuscripts should be as succinct as possible.

For further support when making your submission please refer to the resources available on the [BMJ Author Hub](#).

Here you will find information on [writing and formatting](#) your research through to the [peer review process](#) and [promoting your paper](#). You may also wish to use the language editing and translation services provided by [BMJ Author Services](#).

- [Original research](#)
- [Protocol](#)
- [Cohort profile](#)
- [Communication](#)
- [Rapid response](#)
- [Supplement](#)

- [Original research](#)

Research submissions should have a clear, justified research question.

We strongly encourage you to register your study. Prospective registration is mandatory for any clinical trials.

[Acceptable registries for trials](#) include [clinicaltrials.gov](#). We recommend [Prospero](#) for registration of systematic reviews.

All articles should include the following:

- **The article title should include the research question and the study design.** Titles should not declare the results of the study.
- **A structured abstract** (max. 300 words) including all the following where appropriate (please note that for RCTs there is a specific [CONSORT extension for abstracts](#)):
 - **objectives:** clear statement of main study aim and major hypothesis/research question
 - **design:** e.g. prospective, randomised, blinded, case control
 - **setting:** level of care e.g. primary, secondary; number of participating centres. Generalise; don't use the name of a specific centre, but give geographical location if important
 - **participants:** numbers entering and completing the study; sex and ethnic group if appropriate. Clear definitions of selection, entry and exclusion criteria
 - **interventions:** what, how, when and how long (this can be deleted if there were no interventions)
 - **primary and secondary outcome measures:** planned (i.e. in the protocol) and those finally measured (if different, explain why) – for quantitative studies only
 - **results:** main results with (for quantitative studies) 95% confidence intervals and, where appropriate, the exact level of statistical significance and the number need to treat/harm. Whenever possible, state absolute rather than relative risks
 - **conclusions:** primary conclusions and their implications, suggest areas for further research if appropriate. Do not go beyond the data in the article
 - **where applicable, trial registration:** registry and number (for clinical trials and, if available, for observational studies and systematic reviews)
- **An Article Summary, placed after the abstract, consisting of the heading 'Strengths and limitations of this study',** and containing up to five short bullet points, no longer than one sentence each, that relate specifically to the methods. They should not include the results of the study.
- **The original protocol for the study,** as a supplementary file.
- **A funding statement,** preferably worded as follows. Either: 'This work was supported by [name of funder] grant number [xxx]' or 'This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors'. You must ensure that the full, correct details of your funder(s) and any relevant grant numbers are included.
- **A competing interests statement.** See the [BMJ Author Hub](#) for details on what to include as competing interests.
- **Articles should list each author's contribution individually at the end;** this section may also include contributors who do not qualify as authors. Please visit the [ICMJE](#) website for more information on authorship.
- **Any checklist and flow diagram for the appropriate reporting statement,** e.g. STROBE (see below).
- **A patient consent form:** any article that contains personal medical information about an identifiable living individual requires the patient's explicit consent before we can publish it. We will need the patient to sign our [consent form](#), which requires the patient to have read the article. This form is available in multiple languages.
- **A data sharing statement,** such as: "Technical appendix, statistical code, and dataset available from the Dryad repository, DOI: [include DOI for dataset here]."
- **Word count,** we recommend your article does not exceed 4000 words, with up to five figures and tables. This is flexible, but exceeding this will impact upon the paper's 'readability'. Authors are encouraged to

submit figures and images in colour – there are no colour charges. We require that you upload your figures as separate files rather than embedding them in the manuscript.

- **Supplementary and raw data** can be placed online alongside the article although we prefer raw data to be made publicly available and linked to in a suitable repository (e.g. Dryad, FigShare). We may request that you separate out some material into supplementary data files to make the main manuscript clearer for readers.

We also recommend, but do not insist, that the discussion section is no longer than five paragraphs and follows this overall structure (you do not need to use these as subheadings): a statement of the principal findings; strengths and weaknesses of the study; strengths and weaknesses in relation to other studies, discussing important differences in results; the meaning of the study; possible explanations and implications for clinicians and policymakers; and unanswered questions and future research.

At upload you will be asked to choose one general subject area that applies to your article – it will be published under this banner on the main table of contents. You will also be asked to select further subject headings to be used for the 'Browse by topic' section, and specific keywords for help with identifying reviewers.

Following the lead of The BMJ and its [patient partnership strategy](#), *BMJ Open* is encouraging active patient involvement in setting the research agenda. As such, we require authors of Research Articles to add a [Patient and Public Involvement statement](#) in the Methods section. Please see more details above.

- [Pilot studies](#)

Articles reporting pilot studies should explain the work's wider context and explain why the term 'pilot study' applies. The term 'pilot study' should not be applied to justify reporting a small-scale study. Justifications for a pilot study include:

- trialling a new procedure intended for use in a larger programme of research
- establishing power calculations required for a full-scale study
- establishing how many patients and/or healthcare professionals can be recruited
- evaluating the financial, technical, administrative or logistic feasibility of a full-scale study, including issues of data collection, protocol adherence, and questionnaire design.

The sample/patient size should still be justified. The article should explain the impact that the pilot study had on decisions regarding future research.

- [Reporting guidelines](#)

The guidelines listed below should be followed where appropriate. Please use these guidelines to structure your article. Completed applicable checklists, structured abstracts and flow diagrams should be uploaded with your submission; these will be published alongside the final version of your paper.

- [CONSORT Statement](#)

For reporting of randomised controlled trials: please use the appropriate extension to the CONSORT statement, including the extension for writing abstracts

- [SRQR](#)

For reporting qualitative research

- [COREQ](#)

For reporting qualitative research

- [STARD](#)

For reporting of diagnostic accuracy studies

- [STROBE](#)

For reporting of observational studies in epidemiology

[Checklist for cohort, case-control, and cross-sectional studies \(combined\)](#)

[Checklist for cohort studies](#)

[Checklist for case-control studies](#)

[Checklist for cross-sectional studies](#)

- [PRISMA](#)

For reporting of systematic reviews

- [PRISMA-P](#)

For reporting of systematic review and meta-analysis protocols

- [PRISMA-ScR](#)

For reporting of scoping reviews

- [MOOSE](#)

For reporting of meta-analyses of observational studies

- [SPIRIT](#)

For reporting protocols for RCTs

- [STREGA](#)

For reporting of gene-disease association studies

- [TRIPOD](#)

For reporting of studies developing, validating, or updating a prediction model, whether for diagnostic or prognostic purposes.

- [CHEERS](#)

For reporting of health economic evaluations

The [Equator Network](#) (Enhancing the Quality and Transparency Of Health Research) provides a comprehensive list of reporting guidelines.

- **Protocol**

Protocol manuscripts should report planned or ongoing research studies. If data collection is complete, we will not consider the manuscript. We encourage the submission of protocol manuscripts at an early stage of the study. Protocols nearing completion of data collection will be treated on a case by case basis and the final decision on whether to consider a protocol for publication will rest with the Editor.

Publishing study protocols enables researchers and funding bodies to stay up to date in their fields by providing exposure to research activity that may not otherwise be widely publicised. This can help prevent unnecessary duplication of work and will hopefully enable collaboration. Publishing protocols in full also makes available more information than is currently required by trial registries and increases transparency, making it easier for others (editors, reviewers and readers) to see and understand any deviations from the protocol that occur during the conduct of the study.

The SPIRIT ([Standard Protocol Items for Randomized Trials](#)) statement has now been published. It is an evidence-based tool developed through systematic review of a wide range of resources and consensus. It closely mirrors the CONSORT statement and also reflects important ethics considerations. We encourage investigators to adhere to the SPIRIT recommendations when drafting their protocols and include a completed SPIRIT checklist with their trial protocol submission.

The PRISMA-P (Preferred reporting items for systematic review and meta-analysis protocols) is a new reporting guideline. An article stating the [guideline checklist](#) has now been published. The PRISMA-P checklist contains 17 items considered to be essential and minimum components of a systematic review or meta-analysis protocol. Systematic review authors and assessors are strongly encouraged to make use of PRISMA-P when drafting and appraising review protocols and authors should include a completed PRISMA-P checklist with their protocol submission.

Various other resources exist that list the ingredients of an authoritative trial protocol, e.g. the UK Dept of Health/Medical Research Council [Clinical Trials Toolkit](#) and the US National Institutes for Health provide advice on how to structure a trial protocol. *BMJ Open* will consider for publication protocols for any study design, including observational studies and systematic reviews.

We strongly encourage you to register your study. Prospective registration is mandatory for any clinical trials. [Acceptable registries](#) for trials include [clinicaltrials.gov](#). We recommend [Prospero](#) for registration of systematic reviews.

Following the lead of The BMJ and its [patient partnership strategy](#), *BMJ Open* is encouraging active patient involvement in setting the research agenda. As such, we require authors of Study Protocols to add a [Patient and Public Involvement statement](#) in the Methods section. please see more details above.

General BMJ policies apply (see above) on manuscript formatting, editorial policies, licence forms and patient consent (where applicable to study designs). Protocols should include, as a minimum, the following items.

- **Protocol papers should report planned or ongoing studies.** Manuscripts that report work already carried out will not be considered as protocols. The dates of the study must be included in the manuscript and cover letter.
- **Protocols for studies that will require ethical approval**, such as trials, are unlikely to be considered without having received that approval.
- **Title:** this should include the specific study type, e.g. randomised controlled trial.
- **Abstract:** this should be structured with the following sections. Introduction; Methods and analysis; Ethics and dissemination. Registration details should be included as a final section, if appropriate.
- **An Article Summary, placed after the abstract, consisting of the heading ‘Strengths and limitations of this study’,** and containing up to five short bullet points, no longer than one sentence each, that relate specifically to the methods.
- **Introduction:** explain the rationale for the study and what evidence gap it may fill. Appropriate previous literature should be referenced, including relevant systematic reviews.
- **Methods and analysis:** provide a full description of the study design, including the following. How the sample will be selected; interventions to be measured; the sample size calculation (drawing on previous literature) with an estimate of how many participants will be needed for the primary outcome to be statistically, clinically and/or politically significant; what outcomes will be measured, when and how; a data analysis plan.
- **Ethics and dissemination:** ethical and safety considerations and any dissemination plan (publications, data deposition and curation) should be covered here.
- **Full references.**
- **Authors’ contributions:** state how each author was involved in writing the protocol.
- **Funding statement:** preferably worded as follows. Either: ‘This work was supported by [name of funder] grant number [xxx]’ or ‘This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors’.
- **Competing interests statement.**
- **Word Count:** 4,000 words. Should the word count exceed this number, please state this in the cover letter upon submission.

- **Cohort profile**

The cohort profile is an article type set up in *BMJ Open* to fill the space between a study protocol and a results paper. Cohort profiles should describe the rationale for a cohort's creation, its methods, baseline data and its future plans. Cohorts described should be long-term, prospective projects and not time-limited cohorts established to answer a small number of specific research questions.

If a cohort has yet to complete recruitment or baseline data collection, it should be submitted as a study protocol.

Papers addressing a specific research question using cohort data should be submitted as a Research paper.

Following the lead of The BMJ and its [patient partnership strategy](#), *BMJ Open* is encouraging active patient involvement in setting the research agenda. As such, we require authors of Cohort Profiles to add a [Patient and Public Involvement statement](#) in the Methods section. please see more details above.

Title

Should begin 'Cohort profile: ...'. It should include the full name and any commonly used abbreviation of the cohort, plus its location or whether it is international. Include the type of cohort.

Abstract

Use these headings to provide brief descriptions of the following:

- **Purpose:** describe why the cohort was set up
- **Participants:** describe who is in the cohort
- **Findings to date:** what data has been collected so far and any major results
- **Future plans:** how will the cohort be used in future, including any date for completion of data collection
- **Registration:** if your Cohort Profile is linked to a clinical trial, we require the registry and trial registration number to be included. In line with the recommendations of the [International Committee of Medical Journal Editors](#), we require prospective registration of all clinical trials. For Cohort Profiles not linked to a clinical trial, registration is not required.

Introduction

Describe the rationale for the study, including any specific research questions that motivated the project.

Cohort description

Describe the setting, locations and relevant dates, including periods of recruitment, exposure, follow-up and data collection.

Give the eligibility criteria and how participants were recruited. Report numbers of individuals at each stage of the study, e.g. how many were approached, included in the study and have been retained. Reasons for non-participation should be reported. A flow diagram is recommended to illustrate this.

Describe methods of data collection and follow-up, and any external data sources used.

Give characteristics of study participants (e.g. demographic, clinical, social) and information on exposures and potential confounders. Indicate number of participants with missing data for each variable of interest.

Detailed statistical plans should not be reported.

Findings to date

Include a short explanation of the most notable results from the cohort so far, with references to relevant publications. This section should summarise rather than present results.

Strengths and limitations

These should be specific to the cohort being described. Include any lessons learned from the cohort's creation that can be shared to help future researchers.

Collaboration

Authors should include a section on what data will be available, to whom, how it can be accessed and what restrictions to reuse may apply. (This should be in the text, not the data sharing statement.) Please also state what kind of collaboration you are encouraging.

Further details

Our standard inclusions – Strengths and limitations of this study, a data sharing statement, funding declaration, contributorship statement, etc., should also be included.

- **Communication**

If you are interested in submitting a communication article to *BMJ Open*, we ask that you send us a presubmission enquiry via our [online submission system](#). You will be asked to provide a title, an abstract, and a cover letter explaining why you think your work is appropriate as a communication article for *BMJ Open*.

Communication articles will be considered at the discretion of the editorial team at *BMJ Open* based on the relevance of the topic and the quality of a presubmission enquiry. If your presubmission enquiry is accepted, the editorial team will invite you to submit a full manuscript.

The communication article will cover content that does not fit within our existing article types, but is of interest to *BMJ Open's* readership. Rather than presenting primary research, it is an opportunity to present ideas, examples, and innovations relating to the conduct of clinical research. As with all content in *BMJ Open*, communication articles will relate to key research issues in clinical medicine, public health, and epidemiology.

Examples of topics of relevance to *BMJ Open* include:

- Encouraging patient and public involvement
- Improving research transparency and reproducibility
- Reducing research waste through collaboration
- Improving best practice in publishing and peer review

All communication articles will undergo external open peer review. As with our other content, the peer reviewer reports and previous versions of the manuscript will be posted alongside the final article. We also encourage readers to participate in the discussion by posting comments on the articles.

The communication article should include, as a minimum, the following items:

- **Title:** a clear, concise description of the article's content.
- **Abstract:** we recommend a structured abstract that states the objective of the piece along with a summary of the key data or arguments and an overall conclusion.
- **Full References**
- **Authors' Contributions:** state how each author was involved in writing the article.
- **Funding statement:** preferably worded as follows. Either: 'This work was supported by [name of funder] grant number [xxx]' or 'This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors'.
- **Competing Interests statement.** see the [BMJ Author Hub](#) for details.

The structure and format of the article is at the discretion of the author. However, it should follow a logical form and be divided into subsections.

Communication articles should be kept as brief as possible. We recommend a word count of approximately 2,500 words (not including references). If you expect that your word count will exceed our recommendations, please mention this in the cover letter of your presubmission enquiry along with a justification.

- [Rapid response](#)

We encourage readers to comment on articles published in *BMJ Open*. Please follow our [guidelines](#) set out in our blog and eLetter response terms and requirements.

- [Supplement](#)

BMJ Journals are willing to consider publishing supplements to regular issues. Supplement proposals may be made at the request of:

- The journal editor, an editorial board member or a learned society may wish to organise a meeting, sponsorship may be sought and the proceedings published as a supplement.
- The journal editor, editorial board member or learned society may wish to commission a supplement on a particular theme or topic. Again, sponsorship may be sought.
- BMJ itself may have proposals for supplements where sponsorship may be necessary.
- A sponsoring organisation, often a pharmaceutical company or a charitable foundation, that wishes to arrange a meeting, the proceedings of which will be published as a supplement.

In all cases, it is vital that the journal's integrity, independence and academic reputation is not compromised in any way.

For further information on criteria that must be fulfilled, download the [supplements guidelines](#).

When contacting us regarding a potential supplement, please include as much of the information below as possible.

- Journal in which you would like the supplement published
- Title of supplement and/or meeting on which it is based
- Date of meeting on which it is based
- Proposed table of contents with provisional article titles and proposed authors
- An indication of whether authors have agreed to participate
- Sponsor information including any relevant deadlines
- An indication of the expected length of each paper
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For Article 3

American Journal of Clinical Nutrition

Instructions to Authors

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Statement of Scope

The purpose of *The American Journal of Clinical Nutrition* (AJCN) is to publish original research studies relevant to human and clinical nutrition. Well-controlled clinical studies that describe scientific mechanisms, efficacy, and safety of dietary interventions in the context of disease prevention or a health benefit will be considered. Public health and epidemiologic studies relevant to human nutrition, and innovative investigations of nutritional questions that employ epigenetic, genomic, proteomic, and metabolomic approaches are encouraged. Solicited editorials, book reviews, solicited or unsolicited review articles, invited controversy position papers, and letters to the Editor that relate to prior AJCN articles are essential components of the AJCN. All submitted material with scientific content will undergo peer review by the Editors or their designees before acceptance for publication.

Symposia or workshop articles may be published as supplements to the AJCN and are funded by their sponsors. The AJCN welcomes queries about the publication of supplements. The AJCN uses a 2-part acceptance process for supplements. The first step involves editorial acceptance of the topic and content as provided by the symposium organizer; please send the following material to Christopher.Duggan@childrens.harvard.edu and cc the ASN Editorial Office at ajcn.editorialoffice@oup.com for consideration: title, location, and date of the meeting; the names and affiliations of potential Supplement Coordinators; the sponsor(s) of the meeting; the sponsor(s) of the publication; and the agenda/program from the meeting along with the names of the speakers. The second step involves anonymous peer review of the individual articles. To be considered for publication, supplement articles must be received within 3 mo of each symposium or workshop. Each manuscript should not exceed 15 text pages, exclusive of tables, figures, and references; must adhere to AJCN style and format; and will be reviewed according to the same scientific standards used to evaluate original research articles.

Major Sections of the AJCN

- Editorials
- Review Articles
- Special Invited Articles, including Great Debates in Nutrition, and Controversies & Perspectives
- Original Research Communications (including formal systematic review/meta-analysis)
- Letters to the Editor
- Book Reviews
- ASN Announcements
- Calendar of Events

Letters to the Editor

Letters to the Editor that refer to a recent AJCN original research article must be received within 12 weeks of the article's publication. All letters will be subjected to editorial review and decision before acceptance. The AJCN does not accept letters that are unrelated to a specific, recently published original research article; that contain extensive unpublished data; or that engage in personal slander or invective. The AJCN does not generally print letters to the editor based on opinion pieces such as editorials, perspectives, commentaries, and the like, since these are clearly the opinions of the authors. Letters should be submitted through the Editorial Manager submission site. Letters must be provided as a Word document. They must be double-spaced, include line numbers, should include a title page, should have no more than 10 references, and should not exceed 1000 words. All letters to the Editor and book reviews must include a conflict of interest statement.

Great Debates in Nutrition

Great Debates in Nutrition (GDN) are an invited article series meant to provide a venue for evidence-based and collegial discourse on hot topics in nutrition. The intended audience includes the public with a general knowledge of nutrition – not exclusively experts in the field. Engaging, clear, and accessible writing factors heavily in the decision to

accept a GDM submission for publication. Review of manuscripts will focus on logical strength, clarity, and scientific accuracy. Occasionally, submissions may be rejected.

Each GDN is comprised of 3 linked articles addressing a formal resolution: the argument in favor, the argument opposed, and a conclusion, as illustrated by this example: 1) Consumption of saturated fat should be reduced as much as possible: Yes; 2) Consumption of saturated fat should be reduced as much as possible: No; and 3) Consumption of saturated fat should be reduced as much as possible: Conclusions. Each GDN includes only 2 authors, one representing each side.

Word and reference limits for the primary articles are strict.

- “Yes” article:
 - Main Argument, 2000 words, 20 references – Written by Author 1
 - Refutation, 800 words, 8 references – Response written by Author 2
 - Rebuttal, 400 words, 4 references – Response written by Author 1
- “No” article, to be drafted simultaneously with the “Yes” article, follows a similar format, with the authors’ roles reversed.
- “Conclusions” article – written collaboratively by both authors after completing the two primary articles. The Editor plays an active role guiding manuscript development and mediating disagreements.
 - Summary (narrative), addresses key issues, challenges and opportunities – with a focus on scientific advancement and clinical implications, 750 words
 - Points of Consensus, Table 1
 - Remaining Controversies, Table 2
 - Research Agenda, Table 3

Consistent with the aims of a formal academic debate, participants are instructed to argue for or against the proposition. Views expressed do not necessarily reflect personal opinion. Suggestions for debate topics should be sent to ajcnsubmit@nutrition.org.

Research Categories

The journal considers Original Research Communications that fall under the following research categories:

- Aging
- Alcohol
- Bone metabolism
- Cancer
- Carbohydrate metabolism and diabetes
- Cardiovascular disease risk
- Dietary supplements
- Digestive and liver diseases
- Education
- Energy and protein metabolism
- Food systems and the environment
- Gene-nutrient interactions
- Global nutrition
- Growth, development, and pediatrics
- Infectious Diseases and Immunity
- Lipids
- Nutrigenomics and Precision Nutrition
- Nutritional epidemiology and public health
- Nutritional status, dietary intake, and body composition
- Nutritional support
- Obesity and eating disorders
- Renal disease
- Vitamins, minerals, and phytochemicals
- Women's Nutrition

Criteria for Manuscript Acceptance

The AJCN can publish only about 20% of the more than 1800 original submissions received per year. Submitted manuscripts may be rejected without detailed comments after initial review by at least two AJCN editors if the manuscripts are considered inappropriate or of insufficient scientific priority for publication in the AJCN. All other manuscripts undergo a complete review by at least two consulting editors or other selected experts. Criteria for acceptance by the AJCN include originality, validity of data, clarity of writing, strength of the conclusions, and potential importance of the work to the field of clinical nutrition. Indicate explicitly in your cover letter what is truly new in the present work compared to work already published in the field. Because Cochrane assessments are now readily available on the web via PubMed, AJCN no longer considers it necessary to disseminate them by secondary publication in the Journal. Submitted manuscripts will not be reviewed if they do not conform to standard English usage and to the “Uniform Requirements for Manuscripts Submitted to Biomedical Journals” (Internet: [ICMJE](http://www.icmje.org)), which is also available free of charge from the Secretariat Office, *Annals of Internal Medicine*, American College of Physicians, Independence Mall West, Sixth Street at Race, Philadelphia, PA 19106-1572. As recommended in the Committee on Publication Ethics Code of Conduct for Journal Publishers, and supported by the International

Committee of Medical Journal Editors, when ASN is made aware of cases of suspected research and publication misconduct, ASN holds the right to publish an Expression of Concern during an investigation, and, depending on the outcome of the investigation, to retract articles.

ASN Preprint Policy

Authors retain the right to make an Author's Original Version (preprint) available through various channels. The ASN journals will allow the submission of manuscripts that have been previously posted on preprint servers as long as the site does not require authors to transfer copyright to the preprint server, or sign a closed license. Authors should read the copyright policies of the preprint server prior to depositing. Manuscripts that have been submitted to a pre-print server and submitted to the journal will undergo the same rigorous peer review as manuscripts that were not posted on preprint servers. In addition to meeting the standard requirements for submitted manuscripts (e.g., that the manuscript not be under editorial consideration for publication elsewhere), preprint submissions must satisfy the following conditions:

1. Upon first submission to an ASN journal, the author must inform the journal that the manuscript has been posted to a preprint server by providing a direct link to the preprint.
2. No additional versions of the manuscript may be posted to preprint servers at any time after initial submission to an ASN journal.
3. The copyright terms of the preprint must not be changed after submission of the manuscript to an ASN journal.
4. A preprint DOI must be assigned to the preprint.
5. Once the article has been published in its final form on the journal website, the preprint server on which the preprint is hosted must link to the article on the journal's website (the link should appear as follows: This article has been accepted for publication in [Journal Title] Published by Oxford University Press.) View the Author Self-Archiving policy page for more information.
6. Upon publication in an OUP title, the authors will sign an exclusive license to publish with the society but retain copyright.

Summary of Requirements

ASN journal editors know first-hand the amount of time and effort it takes to generate the highest quality research. That's why we've introduced a streamlined first submission policy. All first submissions are now hassle-free, with limited requirements for formatting. ASN Journal Editors also have worked to create a single ASN Journal style so that authors can seamlessly transfer submissions between ASN journals without the need for formatting edits. Adhering to ASN guidelines for formatting will only be expected for revised submitted manuscripts. We hope that this new approach will reduce the workload for authors that it takes to format a first submission, because we appreciate that the time to publication can be a critical factor in disseminating your important work.

Each manuscript component should begin on a new page in the following sequence:

- Title page
- Abstract
- Text
- Acknowledgments
- References
- Tables: each table on a separate page, complete with title and footnotes
- Legends for figures
- Figures

Identify on the title page the author who will be responsible for correspondence regarding the manuscript. Copies of any documents granting permission needed to reproduce material in print and electronic form or to use illustrations of identifiable subjects should be scanned and uploaded to the [submission system](#) or e-mailed to ajcnsubmit@nutrition.org. As recommended by the Council of Science Editors, when a coauthor dies or is incapacitated during the writing, submission, or peer review process, coauthors should obtain disclosure and copyright documentation from a familial or legal proxy. Authors should keep copies of all submitted material. The *AJCN* encourages authors to provide the names, fields of interest, addresses, telephone and fax numbers, and e-mail addresses of 4–6 unbiased and qualified potential expert reviewers from outside the authors' institutions. All material to be considered for publication in a regular or supplement issue should be submitted electronically on [this website](#). See "Tips for Authors" for helpful advice regarding electronic submission.

A health research reporting checklist must be included with the submission of every research manuscript. Papers will be screened for similarity to previously published papers using [iThenticate](#). The Editor reserves the right to request a copy of the Institutional Review Board approval documents, data, or statistical analyses files upon which the paper is based.

To prevent conflicts of interest from arising during the peer review process, the *AJCN* requires individuals who are asked to review a manuscript to decline the solicitation if they have a possible conflict of interest. [Detailed guidelines.](#)

Research Registration

Clinical Trial Registration

For all research, including research that does not require registration per the below rules, the authors must state explicitly in the Methods section the predeclared primary and secondary endpoints of their research and whether these changed during the course of the research or during post-hoc analyses. In addition, the paper must state explicitly that analyses not prespecified are considered exploratory.

The *AJCN* follows the protocol outlined by NIH for clinical trial registration (see [this page](#) for details) and identification of outcome variables for nutrition research:

1. Did the research involve human participants?

2. Were the participants prospectively assigned to an intervention?
3. Was the research designed to evaluate the effect of the intervention on the participants?
4. Was the effect being evaluated a health-related biomedical or behavioral outcome?

If you respond “yes” to each of these questions, you must register your research at [ClinicalTrials](#) or another ICMJE trial registry ([Clinical Trials](#), [ANZCTR](#), [ISRCTN](#), [UMIN](#), [TrialRegister](#)). In addition to the above registries, the ICMJE will also accept registration in any of the primary registries that participate in the [WHO International Clinical Trials Portal](#). Please note that registration of trials in alternative registries is not acceptable. In addition, trial registration with missing or uninformative fields for the minimum data elements is inadequate even if the registration is in an acceptable registry.

If you respond “no” to one or more of these questions, you do not need to register your research.

Note that after 1 July 2018, AJCN will no longer allow retrospective registration. All studies that fall under the NIH registration rules and that recruited their first participant after 1 July 2018 must be registered prospectively.

Systematic review and meta-analysis registration

All meta-analyses and systematic reviews must be prospectively registered with [PROSPERO](#) if the analysis begins after 1 July 2018.

Required Checklists

Depending on the design of the study, one of the health research reporting checklists referenced at the [Equator Network](#) must accompany the first version of each manuscript as a “supplemental file” in the online manuscript submission system. Page or line numbers must be included to indicate where the checklist items are located in your paper. Participant flow charts should be included whenever possible, especially with manuscripts that require the CONSORT, [STROBE-NUT](#), PRISMA, or ARRIVE checklists. Include the participant flow chart either as part of the full paper or as part of the Online Supporting Material. If your study did not include participants, please use the flowchart to demonstrate your selection process for the data included in your analysis.

If none of the checklists apply to your study, please explain in your cover letter why none is needed.

Please visit [GoodReports](#) to review additional checklist options.

Data Sharing

1. The corresponding author needs to confirm in the cover letter at time of submission that they will make the data (in de-identified form, if human data) used in the manuscript, code book, and analytic code available to editors upon request either before or after publication for checking. This is consistent with the ICMJE statement that ICMJE “The corresponding author should ...cooperate with any requests from the journal for data or additional information should questions about the paper arise after publication.”¹
2. The AJCN encourages authors to share raw data.
3. Authors need to state on the title page of the manuscript one of the following:
 - a. Data described in the manuscript, code book, and analytic code will be made publically and freely available without restriction at [URL link].
 - b. Data described in the manuscript, code book, and analytic code will be made available upon request pending [e.g., application and approval, payment, other].
 - c. Data described in the manuscript, code book, and analytic code will not be made available because [specify reason].
4. Consistent with the ICMJE statement, “Clinical trials that begin enrolling participants on or after 1 January 2019 must include a data sharing plan in the trial’s registration.”¹

Format and Style Requirements

Articles are copyedited according to AJCN style policy, the “Uniform Requirements for Manuscripts Submitted to Biomedical Journals,” and the style manual of the Council of Science Editors (Scientific style and format: the CSE manual for authors, editors, and publishers. 8th ed. Chicago: The University of Chicago Press; 2014).

Authorship

Scientific conduct

Each author must have participated sufficiently, intellectually, or practically in the work to take public responsibility for the content of the article, including the conception, design, and conduct of the experiment, and for the data interpretation. An article with corporate (collective) authorship must specify the key persons responsible for the article; others contributing to the work should be recognized separately. The Editors may require authors to justify the assignment of authorship.

Conflict of interest

Authors must disclose on the title page or in the Acknowledgment section any possible conflicts of interest. [Detailed guidelines](#). Authors who wish to make use of the [ICMJE Form for Disclosure of Potential Conflicts of Interest](#) may copy the Disclosure Statement from that form and paste it in to the Acknowledgments section of the manuscript file.

Instructions for manuscript preparation

Initial manuscript submissions

Prepare your manuscript, including figure legends and tables, in Word format. The manuscript should be formatted as follows: 216 x 279 mm (8½ x 11 in) or ISO A4 (212 x 297 mm), with margins of at least 2.5 cm; use double-spacing and 12-point type throughout. Do not justify the right margin. The abstract and text pages should have consecutive line numbers in the left margin beginning in the abstract and ending before the reference section. Number pages consecutively in the upper right-hand corner of each page, beginning with the title page. Foreign authors are advised to have their manuscripts reviewed by a scientific colleague who is fluent in English so that the manuscripts will conform to US English usage and grammar.

Revised manuscript submissions

Submit manuscript text, including figure legends and tables, in a Word file; tables must be included in the text file; do not submit tables in separate files. Submit each figure in a separate file according to the specifications listed in the

section titled, Figures. Changes in the manuscript text must be marked track changes. Do not highlight or use a colored font. This requirement does not apply to changes made to figures or supplemental material.

Fonts

For optimum legibility we recommend that you use only certain fonts in your document: Times, Times New Roman, Courier, Helvetica, Arial, and the Symbol font for special characters. For review purposes references will be copied from the manuscript file into the submission system and linked to the online source of the cited abstract or article.

Title page

The title page should contain: 1) Title of the article, beginning with a key word if possible, with only the first letter of the first word capitalized; 2) Author Names (first name, middle initial, last name); 3) Author Affiliations (departmental and institutional) at the time the research was done. Indicate which authors are associated with which institutions by listing the appropriate author initials in parentheses after each affiliation listed. 4) Any authors' changed affiliations - should be included in a separate line on the title page. 5) Disclaimers, if any. Note that Conflicts of Interest should be included after the Acknowledgement section. 6) Corresponding Author name, mailing address, telephone number, and e-mail address; 7) the Sources of Support including grants, fellowships, and gifts of materials (eg, chemicals, experimental diets); 8) Short running head of not more than 50 characters (count letters and spaces); 9)

Abbreviations list and their definitions for all abbreviations used in the text if there are 3 or more; and 10) Clinical Trial Registry number and website where it was obtained and 10) Registry and registry number for systematic reviews or meta-analyses. An appropriate registry for systematic reviews and meta-analyses is [PROSPERO](#). Please note that PROSPERO does not allow for post-completion registration of studies..

Abstract A properly constructed and informative abstract is helpful for the initial editorial review of the submitted manuscript. Original research articles must include a structured abstract that contains no more than 300 words, is written in complete sentences, and includes the following headings:

- **Background:** Provide 1 or 2 sentences that explain the context of the study.
- **Objective:** State the precise objective, the specific hypothesis to be tested, or both.
- **Design:** Describe the study design, including the use of cells, animal models, or human subjects. Identify the control group. Identify specific methods and procedures. Describe interventions, if used.
- **Results:** Report the most important findings, including results of statistical analyses.
- **Conclusions:** Summarize in 1 or 2 sentences the primary outcomes of the study, including their potential clinical importance, if relevant (avoid generalizations).

Review articles, special articles, and reports should include an unstructured abstract (no more than 300 words) that states the purpose of the article and emphasizes the major concepts and conclusions. Any abbreviations used in the abstract should be defined in the abstract at first mention.

Below the abstract, provide and identify 5–10 keywords or short phrases, including the subject group, that will help to increase the discoverability of your manuscript; do not use adjectives. Terms that are fundamental to your manuscript but are not included in your manuscript title or abstract are especially important to include to increase discoverability by indexing services such as PubMed.

Please note that during manuscript submission, you will be asked to supply keywords to assist the editors in locating suitable reviewers for your manuscript. Keywords for reviewer searches should include the terms most fundamental to your manuscript, and may differ from your list of keywords for publication.

Text

Use active voice whenever possible. Use past tense when describing and discussing the experimental work on which the article is based. Reserve present tense for reference to existing knowledge or prevailing concepts and for stating conclusions from the experimental work. Clearly differentiate previous knowledge and new contributions. Do not use level when referring to a concentration. Use metric units of measure; SI units are no longer required.

Use "sex" when referring to biological factors and "gender" when referring to gender identity, or psychosocial or cultural factors. The terms "males" and "females" are preferable to "men" and "women".

Race and ethnicity are important concepts in the nutrition literature, but race is essentially a social and not biologic concept, and race and ethnicity should not be conflated. Attempts to describe the racial background of the population studied, or conclusions drawn, should be made accordingly, and should include other social factors that might explain a paper's findings. "Ethnicity" should describe traditions, lifestyle, language, diet, and values, and "ancestry" should describe ancestry informative markers (AIM) based on genetic or genomic data. When race, ethnicity and/or ancestry are described, authors should specify what methods were used for these classifications. For a more detailed description of this topic please see: <https://doi.org/10.1093/ajcn/nqaa341>.

AJCN strongly recommends the use of "people-first language". This includes describing individuals as people with a medical condition rather than as diseases or disabilities. Terms such as "adults with obesity" and "children with diabetes" are preferred over "obese adults" and "diabetic children". For more information consult "[Use of people-first language with regard to obesity](#)" Am J Clin Nutr 2018;108:201 or "[The Effect of Words on Health and Diabetes](#)" Diabetes Spectrum 2017;30:11-16.

The text of observational and experimental articles should be divided into sections with the following headings: Introduction, Subjects (or Materials, for cell or animal studies) and Methods, Results, and Discussion. Long articles may require subheadings within some sections. Authors should consult recent issues of the *AJCN* for guidance on the formatting of other types of articles, book reviews, and editorials.

Introduction

Clearly state the purpose of the article. Summarize the rationale and background for the study or observation, giving only strictly pertinent references. Do not include methods, data, results, or conclusions from the work being reported. The introduction should be limited to 1.5 manuscript pages.

Subjects (or Materials) and Methods

Describe clearly your selection of the experimental and control subjects and provide eligibility and exclusion criteria and details of randomization. Describe the methods for, and success of, any masking (blinding) of observations. Report any complications of experimental treatments. Identify the methods, apparatus (manufacturer's name in parentheses), and procedures in sufficient detail to allow other researchers to reproduce the results. Define all group designations parenthetically at first mention [for example, "control (CON) and high-fat (HF) groups"] and include definitions for these abbreviations in the abbreviation footnote on the title page. Do not use trademark names, such as Teflon, as generic terms. Give references for established methods, including statistical methods; provide references and brief descriptions of methods that have been published but are not well known; and describe new or substantially modified methods, giving reasons for using them and evaluating their limitations. Identify precisely all drugs and chemicals used, including generic names, dosages, and routes of administration. If trade names for drugs and chemicals are included, give the manufacturer's name and location.

Ethics. When reporting experiments on human subjects, indicate that the procedures followed were in accordance with the ethical standards of the responsible institutional or regional committee on human experimentation or in accordance with the Helsinki Declaration of 1975 as revised in 1983. Do not use patients' names, initials, or hospital identification numbers. When reporting experiments on animals, indicate approval by the institution's animal welfare committee and state whether the National Research Council's guide for the care and use of laboratory animals was followed.

Statistics. Describe statistical methods with enough detail to enable a knowledgeable reader with access to the original data to verify the reported results. When possible, quantify findings and present them with appropriate indicators of measurement error or uncertainty (eg, CIs, SDs, or SEs), even for differences that were not significant. Report the numbers of observations. Specify any general-use computer programs used, including the version number and the manufacturer's name and location. Include general descriptions of statistical methods in the Subjects (or Materials) and Methods section and specific descriptions in each table and figure legend. Indicate whether variables were transformed for analysis. Provide details about what hypotheses were tested, what statistical tests were used, and what the outcome and explanatory variables were (where appropriate). Indicate the level of significance used in tests if different from the conventional 2-sided 5% alpha error and whether or what type of adjustment is made for multiple comparisons.

The AJCN prefers replicate analyses. The Journal does not have a specific policy on replicate sample analysis, but we will assess these on a case by case basis. Reviewers and Editors might disagree with the authors' approach, and we cannot guarantee the outcome. The Journal recommends strongly that at least a subset of samples be analyzed at least in duplicate in order that the degree of measurement error can be estimated. Furthermore, we refer the author to established approaches for dealing with such circumstances: Allison, D. B., Allison, R. L., Faith, M. S., Paultre, F., & Pi-Sunyer, F. X. (1997). Power and money: Designing statistically powerful studies while minimizing financial costs. *Psychological Methods*, 2(1), 20-33.

When data are summarized in the Results section, specify the statistical methods used to analyze them. Avoid nontechnical uses of technical statistical terms, such as random (which implies a randomizing device), *normal*, *significant*, *correlation*, *sample*, and *parameter*. Define statistical terms, abbreviations, and symbols not listed under "Abbreviations for statistical terms" below. If there are 3 or more abbreviations used in the text, prepare an abbreviation footnote. The footnote should be associated with the first abbreviated term in the text and should be an alphabetized listing of all author-defined abbreviations and their definitions. Detailed statistical analyses, mathematical derivations, and the like may sometimes be suitably presented as one or more supplemental files.

Results

Present your results in a logical sequence in the text, tables, and figures. Do not present specifics of data more than once and do not duplicate data from tables or figures in the text; emphasize or summarize only important observations. Do not present data from individual subjects except for very compelling reasons. Report losses to observation (such as dropouts from a clinical trial). Use boldface for the first mention of each table or figure.

Discussion

The Discussion should not exceed 4 typewritten pages except in unusual circumstances as approved by the Editor. Emphasize concisely the important aspects of the study and the conclusions that follow from them. Do not repeat in detail data or other material given in the Introduction or Results. Include the implications of the findings and their limitations and relate the observations to other relevant studies. Link conclusions with the goals of the study and avoid unqualified statements and conclusions that are not completely supported by the data. Avoid claiming priority and alluding to work that has not been completed. State new hypotheses and recommendations when warranted by the results and label them clearly as such.

Acknowledgments

1) Acknowledge only persons who have made substantive contributions to the study. Authors are responsible for obtaining written permission from everyone acknowledged by name and for providing to the Editor a copy of the permission, if requested. 2) If not present on title page, Conflict of Interest (COI) Statement: Authors must disclose any financial or personal relationships with the company or organization sponsoring the research at the time the research was done. Such relationships may include employment, sharing in a patent, serving on an advisory board or speakers' panel, or owning shares in the company. If an author or authors have no potential conflicts of interest, please state this. The COI Statement must include all authors. 3) Authors' contributions to the manuscript – Each author is required to list his or her contribution to the work, with a description of the contribution.

Please use the following descriptors: 1) designed research (project conception, development of overall research plan, and study oversight); 2) conducted research (hands-on conduct of the experiments and data collection); 3) provided essential reagents or provided essential materials (applies to authors who contributed by providing animals, constructs, databases, etc, necessary for the research); 4) analyzed data or performed statistical analysis; 5) wrote paper (only authors who made a major contribution); 6) had primary responsibility for final content; 7) other (use only

if categories above are not applicable; describe briefly); 8) for single-authored papers, please state: The sole author had responsibility for all parts of the manuscript.

Please do not include “obtained funding” (the initials of authors who received grants may be included in the footnote regarding support on the manuscript’s title page). Although not all manuscripts will necessarily include all descriptors, all manuscripts, including reviews, must indicate who is responsible for design, writing, and final content. An example of a properly formatted author contribution statement is as follows: “AX, RFG, and PGY designed research; RFG and QC conducted research; PT analyzed data; AX, PGY, and QC wrote the paper; PGY had primary responsibility for final content. All authors read and approved the final manuscript.”

References

Number references consecutively in the order in which they are first mentioned in the text. For a standard journal article with more than 10 authors, please list first 10 authors before using “et al.”; list all authors when 10 or fewer. In the text, identify references by Arabic numerals in parentheses (1), not superscript. References cited in tables or in legends to figures should be numbered according to the first citation of the table or figure in the text. Supplemental Material should have a separate reference section.

It is rarely necessary to cite more than 50 references in an original research article. Try to avoid citing published abstracts as references [if a published abstract is cited, include “(abstr)” at the end of the reference]. Abstracts from scientific meetings not published in peer-reviewed journals may not be used as references. Unpublished observations and personal communications (written, not oral) may not be used as references but may be inserted in parentheses with the names of the responsible researchers and the year of the observation or communication. Authors are responsible for obtaining written permission from everyone so cited and for providing to the Editor a copy of the permission, if requested. Doctoral dissertations may be used as references. Include manuscripts accepted but not yet published; designate journal name followed by “(in press).” Report foreign titles in the original language, identify the language, and provide the English translation in parentheses. The references must be verified by the author against the original documents.

Journals

1. Journal article published electronically ahead of print: Authors may add to a reference, the DOI (“digital object identifier” number unique to the publication) for articles in press. It should be included immediately after the citation in the References.
Bergholdt HKM, Nordestgaard BG, Ellervik C. Milk intake is not associated with low risk of diabetes or overweight-obesity: a Mendelian randomization study in 97,811 Danish individuals. *Am J Clin Nutr* 2015 Jul 8 (Epub ahead of print; DOI: [doi:10.3945/ajcn.114.105049](https://doi.org/10.3945/ajcn.114.105049)).
2. Standard journal article: list all authors when 10 or fewer; when >10, list only the first 10 and add “et al.”
Abbreviate journal titles according to *Index Medicus* style, which is used in MEDLINE citations. Jeffery RW, Wing RR, Sherwood NE, Tate DF. Physical activity and weight loss: does prescribing higher physical activity goals improve outcome? *Am J Clin Nutr* 2003;78:684–9.
3. Corporate author
National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation* 2002;106:3143–421.

Books and other monographs

4. Personal authors
Shils M, Shike M, Olson J, Ross AC. *Modern nutrition in health and disease*. 9th ed. Baltimore: Lippincott Williams & Wilkins, 1998.
5. Committee report or corporate author
National Research Council. *Recommended dietary allowances*. 10th ed. Washington, DC: National Academy Press, 1989.
Food and Nutrition Board, Institute of Medicine. *Dietary reference intakes for vitamin C, vitamin E, selenium and carotenoids*. Washington, DC: National Academy Press, 2000.
6. Chapter in book
Young VR, Tharakan JF. Nutritional essentiality of amino acids and amino acid requirements in healthy adults. 2nd. ed. In: Cynober LA, ed. *Metabolic and therapeutic aspects of amino acids in clinical nutrition*. Boca Raton, FL: CRC Press, 2004:439–70.
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US Department of Agriculture, US Department of Health and Human Services. *Nutrition and your health: dietary guidelines for Americans*. Washington, DC: US Government Printing Office, 2000. [USDA Home and Garden Bulletin no. 232.]

Internet references

8. Website
National Center for Health Statistics. *National Health and Nutrition Examination Survey*. Version current 1 October 2003. Internet: <http://www.cdc.gov/nchs/nhanes.htm> (accessed 13 October 2003).
9. Online journal article
Sinha A, Madden J, Ross-Degnan D, Soumerai S, Platt R. Reduced risk of neonatal respiratory infections among breastfed girls but not boys. *Pediatrics* [serial online] 2003;112:e303. Internet: <http://pediatrics.aappublications.org/cgi/content/full/112/4/e303> (accessed 14 October 2003).

Tables

Tables must be included in the text file, and each table should begin on a new page. Please do not upload tables as separate files. Double-spacing of tables is preferred but not required. Number tables consecutively with Arabic

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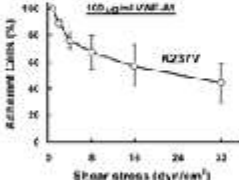
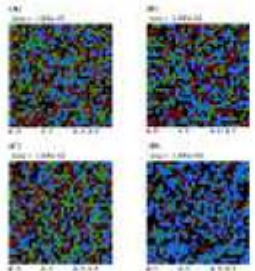
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Figure 1

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Abbreviations for statistical terms

- analysis of variance, ANOVA
- analysis of covariance, ANCOVA
- coefficient of correlation, sample, *r*
- coefficient of multiple correlation, *R*
- coefficient of variation, CV
- confidence interval, CI
- degrees of freedom, df
- hazard ratio, HR
- interquartile range, IQR
- not significant, NS
- number of observations, *n*
- odds ratio, OR
- probability, *P*
- risk ratio, RR
- standard deviation, SD

- standard error of the estimate, SEE
- standard error of the mean, SEM
- variance ratio, *F*

Metric units are required and the use of the International System of Units (SI units) is optional. For a comprehensive listing of SI conversion factors, consult *SI Units for Clinical Measurement* (Young DS, Huth EJ. Philadelphia: American College of Physicians, 1998), Am J Clin Nutr 1998;67:166–81 or J Nutr 1990;120:20-35. Dosage forms and dietary ingredients may be expressed in gram or mole quantities. Energy may be expressed in kilocalories or joules; the conversion factor for converting kilocalories to kilojoules is 4.184. Do not report energy in Calories with a capital C; use kcal, MJ, or kJ instead. Temperatures should be reported in degrees Celsius. Blood pressures should be reported in millimeters of mercury. Use of katal to report enzyme activity is optional.

Commonly used approved abbreviations

Standard units of measurement

- ampere, A
- becquerel, Bq
- coulomb, C
- curie, Ci
- day, d
- degree Celsius, °C
- farad, F
- gram, g
- hertz, Hz
- hour, h
- joule, J
- katal, kat
- kelvin, K
- kilocalorie, kcal
- liter, L
- meter, m
- minute, min
- mole, mol
- month, mo
- ohm, Ω
- pascal, Pa
- second, s
- sievert, Sv
- volt, V
- watt, W
- week, wk
- year, y

Acceptable standard units

- *length*: m, mm, μm
- *area*: m², mm², μm²
- *volume*: L, mL, μL, pL
- *mass*: kg, g, mg, μg, ng, pg
- *mass concentration*: kg/L, g/L, mg/L, μg/L
- *substance concentration*: mol/L, mmol/L, μmol/L, nmol/L

Unacceptable units

- *length*: not acceptable: in, ft, yd, Å, mμ
- *area*: not acceptable: sq in, in², μ²
- *volume*: not acceptable: pint, gallon, cc, ccm, λ, μμL
- *mass*: not acceptable: gr, gm, gms, mgm, mgms, mgs
- *mass concentration*: not acceptable: mg %
- *substance concentration*: not acceptable: M, N

Combining prefixes

- tera- (10¹²), T
- giga- (10⁹), G
- mega- (10⁶), M
- kilo- (10³), k
- milli- (10⁻³), m
- micro- (10⁻⁶), μ

- nano- (10^{-9}), n
- pico- (10^{-12}), p
- femto- (10^{-15}), f
- atto- (10^{-18}), a

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As recommended by the American Society for Microbiology, the spelling of bacterial names should follow the *Approved Lists of Bacterial Names (Amended) & Index of the Bacterial and Yeast Nomenclatural Changes* (V. B. D. Skerman et al. ed., ASM Press, Washington, DC, 1989) and the validation lists and notification lists published in the *International Journal of Systematic and Evolutionary Microbiology* (formerly the *International Journal of Systematic Bacteriology*). Further information on currently approved bacterial names can be found at: [Bacterial Nomenclature Up-to-Date](#) and at [List of Prokaryotic Names with Standing in Nomenclature](#). If authors must use a name that does not have standing in nomenclature, the name should be enclosed in quotation marks in the title, when appropriate, and at its first use in the abstract and the text. Correspondingly, an appropriate statement concerning the nomenclatural status of the name should be made in the text.

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- Number references consecutively in the order in which they are first mentioned in the text, and list 10 authors' names before using "et al."

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