VALIDATION OF BIOIMPEDANCE AGAINST ISOTOPE METHODS FOR DETERMINATION OF BODY COMPOSITION IN HIV-INFECTED SOUTH AFRICAN CHILDREN

Submitted to:

NELSON R MANDELA SCHOOL OF MEDICINE

UNIVERSITY OF KWAZULU-NATAL DURBAN

SOUTH AFRICA

Submitted in partial fulfilment of the academic requirements for the degree:

Master in Medical Science

(Paediatrics and Child Health)

by

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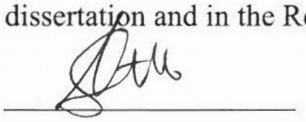

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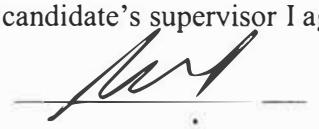
As the candidate's supervisor I agree to the submission of this dissertation



29-03-2011

Dr M.K Chaggan ***Date***

(Supervisor)

To my darling parents

...love you much

ACKNOWLEDGMENTS

Firstly, I would like to thank the children and their parents who availed their time and effort to participate in this study. I acknowledge King Edward VIII Hospital and Clairwood Hospital, their respective medical management and staff for approval and assistance.

I would like to express my gratitude to my supervisor Dr M.K Chhagan whose talks, patience, understanding, guidance, teachings and being such an inspiration added to my graduate research experience. You have transformed me as a young scientist by making me see beyond the lab bench and having a supervisor like you is one in a million...

To my parents, I thank you for the love and support always I am who I am today because of both of you

To the one whose presence I always feel around me, and who has guided me in my life ...Jay Durge Mata...

In summary the following persons have assisted me in this study.

Action/Procedures used in Study	Colleagues
Intellectual design of study, statistical assistance, assistance with ethics submission, procurement of funding	Dr M.K Chhagan
Translation during interviews	Portia Nhlangulela, Slindile Phakathi
Patient recruitment	Alesha Sewnath
Collection of data, performance of anthropometry and BIA, immediate processing, storage, shippment of samples	Alesha Sewnath
Clinical assessment of co-morbidities	Attending doctors
Phlebotomy	Dr Ramjee, Dr Pillay, Dr Khan, Sister Dlamini, Dr Bisetty, Alesha Sewnath
Database design, entry of patient forms	Alesha Sewnath
Laboratory analysis (TBW/ECW)	Alesha Sewnath/ Mr Wen wen Yu
Collection of laboratory results for analysis	Alesha Sewnath
Literature searches, writing up of thesis	Alesha Sewnath
Editing of thesis	Dr M.K Chhagan
Further teaching in Body Cmposition	Dr Stephen Arpadi and staff at the Body Composition Laboratory in St Lukes Roosevelt Hospital, New York
Sources of Support	International Atomic Energy Agency (Grant number 13464) Postgraduate award given by the Medical Research Council of South Africa.

ABBREVIATIONS

A	-	Cross sectional area of the cyclinder
ADP	-	Air Displacement Plethysmography
AIDS	-	Acquired Immunodeficiency Syndrome
ARV	-	Antiretroviral
BIA	-	Bioelectrical impedance analysis
BM	-	Body Mass
BMI	-	Body mass index
Br	-	Bromide
CT	-	Computer Tomography
D_2O	-	Deuterium oxide
DEXA	-	Dual X-ray Absorptiometry
ECW	-	Extracellular water
FFM	-	Fat Free Mass
FM	-	Fat Mass
FTIR	-	Fourier Transform Infrared Spectrometry
HIV	-	Human Immunodeficiency Virus
HPLC	-	High performance liquid chromatography
Ht^2	-	$Height^2$
I	-	Current
ICW	-	Intracellular water
L	-	Cylinder length

LBM	-	Lean Body Mass
MDG	-	Millennium Development Goal
MRI	-	Magnetic Resonance Imaging
MUAC	-	Mid Upper Arm Circumference
p	-	Resistivity
PEM	-	Protein energy malnutrition
R	-	Resistance
SAM	-	Severe acute malnutrition
SD	-	Standard Deviation
TBW	-	Total body water
V	-	Volume of water
X	-	Reactance
Z	-	Impedance

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ABSTRACT

Bioelectrical impedance analysis (BIA) is a simple tool for assessing body composition. Equations used in BIA are developed and validated against a reference method involving isotope dilution. **Aim:** To validate bioimpedance (BIA) against isotope methods for determination of body composition in HIV-infected South African children. **Objectives**: 1) To establish whether BIA equations currently used for deriving total body water (TBW) and Fat Free Mass (FFM) in Nigerian and HIV-infected American children are precise to be applied to young HIV-infected children in South Africa . 2) To determine feasibility of blood and saliva specimens in isotope studies. 3) To compare performance of blood and saliva using the isotope dilution method in children aged 3 to 6 years. **Methods:** Anthropometry and Bioelectric impedance measurements were performed on 42 children. Bioelectric impedance measurements were done using a 50-kHz tetrapolar BIA device (model 101 A; RJL, Detroit). TBW was measured by deuterium oxide (D_2O) dilution. TBW enrichment in blood and saliva was assessed using a Fourier–Transfer Infrared Spectrometer. Extracellular water was measured by sodium bromide dilution and was assessed using the Perkin–Elmer HPLC system. **Results:** There was good correlation between measured and predicted values for TBW and FFM, and between saliva and blood assays of TBW. Despite good correlations, Bland-Altman plots showed poor agreement between measured and predicted values of TBW. Bland-Altman plots also showed saliva overestimated TBW. **Discussion:** Children with poor agreement between methods appeared to have co-morbidities more frequently than children with good agreement between methods. Co-morbidities included oral problems coupled with opportunistic diseases which may have caused measurement variation in salivary samples. Altered mucosal permeability may have affected our results by altering absorption and/or equilibration time of the isotope. Addition of variables such as reactance, resistance, Z-score height and height^2 (Ht^2) improved TBW prediction in our study cohort.

CHAPTER ONE

INTRODUCTION

Since the first discovery of Acquired Immunodeficiency Syndrome (AIDS) in 1981, millions worldwide have been affected by this disease. Sub-Saharan Africa lies amongst the most severely affected regions with more than 65% of people infected with the Human Immunodeficiency Virus (HIV) residing here. In South Africa, the HIV prevalence rate in the 0-5 year age group increased from 2.2% in 2000 to 3.6% in 2006. Common manifestations such as wasting and malnutrition seen in diseases such as HIV/AIDS and cancer cause alterations in Fat Mass (FM), Fat Free Mass (FFM) and Extracellular Water (ECW) (Forbes, 1990). Monitoring of body composition during disease provides an effective tool for medical evaluation and a predicator of disease progression (Wells and Fewtrell, 2006). Techniques such as anthropometry and other sophisticated methods of assessment have been used in body composition studies involving children.

FFM and FM changes can be determined by bioelectrical impedance analysis (BIA). This body composition technique determines body fluid volume by measuring resistance to a frequency at 50 kHz at a low amplitude alternating electric current of 800μA. The resistance is inversely proportional to the liquid volume in the body (Kyle *et al.*, 2004). Statistical methods such as regression analysis are used to formulate body composition predication equations (Guo *et al.*, 1996). Reference measurements such as isotope dilution and variables such weight, height etc, are utilized for the development of predication equations.

Horlick *et al* (2002) developed prediction equations for total body water (TBW) and FFM in healthy HIV-infected children between the ages of 4 and 18 years. Variables such as age, race, sex and HIV-infected / uninfected were included to improve predictive accuracy of the equations. Arpadi *et al* (1996) tested existing BIA equations in HIV-infected Hispanic children aged between 4 and 11 years old. Two prediction equations were developed from this study.

Measurement of TBW allows determination of FFM. This is based on the principle that FM is anhydrous and FFM contains a constant proportion of water. Isotope dilution provides measurement of TBW in the body. The volume of TBW can be defined as the ratio of a tracer dose to the concentration of TBW within a time interval. Various tracers have been developed for use in isotope dilution studies such as oxygen-18, tritium and deuterium. However, deuterium is frequently used. Collection of body fluid samples such as blood plasma, saliva and urine allow determination of TBW (Ellis, 2000).

STUDY AIM

- To validate bioimpedance (BIA) against isotope methods for determination of body composition in HIV-infected South African children.

STUDY OBJECTIVES

- To determine whether equations currently used for determination of TBW and FFM in Nigerian and HIV-infected American children from bioimpedance assessment are precise enough to be applied for use on young HIV-infected children in South Africa using isotope dilution as the reference method.

- To determine the feasibility of blood and saliva specimens for body composition analysis.

- To compare the performance of blood and saliva using the isotope dilution method in children aged 3 to 6 years.

CHAPTER TWO

LITERATURE REVIEW

2.1 INFECTION AND NUTRITION

Nutritional status can be viewed as an interplay between body composition, body energy balance and body functioning (Bedogni *et al.*, 2006). Without proper nutritional intake, the immune system lacks essential components that are needed to generate an effective immune response, causing a constriction like effect on immune functions, body composition and body functioning (Mendez and Adair, 1999). Linings of the nasoesphageal, gastrointestinal and the genitourinary system behave as barriers against infection. Response to infection requires rapid increases in cell differentiation, replication, peptide production and lipid mediators (Scrimshaw and Sangiovanni, 1997).

A synergistic relationship is observed between nutrition and infection, malnutrition makes the body more susceptible to infection. Hence, infection reduces nutrient intake, thus leading to malnutrition (Scrimshaw and Sangiovanni, 1997).

In HIV-infected children we would therefore anticipate both nutritional and infection-mediated influences on metabolism and body composition.

2.2 BODY COMPOSITION

2.2.1 Definition

The human body consists of five essential levels, atomic, molecular, cellular, tissue system and whole body. The sum of these five levels is equivalent to the body mass (BM) shown in (Fig 2.1).

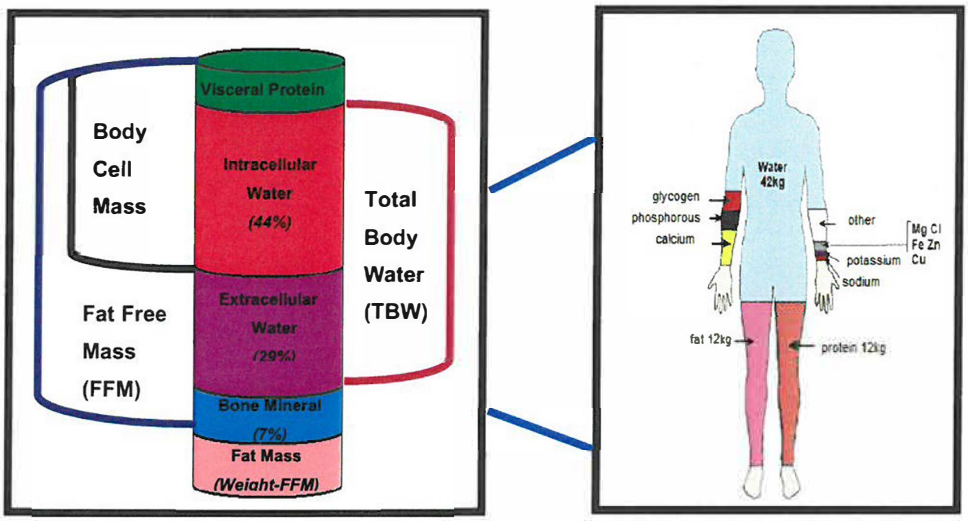

Figure 2.1: The five levels in relation to body composition. Adapted. from:

http://www.rowett.ac.uk/edu_web/sec_pup/body_comp.pdf. Accessed 19April 2009

BM is divided into two or more compartments according to various components (Heymsfield and Waki, 1991) (Wang *et al.*, 1992). The most common model used to describe body composition is the two compartment model whereby body weight is devided into two compartments, FM and FFM (Wang *et al.*, 1992). The FM contains extractable lipids from adipose tissue and other tissues. The FFM contains water, muscle, bone and internal organs. This is the metabolically active constituent of the body, linked to survival in various illnesses (Wang *et al.*, 2000). Quantifying body composition into FM and FFM is especially important during disease states. Infectious disease is associated with changes in relative proprtions of FM and FFM. A lack of nutritent intake during disease more specifically in HIV-infected people may implicate development of malnutrition which in turn increases the severity of disease.

2.3 MALNUTRITION

2.3.1 Background

Childhood malnutrition and more specifically, undernutrition, is a public health concern in low to middle income countries. Although much progress has been in this field, it is still a problem in many parts of the world. Malnutrition is defined as a sub-acute or chronic state of nutrition (Soeters *et al.*, 2008). In the context of this thesis malnutrition will be used to describe undernutrition. Malnutrition in adults and children is very prevalent in low and middle income countries thus increasing mortality and increased disease burden.

Undernutrition arising from starvation or reduced intake leads to reduced adipose tissue and lean body mass (LBM) though LBM loss is more prevalent. During periods of starvation body proteins break down to compensate for amino acid requirements, resulting in loss of LBM. Prolonged periods of malnutrition cause linear decline in child growth leading to muscle mass retardation (Blossner and de Onis, 2005). As severe loss of LBM occurs, the body's response to disease decreases (Suttman *et al.*, 1995).

There are many different classifications for malnutrition. The WHO classification uses wasting as an indicator in disease (WHO, UNICEF, 2009). This is applied in children with a weight for height z-score of -3 or less, a mid-upper arm circumference of less than 11.5 cm or having severe visible wasting or bipedal oedema (WHO, UNICEF, 2009). The Wellcome classification is used to classify marasmus, kwashiorkor, and marasmic kwashiorkor as severe malnutrition. The Waterlow classification system assesses the degree of malnutrition. This classification is based on wasting (inadequate weight for

height) and degree of stunting (inadequate height for age) in children.

Globally, undernutrition is an underlying factor for childhood deaths caused by diarrhoea (61%), malaria (57%), and measles (45%) (Black *et al.*, 2003)

(Bryce *et al.,* 2005).

Comparatively, Sub-Saharan Africa has the highest child mortality rate and largest malnutrition prevalence in the world as shown in (Fig 2.2). In South Africa malnutrition is a factor in 12.3% of deaths (Kibel *et al.*, 2010). The most significant causes of malnutrition include inadequate food intake and illness. In 2005, 9.3% of children aged 1 – 9 years were underweight, 18% were stunted and 4.5% were wasted in South Africa (Kibel *et al.*, 2010). The percentage of children in each province affected by stunting, wasting and underweight is presented in (Table 2.1).

Table 2.1: Proportion of children affected by stunting, wasting and underweight in each province - 2005 (Kibel *et al.*, 2010).

Province	Stunting %	Wasting %	Underweight %
Eastern Cape	**18.0**	**4.1**	**7.8**
Free State	**28.2**	**2.8**	**14.1**
Gauteng	**16.8**	**3.3**	**14.1**
KwaZulu Natal	**15.1**	**1.3**	**5**
Limpopo	**23.8**	**4.4**	**12.3**
Mpumalanga	**17.8**	**7.5**	**10.9**
North West	**15.1**	**3.2**	**12.4**
Northern Cape	**27.7**	**19.1**	**38.3**
Western Cape	**12**	**11.5**	**8.2**
South Africa	**18**	**4.5**	**9.3**

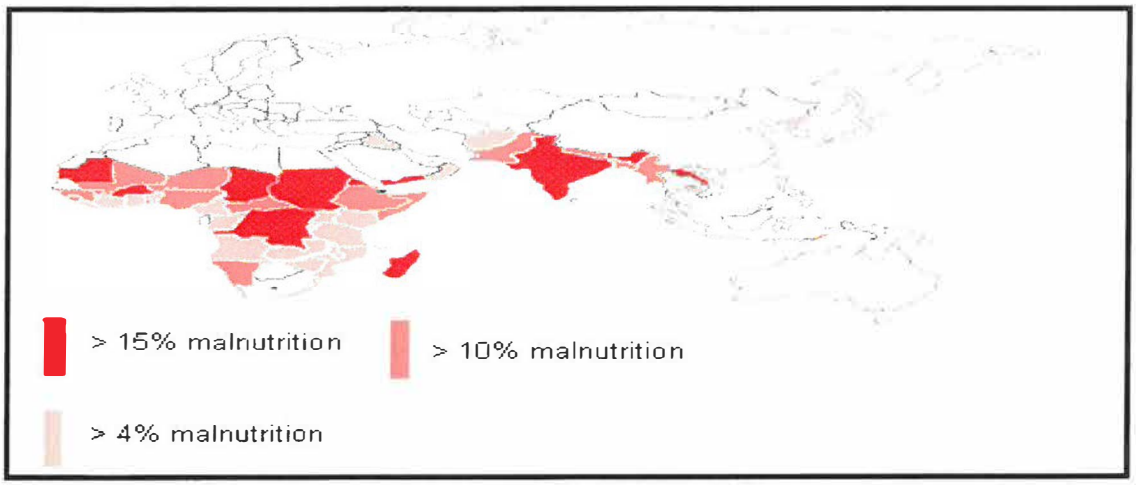

Figure 2.2: Prevalence of child malnutrition. Available. From http://emedicine.medscape.com /article /984496-overview. Accessed 24 May 2010

Malnutrition has been highlighted by the United Nation's Millennium Development Goal (MDG) initiative. The first MDG is to halve the amount of people suffering from hunger and poverty and the fourth MDG is to reduce under-five mortality by two thirds in the same time period (South African MDG Report, 2010). While poverty alleviation programs are crucial in the long term, in the short term reduction of mortality from infectious disease and undernutrition may be improved by encouraging breastfeeding, promoting complementary feeding, vitamin A, zinc, iron and iodine supplementation (Kibel *et al.*, 2010).

Malnutrition often arises as micronutrient deficiencies and protein energy malnutrition (PEM) (Emery, 2005) which are further expanded in the proceeding sections. The degree and spread of PEM and micronutrient malnutrition depend on factors such as prevalence of infectious diseases, breastfeeding, sanitation, availabilty and quality of health services (Emery, 2005). In HIV-infected persons, micronutrient deficiencies and PEM are causative factors for altered growth and development.

2.3.2 Micronutrient malnutrition

Deficiencies in iodine, vitamin A, zinc and iron are common in malnourished children. Iodine deficiency is responsible for a large number of infant mortalities, however, this is reversible with iodine treatment (Cobra *et al.*, 1997).

Findings show in-utero exposure to maternal iodine deficiency has damaging effects on the developing brain leading to permanent cognitive disability (Durkin, 2002). Vitamin A plays a role in the immune system whilst iron deficiencies have a detrimental effect in development (Bobonis *et al.*, 2006). Severe Vitamin A deficiency can result in xerophthalmia. Studies show, this is one of the leading causes of blindness in children (WHO, 2002). Apart from effects to the vision, it may also increase the risk of measles and other infections resulting in long term disability (Sommer, 1993). Low levels of Vitamin A and E are associated with lower CD4+ cell counts in HIV-infected people (Tang *et al.*, 1997).

Within the body, zinc assists in metabolic processes such as Ribonucleic acid (RNA) and Deoxyribonucleic acid (DNA) synthesis and plays a vital role in balancing T helper cell functions. Deficiency of zinc affects key functions such as gene expression, skeletal growth and appetite (Shankar and Parsad, 1998). Mild zinc deficiencies results in decreased LBM and severe zinc deficiency leads to weight loss and infections caused by cell mediated immune dysfunctions (Prasad, 2003). In a cross sectional study in Malawi, findings revealed a high HIV load in pulmonary tuberculosis was linked to micronutrient malnutrition (Van Lettow *et al.*, 2004). In HIV-infected children an altered metabolism is often linked to micronutrient deficiencies.

2.3.3 Protein Energy Malnutrition (PEM)

In children, FFM hydration ranges between 80% in newborns to 75% in 10 year old children (Hoffer, 2001). PEM occurs when protein and energy intake are insufficient (Hoffer, 2001). Loss of FFM is the main determinant of weight loss in PEM. FFM Components such as the skeletal muscles, viscera, blood cells and immune system comprises approximately 35 to 50 % of total weight in an adult. Abnormalities in the humoral, cellular subsystems, metabolic changes and lack of immune mediators such as tumor necrosis factor are common in PEM.

In children, PEM manifests between the ages of 6 months to 2 years. Severe PEM in children is clinically defined as less than 70% weight-to height and/or the appearance of pitting edema on both feet which is described as either marasmus, or kwashiorkor (Hoffer, 2001). HIV- infected children with PEM often suffer from growth retardation. Marasmus occurs more frequently than kwashiorkor among HIV-infected children below the age of 5. Common features of Marasmus include wasting and growth retardation. These features are frequently seen in HIV-infected children.

2.4 HUMAN IMMUNODEFICIENCY VIRUS (HIV)

2.4.1 Background

HIV/AIDS advanced into one of the most disastrous pandemics worldwide. The greatest impact of this virus is felt in countries ridden with high levels of poverty. Worldwide, countries such as South Africa and China have the highest incidence of HIV/AIDS (Kilmarx, 2009).

Early reports of the disease were discovered in young homosexual men with rare diseases then such as Kaposi's sarcoma, persistent Lymphadenopathy and *Pneumocystis carinii* (PCP) (Siliciano, 2001). The virus is transmitted via blood transfusion, sexual activity, breastfeeding, perinatal mother to child transmission and accidental inoculation (Siliciano, 2001).

HIV can be classified either into HIV-1 and HIV-2. They belong to the subfamily *Lentivirinae* of the family *Retroviridae.* A characteristic feature of this family is the presence of a Ribonucleic acid (RNA) genome and reverse transcriptase. They have similar genetic makeup, origins and cell tropism. HIV-2 has a lower replication rate, cell dissemination and CD4 interaction. Only two subtypes of HIV-2 have been characterized with subtype A being the major variant. HIV-2 has a smaller virulence factor and a longer progression time compared to HIV-1 (Whittle *et al.*, 1994).

Worldwide HIV-infection continues to increase in children. Globally HIV/AIDS is one of the main leading causes of death in children.

2.4.2 Epidemiology Worldwide

In 2008, 2.0 million people died due to AIDS and a total of 33.4 million adults and children below 15 years were infected with HIV/AIDS (AIDS epidemic update, 2009. UNAIDS). Approximately 90% of infected children reside in sub-Saharan Africa.

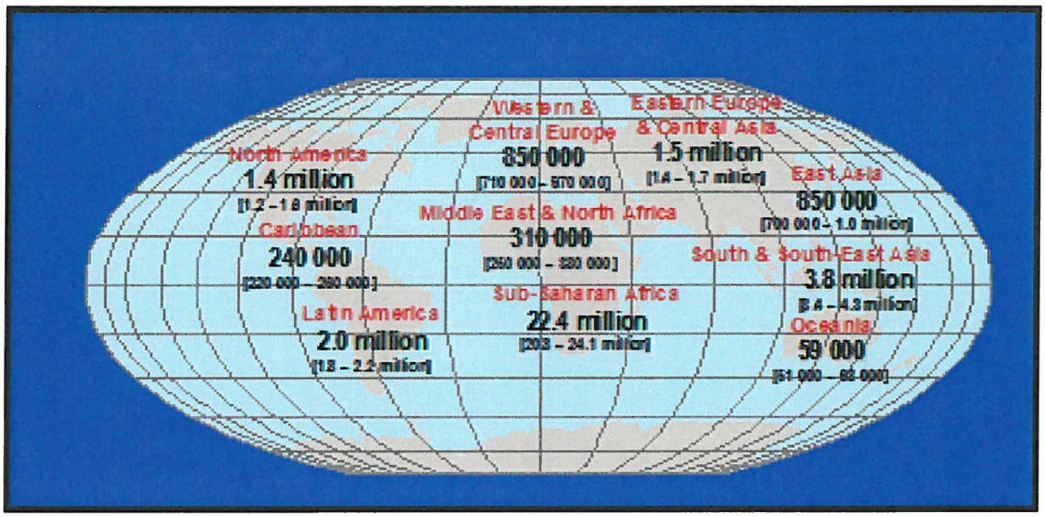

Figure 2.3: Number of people living with HIV. Available. From http://data.unaids.org/pub/EPISlides/2009/2009_epiupdate_ core_en.ppt#264, 4, Slide 4. Accessed 13 June 2010

2.4.3 South Africa

The growth of HIV prevalence in South Africa took place primarily between 1993 and 2000, during which time major political changes were taking place. Whilst attention was focused on the transition from apartheid, HIV was becoming more prevalent. In 2008, 5.6 million South Africans were HIV-infected. HIV infections and AIDS related deaths differs within each province in South Africa as depicted in (Fig 2.4). Kwazulu-Natal has approximately 1.6 million people whom are HIV-infected and 300 000 are still requiring antiretroviral (ARV) treatment (Nathea, 2008).

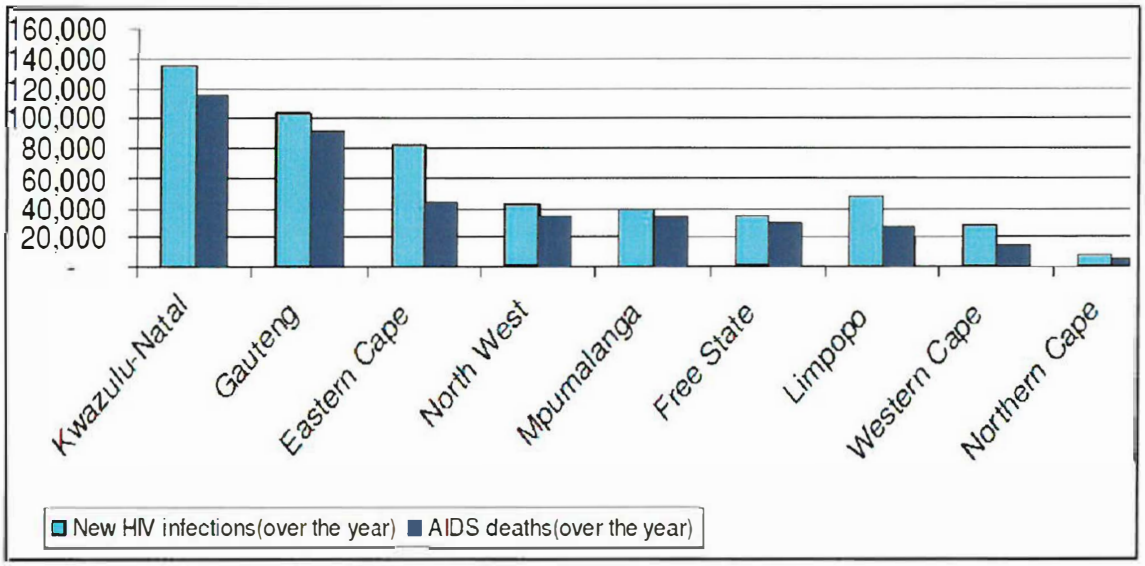

Figure 2.4: New HIV infections and AIDS related deaths in 2008 (Nathea, 2008)

Children have a short window period between the transition from HIV-infection to AIDS. Thus many childen that are unable to access treatment often die befor the age of two (Monson *et al.*, 2006). Between 2008-2009, the number of children in Sub-Saharan Africa receiving ARVs increased from 224100 to 296000 as shown in (Table 2.2) (WHO, UNICEF,UNAIDS, 2010). Factors such as poor access to health care facilities, poverty and lack of availability of ARVs contribute to children not receiving ARVs on time (Posse *et al.*, 2008). Rights of children especially the right to health treatment is often undermined in many areas in Sub-Saharan Africa.

Table 2.2: Number of children living with HIV younger than 15 years receiving antiretroviral therapy, needing antiretroviral therapy and percentage coverage according to region (WHO, UNICEF,UNAIDS, 2010).

Geographical region	Reported number of children (0-14 years) receiving antiretroviral therapy	Estimated number of children needing antiretroviral therapy [range]	Antiretroviral therapy coverage among children [range]
Sub-Saharan Africa	296 000	1 140 000 [710 000-1 500 000]	26% [19%-42%]
Eastern and southern Africa	254 900	790 000 [530 000-1 000 000]	32%[25-48%]
West and central Africa	41 000	350 000 [180 000-510 000]	12% [8%-22%]
Latin America and the Caribbean	18 600	32 200 [23 000-42 000]	58% [45%-80%]
Latin America	16 300	24 100 [19 000-31 000]	68% [52-87%]
The Caribbean	2400	8100 [4100-12 000]	29% [19%-57%]
East, South and South-East Asia	36 500	83 400 [61 000-140 000]	44% [27%-59%]
Europe and Central Asia	4800	9700 [5700-15 000]	49% [31%-58%]
North Africa and the Middle East	560	10 000 [5200-15 000]	6% [4%-11%]
Total	356 400	1 270 000 [830 000-1 700 000]	28% [21%-43%]

2.4.4 Prevalence of HIV/AIDS in children in South Africa

HIV prevalence rate in the 0-5 year age group increased from 2.2% in 2000 to 3.6% in 2006. As shown in (Table 2.3) HIV prevalence rates differ amgonst all the provinces in South Africa with KZN having the highest rate (Monson *et al.*, 2006).

Table: 2.3: The HIV-prevalence rate among children in South Africa from 2000 to 2006 (Monson *et al.*, 2006).

Province	2000 %	2001 %	2002 %	2003 %	2004 %	2005 %	2006 %
Eastern Cape	1.0	1.2	1.4	1.6	1.7	1.9	2.0
Free State	1.5	1.7	2.0	2.2	2.3	2.5	2.6
Gauteng	1.4	1.7	1.9	2.1	2.2	2.4	2.5
KwaZulu-Natal	2.1	2.4	2.6	2.8	3.0	3.1	3.2
Limpopo	0.8	0.9	1.1	1.2	1.3	1.3	1.4
Mpumalanga	1.8	2.0	2.2	2.4	2.5	2.6	2.6
Northern Cape	0.5	0.6	0.7	0.8	0.9	1.0	1.1
North West	1.3	1.5	1.7	1.9	2.0	2.1	2.2
Western Cape	0.4	0.4	0.5	0.6	0.7	0.7	0.8
South Africa	**1.2**	**1.5**	**1.6**	**1.8**	**1.9**	**2.0**	**2.1**

2.4.5 Morbidity and Mortality in children

The number of child deaths in South Africa is still extremely high with most being preventable. An important factor that influences the child mortality rates is the HIV/AIDS pandemic (South African MDG Report, 2010). Studies done show that post-neonatal death rates are increasing the infant mortality rate (IMR), thus increasing the under-five mortality rate (U5MR) (Sanders *et al.*, 2010). The main cause of these deaths is HIV/AIDS (South African MDG Report, 2010).

The Millennium Development Goal 4 (MDG 4) commits countries to reduce the under-five mortality rate by two thirds between 1990 and 2015. Comparatively to other countries child mortality trends in South Africa have showed no major improvement over the past 15 years. The estimated under five mortality rate in 1990 for South Africa was 60 deaths per 1000 live births. However the MDG 4 target is to reduce under five mortality to 20 deaths per 1000 live births (South African MDG Report, 2010). This is unlikely to be achieved as shown in the table (Table 2.4).

Table 2.4: Millenium Development Goal 4 – Reduce Child Mortality in South Africa by 2015. (South African MDG Report, 2010).

GOAL 4: REDUCE CHILD MORTALITY

Goal and Indicators	1994 Baseline (or closest year)	Current Status 2010 (or nearest year)	2015 Target	Target Achievability	Indicator Type
Under-five mortality rate	59 *(1998)*	104 *(2007)*	20	Unlikely	MDG
Infant mortality rate	54 *(2001)*	53 *(2007)*	18	Unlikely	MDG
Proportion of 1 year-old children immunised against measles	68.5 *(2001)*	98.3 *(2009)*	100	Likely	MDG
Immunisation coverage under 1 year of age	66.4 *(2001)*	95.3 *(2009)*	100	Likely	Domestic
Life expectancy at birth for males and females	57.6 *(2001)* 64.8 *(2001)*	55.3 *(2007)* 60.4 *(2007)*	70	Unlikely	MDG
Diarrhoea incidence under 5 years of age (per 1,000)	138.0 *(2001)*	132.6 *(2009)*	No target	Not applicable	Domestic
Pneumonia incidence under 5 years of age (per 1,000)	21 *(2003)*	102.1 *(2009)*	No target	Not applicable	Domestic

2.4.6 HIV infection in children

More than 90% of children living with HIV are infected through mother-to-child transmission (MTCT) during pregnancy or via breastfeeding (Newell, 2001). MTCT of HIV can occur in-utero, intrapartum and postpartum. During pregnancy a child has a 5-10% risk of getting infected, during labor they have a 10-20% risk of getting infected and a 10-20% risk of getting infected via breatfeeding (de Cock *et al.*, 2000). Transmission during pregnancy may occur due to membrane rupture or via placental infection. Findings show a 2% increased risk of HIV infection occurs during the duration of every hour of a membrane rupture (The International Perinatal HIV Group, 2001).

These findings emphasize the need of cesarean section prior to labor and membrane rupture. A randomized clinical trial in European women showed efficacy of cesarean section as a preventive measure for MTCT. The transmission rate was lower in cesarean section (1.8%) versus vaginal delivery (10.5%). An 83% efficacy was found in this trial. (The European Mode Of Delivery Collabration, 1999).

Prolonged duration of breastfeeding increases the risk of HIV infection in a child (The breastfeeding and HIV International transmission study group, 2004). Risk of HIV infection via breastfeeding depends on factors such as breast infection (John *et al.*, 2001), pattern of breast feeding (exclusive versus mixed) (Coutsoudis *et al.*, 1999) (Coutsoudis *et al.*, 2001) and maternal viral load (Dunn *et al.*, 1992).

In a prevention of mother to child transmission (PMTCT) study carried out in South Africa, children born to HIV-infected women had a higher mortality than those born to uninfected mothers (Newell *et al.*, 2004). Immunosupression and opportunistic infections are apparent in HIV-infected children within their first year. Children with rapid progression are infected in-utero and are unable produce an effective immune response (Lambert *et al.* 1997). Decreased weight and linear growth are common features of growth failure in children. Prolonged periods of weight loss are often linked with secondary infections (Macallan, 1993).

HIV infection in children differs from that of adults, disease progression occurs rapidly and their viral loads are much higher. Opportunistic infections arise frequently in children often affecting nutritional status in children. Other important factors such as alterations in food intake, nutrient absorption and energy expenditure also affect the nutritional status in HIV-infected children (Andiman *et al.*, 1994). These are further expanded in the following section.

2.5 NUTRITIONAL CHANGES ASSOCIATED WITH HIV

2.5.1 The wasting syndrome

HIV associated wasting is often seen in the advanced stages of HIV (Clinical stages 3 and 4). In adults it is defined as more than 10% loss of body weight and a decline greater than 2 percentile lines with chronic diarrhoea or fever in children older than a year (Bailey *et al.*, 1999). The wasting syndrome is an AIDS defining illness and plays a role in mortality and morbidity. In adults severe wasting is identified by a body mass index (BMI) less than 18.5 kg or an unintentional weight loss of more than 5% body weight within a 6 month period (Grinspoon *et al.*, 2003). In young children, wasting is linked with LBM loss (Arpadi *et al.*, 1996).

In HIV associated wasting, two forms of malnutrition exist, starvation and cachexia. Starvation is defined as the deprivation of food leading to weight loss. Physiologic and biochemical changes that occur during starvation include decreased energy expenditure and increased lipid oxidation. Cachexia is defined as a disproportionate loss of LBM as compared to FM (Kotler, 2000). The loss of LBM and poor linear growth in HIV-infected children is closely associated with poor survival (Morley *et al.*, 2006).

Paeditric HIV-infected patients with HIV associated growth failure have an increased risk of morbidity and mortality (Bobat *et al.,* 2001). In a cross sectional study, before commencement of ARV therapy, 50% of HIV-infected children were stunted or underweight and one in five had developed wasting (Eley *et al.*, 2006). In a cohort of HIV positive children in South Africa, a high percentage of children were stunted compared to being underweight (Bachmann *et al.*, 2006). Elevated levels of HIV replication is linked with growth failure (Arpadi *et al.*, 2000).

Contributing factors to the HIV wasting syndrome include alterations in food intake, nutrient absorption and energy expenditure (Melchior *et al.,* 1991). Decreased intake of nutrients is often associated with various factors such as gingivitis, esophagitis and enteritis (Fox, 1991). This was evident in our study cohort. Inflammation of the upper gastrointestinal tract caused by various infections and ulcers promote the development of anorexia (Kotler *et al.,* 1992).

Side effects caused by certain medications exacerbate the development of anorexia. HIV-infected children have a higher percentage of abnormalities caused by infections such as cytomegalovirus, Candida albicans or herpes (Miller *et al.*, 1993). Central nervous system (CNS) processes such as dementia or tumors also affect nutrient intake in HIV-infected patients (Mintz, 1996).

Intestinal malabsorption is a common feature in HIV-infected patients and a contributing factor of malnutrition in AIDS (Koch *et al.,* 1996). Causative factors of malabsorption include HIV induced atrophy, enzyme deficiencies and opportunistic infections such as cryptosporidiosis, *Mycobacterium tuberculosis* and salmonella (Winter and Chang, 1993). Resting metabolic rate is a pertinent factor in individuals with HIV/AIDS. Rapid weight loss associated with secondary infections cause increased resting energy expenditure (Grunfeld *et al., 1995*). Cytokines, such as TNF, IL-1, and IL-6, cause increased metabolism, muscle catabolism and negative nitrogen balance during infection (Ridkr *et al.*, 1997).

In a comparative study done by Melchoir *et al* (1993) resting energy expenditure was 11% higher in HIV-infected patients compared to control subjects and in HIV patients with co-infections, resting energy expenditure was 34% higher than in control subjects. Suttaman *et al* (1993) made a correlation between resting energy expenditure and weight loss in HIV-infected adults. All clinical stages displayed increased resting energy expenditure.

HIV-infected children rapidly lose FFM during the initial stages of disease (Fontana *et al.*, 1999). This may have consequences in terms of prognosis and response to therapy. Assessment of body composition in HIV-infected children provides insight in growth and development in relation to disease progression.

2.6. BODY COMPOSITION CHANGES IN DISEASE

Paediatric diseases negatively impact body areas such as fat, bone mineral density and muscle mass. Body composition analysis allows quantification of vital tissue components such as LBM and FM. Therefore, understanding the principles of total body composition assessment allows health care workers to 1) Monitor body composition changes associated with disease 2) To assess nutritional interventions in altering body composition 3) To monitor growth 4) To monitor development and age-related changes in body composition (Heyward and Wagner, 2004).

Various studies illustrate changes in body composition that occur in relation to disease. A Study done on paediatric patients on artificial ventilation showed LBM depletion (Wells *et al.*, 2002). ICW and ECW is higher in stunted children, this is revealed in a study that compared techniques for evaluation of adiposity in stunted and nonstunted children (Hoffman *et al.,* 2006).

Common body composition changes in HIV-infected individuals include fat redistribution, increased visceral adipose tissue and decreased subcutaneous adipose tissue. Loss of muscle mass reduces functional status and strength in HIV positive individuals (Grinspoon and Mulligan 2003). Children with HIV associated growth failure have a lower percentage of FFM as compared to the FM component (Arpadi *et al.*, 2001).

The use of appetite stimulants present an alternative for increasing caloric intake in HIV infected persons. An increase in nutritional intake in children with HIV-associated growth failure using appetiate stimulants improves weight without any improvement on FFM. This is shown by findings from a double-blinded, placebo-controlled trial of megestrol acetate (Megace®,Bristol Myers Squibb, Princeton, NJ), which revealed an increase in weight gain. However, BIA analysis showed a larger increase in FM as compared to FFM (Kotler and Grunfeld,1996). ARV therapy has a positive effect on growth and body composition (Miller *et al.*2001) (Verweel *et al.* 2002). This was observed in a paediatric group on Highly Active Antiretroviral Therapy (HAART) in South Africa. The findings are illustrated in the table below (Table 2.5) (Reddi *et al.* 2007).

Table 2.5. Growth changes in HIV infected children <15 years after 1 year on HAART (Reddi *et al.* 2007).

Parameter	***Baseline number (%)***	***1 year number (%)***
Wasting (n=254)	52 (20.5)	6 (2.4)
Underweight (n=266)	149 (56.0)	51 (18.2)
Stunting (n=264)	178 (67.4)	123 (46.6)

A number of body composition techniques have been developed for use in various disease settings such as anthropometry, BIA, isotope dilution and imaging methods. These will be discussed further in the subsequent section.

2.7 BODY COMPOSITION TECHNIQUES

2.7.1 Anthropometry

Anthropometry is a common body composition technique used in clinical studies. It is an inexpensive and non-invasive technique. Recent developments in this field involve calibration of anthropometric measurements against reference estimates of body composition to develop various prediction models. Basic anthropometric measurements such as body height, weight and skinfold thickness are utilized for determining nutritional status as shown in (Fig 2.5) (Wang *et al.*, 2000).

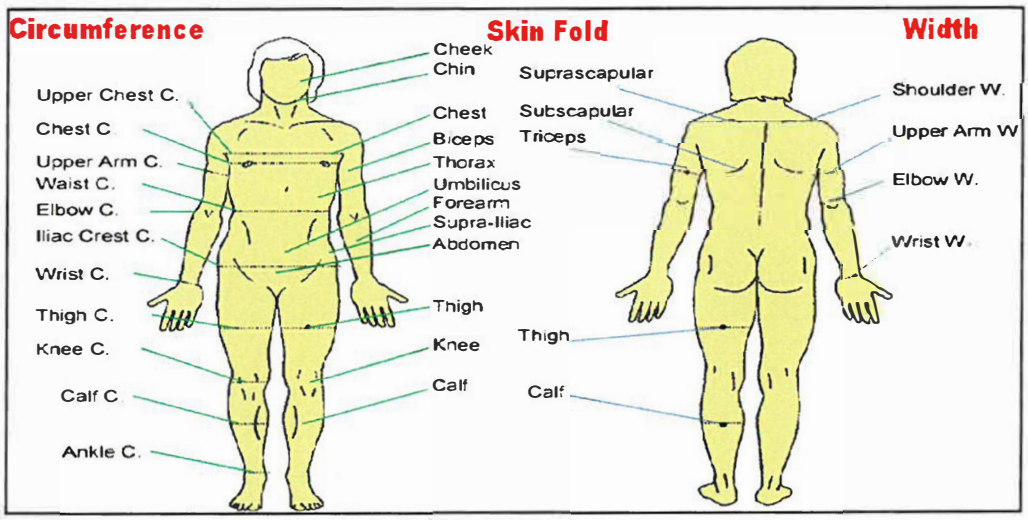

Figure 2.5: Anthropometry measurement areas (Wang *et al.*, 2000)

Studies using anthropometry provide vital background of nutritional disorders reflected in diminished triceps, skinfold thickness or other measurements illustrating body fat (Miller, 1993). Skinfold thickness is used as a predictor for body fatness since 40–60% of total body fat is located in the subcutaneous region within the body (Wang, 2000).

BMI defined as Wt/Ht^2 provides childhood fat estimates in large epidemiological studies (Brodie *et al.*, 1998). A variation in BMI during childhood emphasizes the need for age and gender specific reference standards (Cole *et al.*, 2000).

The sub-scapular, triceps and MUAC illustrate LBM and fat stores in children older than one year. Measurements are applied in multiple regression models to derive equations for calculation of body density, body fat (adiposity) and FFM (Miller, 1993). Skin fold measurements used in clinical or field settings can then be used to estimate body composition (Wells *et al.,* 1999).

2.7.2 Bioelectrical Impedance Analysis (BIA)

BIA is a body composition technique that is used to derive TBW measurement and hence estimate FFM and adiposity. It is a quick, simple, portable and non-invasive technique that could potentially provide accurate results with small interobserver variability (Segal *et al.,* 1991). The history of BIA goes back since 1871. Today it is widely used in various research settings for a number of diseases.

2.7.2.1 Principle of BIA

An electrical current is able to pass easily through blood and urine while bone and fat have large impedance (Z) and do not conduct significant current. Current thus flows through materials with higher conductivities (O'Brien *et al.*, 2002). BIA is based on the relationship between volume of the human body (conductor), length (height), components (FM/FFM) and Z. It is possible to estimate body composition by assuming the conductor is a perfect cylinder as shown in (Fig 2.6). Voltage (V) the parameter measured with BIA is produced between two electrodes. It is often expressed as a ratio V/I. This ratio known as Z is the function of two components, resistance (R) which is normally 250Ω and reactance (X) which is 10% the amount of resistance (Kyle *et al.*, 2004). Magnitude of Z is similar to R. Z and R can be applied as if they are interchangeable although

$$Z=(R^2+X^2)^{1/2}$$ **(Equation 2.1) (Dittmar and Reber, 2001)**

Resistance of a conductor is proportional to its length (L) and inversely proportional to its cross sectional area (A, cm^2)

$$R = \rho \frac{L}{A}$$

(Equation 2.2) (Kyle *et al*,, 2004)

*Where **R** is the resistance, **ρ** is the resistivity, **L** is cylinder length, and **A** is the cross sectional area of the cylinder*

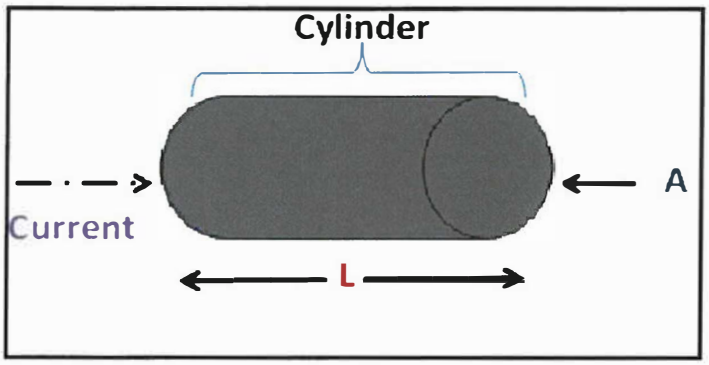

Figure 2.6: A cylinder model for Bioimpedance Analysis. Adapted from: http://www.jawon.co.kr/eng/technology/body-composition/principles-of-bia.php. Accessed 12 April 2009

Since conductivity and resistivity is not constant, body water volume can be determined by **(Equation 2.3)** (Kyle *et al*., 2004). The resistance index (Ht^2/R) is often applied to predict FFM and TBW as shown in **(Equation 2.4)** (Dittmar and Reber, 2001).

$$V = \frac{\rho L^2}{R}$$

(Equation 2.3)

$$TBW = \frac{Ht^2}{R}$$

(Equation 2.4)

*Where **R** is the resistance of the body, **ρ** is the resistivity, **L** is the conductor length, **V** is the volume of water and Ht^2 is $Height^2$*

BIA measures the opposition of body tissues to the flow of an electric current. A current (*I*), of about 800 mA at a frequency of 50 kHz is introduced into the body. The current passes between two electrodes, called the source and detector. This generates voltages between different points in the body volume according to Ohms law. The electrodes are usually located on the wrist and ankle shown in (Fig 2.7) below.

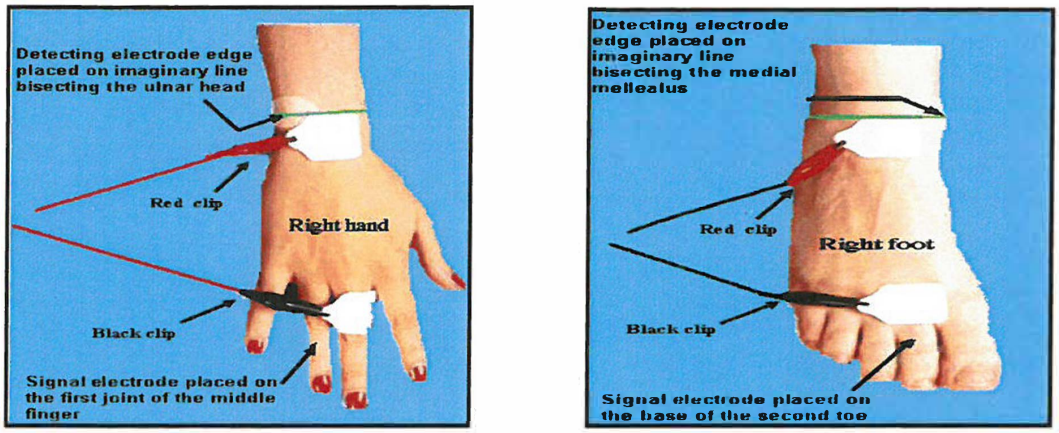

Figure 2.7: Electrode placement for Bioimpedance Analysis. Adapted from:

http://www.rjlsystems.com/support/docs/analyzers/general/placement/. Accessed 12 April 2009

2.7.2.2 Background of Validation

In body composition studies, a new or existing body composition method is valid when it provides an estimate that is close to the value obtained in a criterion method. However accuracy of the method tested is often determined by size of the prediction error (Lohman, 1992). Often cross validation is the final aspect of validating a new or existing body composition assessment method. In the cross validation process, the assessment method being evaluated is often applied to estimate body composition in an independent group in order to determine how strongly the method predicts body composition as compared to the criterion method. An independent group often comprises of a new, representative sample of individuals (Manore *et al.*, 2009).

The body composition assessment method being tested is considered accurate and precise if results from the cross validation study indicate prediction errors and reliability estimates for the body composition variables are within acceptable ranges (Manore *et al.*, 2009).

2.7.2.3 Prediction equations

To achieve an accurate prediction of TBW in an individual a prediction equation validated for subjects of overall body composition similar to that subject is required (Kyle *et al.*, 2004). Errors in calculation of FFM from BIA assessment arise from the assumptions that: 1) A 50-kHz current passes freely through cell membranes and body fluids and 2) Body water distribution is uniform throughout the body (Kyle *et al.*, 2004). This may result in inaccuracies in conditions with disturbances in extracellular vs. intracellular water distribution or in instances such as wasting conditions or short stature.

BIA prediction equations are either population-specific or generalized (Ainsworth *et al.*, 1997). Theoretical and empirical relationships between FFM, TBW and bioimpedance measures estimate FFM and TBW. BIA equations developed that are population-specific account for differences in age, ethnicity, gender, physical activity and level of body fat. These equations are valid and can be applied to individuals with similar characteristics to those in the specific study group (Wells. 2006). Generalized BIA equations vary in age, gender, body fatness and ethnicity. These equations account for variability between populations via inclusion factors such as age and gender used as predictor variables in estimating FFM or TBW. R is a better predictor of FFM and TBW than Z since the size of R is larger than X (at a 50 kHz frequency) when measuring whole body impedance (Houtkooper *et al.*, 1989).

In order to account for the complex body shape, body weight and individual differences in body size are included in BIA prediction equations. Predictive accuracy of BIA equations is improved by inclusion of body weight, Ht^2 and R in BIA prediction equations (Kushner *et al.*, 1992). A number of regression equations have been developed using BIA alongside other techniques such as dual-energy X-ray absorptiometry (DEXA), anthropometry and isotope dilution to predict FM, FFM and TBW.

Goran *et al* (1993) incorporated age into a prediction equation using 26 preschool children to obtain FFM. Arpadi *et al* (1996) tested existing BIA equations in HIV-infected children. Addition of sex, weight and height had no improvement on the predication of TBW and FFM. Majority of the study subjects were Hispanic and ages ranged between 4 and 11 years.

BIA prediction equations have been developed in HIV-infected prepubertal children (Arpadi *et al.*, 1996). Prediction equations for estimating body cell mass, FFM and TBW in HIV-infected adults have been developed (Kotler *et al.*, 1996). Horlick *et al* (2002) developed prediction models for TBW and FFM in healthy HIV-infected children aged between 4 and 18 years. Variables such as age, race, sex, HIV-infected/ uninfected were added to improve the predictive accuracy of the equations.A study carried out by Faisy *et al* (2000) showed good correlation between BIA and DEXA in paediatric patients with chronic inflammatory disorders. In a longitudinal study involving obese women, correlation was observed between BIA, FFM and deuterium dilution (Kushner *et al.*, 1990).

2.7.3 Isotope dilution

2.7.3.1 Introduction

Water is located in all tissues, organs, cells and compartments such as the ICW, plasma, interstitial water, dense connective tissue and transcellular water (Mattsson and Thomas, 2006). TBW makes up 73% of the FFM in normal healthy individuals (Kotler, 1999). In infants TBW consists of 80-83% FFM, however this declines till hydration becomes comparable to that of adults (Fomon and Nelson, 2002). Measurement of BM and TBW permits calculation of FFM. The amount of TBW within the body alters during various conditions (Kotler, 1999).

Measurement of TBW is carried out using isotope dilution. Tracers used for TBW analysis include titrated water, deuterium water and ^{18} O – labeled water. Assumptions that arise with TBW measurement by isotope dilution are 1) The tracer is distributed only in body water. 2) The tracer is equally distributed in all anatomical water compartments. 3) Equilibration rate of the tracer is rapid. 4) Neither tracer nor body water is metabolized during the time period of tracer equilibration. The intercept and the equilibration methods are often used to measure TBW. The intercept method requires a longer time period between 7 to 14 days (Haisma *et al.*, 2003).

The equilibration method is more advantageous as it requires a considerably shorter time period as compared to the intercept method. Equilibrium after oral administration requires a time period of 3-4 hours.

2.7.3.2 Principle

The volume of a compartment is defined as the ratio of a tracer dose given orally to its concentration in the body compartment within a time period after dose administration. The tracer enrichment in the body water pool is carried via collection of body fluids such as saliva, blood or urine samples. The first sample is collected before the dose is given. The second sample is collected after a certain time period. This time period allows for sufficient equilibration of the tracer within the body compartment. This process is depicted in (Fig 2.8) (Smith *et al.*, 2002).

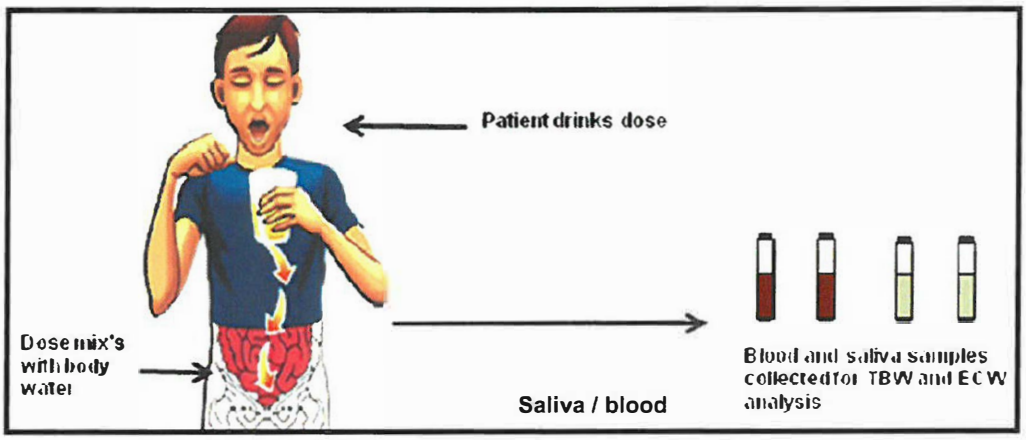

Figure 2.8: Schematic diagram depicting isotope dilution. Adapted from: http://www.iaea.org/NewsCenter/Features/Nutrition/energyintake.html. Accessed 26 October 2009

As isotopes enter the body, H isotopes and O isotopes undergo non-aqueous exchange. Specifically, hydrogen exchanges with exchangeable atoms in body protein and other non-aqueous components, but most of the exchange is with protein. By labelling water molecules it is possible to measure body water. If the water molecule is labeled in one of the hydrogen positions with the stable isotope deuterium, it will distribute throughout the water pool and reach a near steady state concentration (Racette *et al.*, 1994).

TBW is composed of two compartments, intracellular water (ICW) and ECW. ICW is the fluid phase found in organs and muscles whilst ECW is distributed in interstitial fluid, connective tissue fluids and plasma. Alteration of the ECW compartment occurs during disease and is characteristic of various diseases such as endocrine disorders, congestive heart failure, cirrhosis and liver disease (Geerling *et al.,* 1999).

Infection and various inflammatory illnesses lead to lowered serum albumin levels by decreasing albumin synthesis and increasing ECW. In conditions such as heart failure, arterial circulation is the main determinant of water retention in the body thus expanding ECW (Uszko *et al.*, 2006). Renal problems also lead to alterations in water metabolism. Increase or decrease in water and sodium is associated with metabolic alterations followed by oedema (Woodrow *et al.*, 2005).

A number of factors such as sex, race, age, weight, and height influence interpretation of ECW. In disease, ICW changes are less obvious as compared to ECW. Usefulness in ECW monitoring with other nutritional measurements allows determination of nutritional status in those with acute and chronic diseases (Kim *et al.*, 1999). ICW is calculated as the difference between TBW and ECW (Bedogni *et al.*, 1997).

Various tracers such as Bromide (Br), chloride (Cl), thiocyanate, thiosulphate, sulphate, insulin, sucrose and mannitol have been used for measuring ECW. Br and Cl approximate ECW well however, no suitable radioisotope for chlorine exists. Thus Br as sodium bromide (NaBr) may be used as a tracer to determine ECW (Mattsson and Thomas, 2006).

The administration of Br allows rapid exchange with body chloride and the volume distribution of Br is equivalent to that of Cl. The use of Br has a good absorption, slow excretion and slight penetration into tissues (Mattsson and Thomas, 2006). A study by Snel *et al* (1995) determined body composition in adults with growth hormone deficient (GHD) by measuring ECW directly using Br dilution. ECW was estimated in children with malaria (Planche *et al*., 2004) and in low birth weight infants (Mayfield *et al*., 1991).

2.7.4 Measurement of Total Body Water by Deuterium

2.7.4.1 Fourier Transform Infrared Spectrometry (FTIR)

Figure 2.9 The Thermo Scientific Nicolet 380 FTIR Spectrometer. Available from: www.thermonicolet.com. Accessed 26 October 2009

FTIR is an infrared spectrometry technique used in various research areas including body composition research. Data obtained from measuring deuterium concentration in saliva or plasma samples enables TBW measurement. In FTIR, absorption in the infrared region of the electromagnetic spectrum is linked with the vibrational energy of the O-H and O-D bonds. The absorption band at $2504cm^{-1}$ is used to determine the deuterium content of an aqueous sample. Thus, the intensity of this band is linearly related to the concentration of deuterium in the sample (International Atomic Energy Agency (IAEA), human health series, 2009).

2.7.4.2 FTIR instrumentation

Inferometers composed of a beam splitter, splits incoming infrared beams into two optical beams. One beam reflects a fixed flat mirror whilst the other beam reflects off a flat mirror placed on a mechanism. The two beams are recombined when they meet again at the beam splitter (Introduction to Fourier Transform Infrared Spectrometry). Available from: www.thermonicolet.com.[Accessed 23 January 2010]. The signal exiting the inferometer is called an interferogram.

The interference patterns of the modulated signals from the interferogram are amplified, digitised, electronically stored and transformed into a spectrum via a mathematical technique known as the Fourier transformation. This decodes the individual frequencies. A computer performs the Fourier transformation and the spectral information is obtained (Beekes *et al.*, 2007).

2.7.5 Measurement of extracellular water (ECW) by NaBr dilution

2.7.5.1 High performance liquid chromatography (HPLC)

High performance liquid chromatography (HPLC) can be used to assess Br concentrations in plasma and saliva. HPLC is widely used in body composition for ECW determination. This technique has already been implemented in various research areas. The eluent is filtered and pumped through a chromatographic column. The sample is loaded and injected onto the column and the effluent is monitored using a detector and recorded as peaks.

Figure 2.10: The PerkinElmer HPLC system, Series 200 liquid chromatograph. (PerkinElmer Series 200 HPLC System) Available from: www.perkinelmer.com. Accessed 26 October 2009

Analysis of Br using the PerkinElmer HPLC system is performed as follows: There are four solvent reservoirs located in a closed chamber. The Series 200 Liquid Chromatograph delivers the solvents. The pump system connected with a helium gas tank moves solvents through the system. The autosampler deposits samples at the top of the column. The Br is separated from other compounds by the anion column. The detector of the PerkinElmer Model series 200 spectrophotometer measures absorbance in the wavelength range 190nm-700nm. The Series 200 Laboratory computing integrator digitises the analog signal from the chromatograph, plots the chromatogram, detects, integrates the peaks and produces a report. (The PerkinElmer Series 200 HPLC System). Available from: www.perkinelmer.com.[Accessed 23 January 2010].

2.7.6 Air Displacement Plethysmography (ADP)

Air displacement plethysmography(ADP) is body composition technique that has replaced underwater weighing. The subject in placed in a device composed of an air filled chamber. The device consists of two chambers, the subject is placed in one chamber and the other is a reference chamber. When the subject is placed inside the chamber, pressure is increased and the diaphragm separating the two chambers is oscillated to produce a volume and pressure change in each chamber (Ellis, 2000). Comparative analyses between ADP and underwater weighing have shown good results in adults and children (Lockner *et al.* 2000).

2.7.7 Neutron Activation

This method applies the principle of activating the body with a known amount of neuron energy. When the body is exposed to neutrons, y- rays are emitted. The induced radioactivity is counted in a whole body counting chamber. A known dose of neutrons generates a known amount of activity within a defined mass of a substance (Ellis, 2005). Various factors such as the detection system used and body position within the neutron field influence the validity of the technique. Common elements measured are total body calcium and total body nitrogen. The determination of total body nitrogen allows estimation of LBM. This technique is limited due to radiation exposure to children and pregnant women (Ellis, 2000).

2.7.8 Imaging methods

Common body imaging methods include computer tomography (CT), magnetic resonance imaging (MRI) and DEXA. However these methods cannot be considered as valid field methods for body composition due to high investment, highly trained technical staff, expensive maintenance and service costs.

2.8 BODY FLUIDS AND ISOTOPE DILUTION

In adults, equilibrium of ICW and ECW occurs within 3 hours. The most frequent body fluid collected for isotope dilution is plasma as it allows rapid equilibration of the tracer. Urine however, has shown to be less reliable as compared to blood and saliva due to the longer isotope equilibration time in the bladder (Schoeller *et al.*, 1980).

The usage of urine or saliva samples are non-invasive and can be used in children. In a comparative study by Schoeller *et al* (1982) saliva and serum were compared after isotope ingestion in obese children. There was no difference in saliva samples collected at the 3 hour interval compared to the 4 and 6 hour blood samples. The isotope equilibration was found to be rapid between serum and saliva samples. In another study by Horlick *et al* (2002) who evaluated predictive BIA equation in children aged 4 to 18 years old, saliva samples were collected for isotope dilution analysis.

CHAPTER THREE

METHODS AND MATERIALS

3.1 STUDY COHORT

We recruited a total of 42 children from the King Edward VII Hospital paediatric HIV clinic, the paediatric wards at King Edward VII Hospital and Clairwood Hospital in Durban. A number of body composition studies used children above the age of 4 years, thus excluding earlier periods when more dramatic changes occur in body water and body composition. In order to address this gap in knowledge, this study included male and female children between the ages of 3 to 6 years. Due to the 3 hour fasting period and collection of saliva, inclusion of infants in this study would have been problematic.

All study subjects were attending routine follow up for preparation for ARV treatment. There were no restrictions on ethnicity of subjects. Subjects at different stages of HIV disease severity and differing nutritional status were included. Children were included into the study only when their parent, or legal guardian provided informed consent/assent and they were between 3 and 6 years of age. Children were excluded from the study if they were on any current or previous treatment with megestrol acetate or corticosteroids. Glucocorticoids were allowed if used more than 2 weeks prior to study entry, and duration of treatment in past 6 months did not exceed 2 weeks. Other exclusions were any current febrile illness, cardiac or renal failure, abnormal losses through diarrhoeaor vomiting at time of assessment, clinical features of dehydration, on diuretic therapy, presence of edema, hydrocephalus, or any condition that may limit ability to completely ingest isotope dose.

3.2 ETHICS

Ethical approval was obtained from the Biomedical Research Ethics Committee, Nelson R Mandela School of Medicine, South Africa (***Ref number: E049/05***) (Appendix F), and hospital management approval from King Edward VIII Hospital and Clairwood Hospital. Prior to assessment, all patients were given consent forms (Appendix D) that were translated into Zulu and study was explained to parents and caregivers before they signed. Standard case record forms were used to document assessments in this study (Appendix E).

3.3 SAMPLE SIZE

Our original crude sample size estimates were based on the sample sizes used in equivalent studies in the literature as well as the project's budgetary constraints. Arpadi *et al* (1996) studied 20 prepubertal children for a similar study design and aim, Leman *et al* (2003) studied 39 children. We calculated that using an average of these two sample sizes, a sample size of 30 children would allow us to account for 97% of the variance observed in TBW. We further anticipated 20% loss of usable data arising from inadequate specimen quality which could be due to inadequate volumes, breakage during transport or accidental contamination of specimens in transit (although standard precautions were in place to avoid this). Thus a total sample size of 36 was targeted. The development of new equations requires much larger sample sizes so this activity would be purely exploratory and provide the preliminary data for subsequent research.

3.4 STUDY PROCEDURES

Study reruitment and assessment was done between August 2008 to November 2009. After obtaining informed consent participants were given an appointment and instructed to arrive at the study site on the scheduled morning. They were instructed to omit morning meals but to ingest any prescribed medication as per schedule. All anthropometric measurements were recorded in duplicate using standardized technique and the average of both measurements used.

3.5 WEIGHT

Weight was recorded using a portable digital scale and measured to the nearest 0.1kg. Calibration of the scale was done on a daily basis using standard weights and checking the zero value. All footwear and clothing except a light vest and undergarment was removed prior to being weighed.

3.6 HEIGHT

A fixed wall-mounted stadiometer was employed for measuring standing height. All subjects were required to stand on the stadiometer (Seca, Kraaifontein, South Africa) without shoes with the back of the head, shoulder blades, buttocks, calves and heels touching the posterior board. The head was positioned so that the child was looking ahead thus, the external auditory meatus and lower margin of the orbit were aligned horizontally (the Frankfort plane). The headboard was pulled down firmly onto the crown of the head and height was recorded to the nearest 0.1cm. All measures were done in duplicate to increase accuracy.

3.7 MID UPPER ARM CIRCUMFERENCE (MUAC)

Study subjects were asked to stand upright with their right arm bent 90 degrees at the elbow. All clothing such as jerseys, long sleeved shirts covering the arm was removed prior to measurement.

A mark was made on the uppermost edge of the posterior border of the spine extending from the acromion process. A measuring tape was held at this mark and extended down the posterior surface of the arm to the tip of the bony part of the mid-elbow. A midpoint was recorded and marked. The measuring tape was wrapped around the arm at the level of the upper arm mid-point mark and a measurement was taken in duplicate.

3.8 BIO-ELECTRICAL IMPEDANCE ANALYSES (BIA)

For BIA measurements, the single-frequency 50-kHz tetrapolar BIA device (model 101 A; RJL, Detroit) was utilized. All study subjects were placed supine with their arms and legs slightly abducted. Skin sites were disinfected first with alcohol swabs prior to placement of the electrodes. All measurements were performed on the right side of the body. Two pairs of electrodes were placed on the right hand and foot. Measurements were taken when subjects were quiet and not moving. Subjects were measured within 10 minutes of lying supine and measurements had to be done at least 4 hours after the last meal. Direct measurements of resistance and reactance values that were recorded were later used to compute TBW using the prediction equations found below in section 3.8.1.

3.8.1 BIA prediction equations

Leman *et al* (2003) developed and validated the use of regression equations to predict TBW in Nigerian children aged 5-18 years. It was found that impedance index was the most significant predictor of TBW and weight and gender explained much of the remainder of the variability. The limitation in generalizing this to our population of interest is that these children were older than 4 years of age and did not appear to be HIV-infected. The difference between HIV infection and starvation is that the former is linked with FFM loss early in its course with concurrent loss of FM in more advanced disease. The latter usually has a reversed pattern of alteration in body compartments.

Arpadi *et al* (1996) was among the first to test existing BIA equations in HIV-infected children. They developed two regression equations that could be employed in their sample cohort because none of the equations they tested adequately predicted TBW or FFM.

Unlike the Nigerian group they found that gender and weight did not significantly improve predictive value. Most of the children in their sample had moderate to severe symptoms of HIV infection; which is similar to our study cohort. The limitation is the predominant Hispanic background of these children and, once again, the age range did not include children below the age of 4 years. Thus, after considering the equations below, we decided to test our measurements against the equations developed by Arpadi, Horlick and Leman on our study population.

a) Log ($TBWD_20$) = 1.65 + 0.05 ht^2 (cm)/Resistance (ohms) **(Equation 3.1)**

(Arpadi *et al.*, 1996)

b) TBW = 1.18 + 0.42 (ht^2/resistance) + 0.18 (wt) **(Equation 3.2)**

(Leman *et al.*, 2003)

c) TBW = 1.00 + 0.60 (ht^2/resistance) **(Equation 3.3)**

(Leman *et al.*, 2003)

d) TBW = 0.725 + 0.475 ht^2/resistance + 0.140(wt) **(Equation 3.4)**

(Horlick *et al.*, 2002)

e) $FFM = \frac{[3.474 + 0.459\ ht^2/resistance + 0.064wt]}{[0.769 - 0.009A - 0.01S]}$ **(Equation 3.5)**

(Horlick *et al.*, 2002)

f) TBW = 0.76 + 0.18 ht^2/I + 0.39 wt **(Equation 3.6)**

(Fjeld *et al.*, 1990)

g) TBW = 0.7 ht^2/resistance – 0.32 **(Equation 3.7)**

(Kushner *et al.*, 1992)

h) TBW = 0.593 ht^2 resistance + 0.065 wt + 0.04 **(Equation 3.8)**

(Kushner *et al.*, 1992)

Where ***TBW*** is total body water, ***ht*** is height in cm, ***wt*** is weight in kg, ***I*** is impedance, ***A*** is age in years, ***S*** is sex with male=1 and female=0.

3.9 ISOTOPE DILUTION

TBW was measured by 2 hour D_2O dilution and ECW by a 2 hour Br dilution. Body composition study packages were supplied by the Body Composition Unit of St Luke's Roosevelt Hospital, New York. Each package contained 1 dose vial containing a predetermined fixed dose (0.5 ounce) of deuterated water, 2 green top tubes, 2 plastic specimen tubes with cap and 1 study information sheet. Each patient's initials, the date of study and the study name were recorded on the patient information sheet. A unique number was allocated to each child and this number was used to label all samples obtained. Participants were advised not to eat at least two hours before the test.

At time zero (after the BIA measurement) and before dose administration, two dental rolls were placed at the side of the inner cheeks for a time period of 10-15 minutes for saliva collection. Saliva was collected in a specially marked tube called "pre dose saliva". Thereafter venipuncture was performed and blood (4ml) was collected in a specially marked green top collection tube labeled "pre dose blood".

The dose vial was opened and poured into an empty cup to avoid spillage. The empty dose vial was tapped against the cup to allow complete emptying of the dose vial. To ensure complete emptying of the dose vial 5ml of fruit squash was added to the dose vial and the cap replaced, shaken and poured into the cup. Subjects were asked to drink the dose as quickly as possible. All dose cups were rinsed 3 to 5 times with fruit squash to ensure complete ingestion. After 2 hours a second blood sample (4ml) was collected in specially marked green top collection tubes labeled "post dose blood". All pairs of blood samples were marked with a study number. Blood collected in pre and post dose blood tubes were

centrifuged (ALC multispeed refrigerated centrifuge (PK121R)) at 3000rpm for 10 minutes. Thereafter plasma was transferred to pre and post dose plasma tubes and stored at -20°C until shipment. At 3 hours, saliva (2ml) was collected by placing two dental rolls at the side of the mouth for a time period of 10-15 minutes to ensure sufficient collection of sample in specially marked tube called "post dose saliva". Saliva collected in pre and post dose saliva tubes were centrifuged (ALC multispeed refrigerated centrifuge (PK121R)) at 3000 rpm for 10 minutes. Thereafter saliva was transferred to pre and post dose specimen tubes and stored at -20°C until shipment.

At the end of the study all samples were shipped on dry ice to the body composition laboratory at St Luke's Roosevelt Hospital in New York. D_2O enrichment was measured with a Fourier – Transfer Infrared Spectrometer (Appendix C). TBW was then calculated by a standard formula and pre-/post- status. The HPLC system (Perkin – Elmer HPLC system, and the series 200 Liquid Chromatograph) was used for the measurement of extracellular water (Appendix C).

3.10 MEASUREMENT OF TOTAL BODY WATER BY DEUTERIUM OXIDE

3.10.1 Stock and standard dose preparation and storage

D_2O (99.8 % atom.100 grams/bottle, ID 2084, ICON, NJ) was used as the stock dose and stored in a refrigerator. The stock and working standards (Appendix A) were prepared and stored in a refrigerator.

3.10.2 Procedure

The Fourier – Transfer Infrared (FTIR) Spectrometer (Avatar 360 with software OMNIC ESP 5.0, Nicolet Wisconsin) was used for spectrometric analysis. Prior to spectrometric analysis approximately 1ml of sample was lyophilized to total dryness and residuals were condensed in a test tube, sealed and stored under refrigeration until spectroscopic analysis. Quality control (Appendix A) and calibration was performed prior to reading samples. The FTIR system was calibrated with working standards before reading of samples. The concentration of D_2O in each specimen was calculated from the standard calibration. TBW was calculated using the following equation:

TBW (Liter) = D_2O / (post dose-pre dose) / 1.04 **(Equation 3.9)**

(Ellis and Wong, 1998)

3.11 MEASUREMENT OF EXTRACELLULAR WATER (ECW) BY SODIUM BROMIDE DILUTION

3.11.1 Stock and standard dose preparation and storage

Measurment of ECW was undertaken by Sodium Bromide Dilution using an HPLC system (Perkin – Elmer HPLC system, and the series 200 Liquid Chromatograph) . Stock solutions (Appendix B) were prepared and stored in a refrigerator. Standard preparations (Appendix B) were also prepared and stored in a cabinet at room temperature. Quality control of newly prepared stock solutions were carried out (Appendix B).

3.11.2 Procedure

The following preparations were carried out before the HPLC readings:

(1) 0.1ml Pre sample + 0.9ml water

(2) 0.1ml Post sample + 0.9ml water

(3) 0.1ml Pre sample + 0.8ml water + 0.1ml 800uM Standard.

Each sample was vortexed and filtered through an ultra free filter unit (cat# UFP1TGCBK, Millpore). The sample (3) was the spiked sample used for calculating post Br concentration. 100ul of filtered sample was injected into the sampler with a microsyringe. The injection syringe was washed with acetone thoroughly after every sample.

3.12 DATA ANALYSIS

Data analysis was performed using the SPSS statistical software version 15.0 and SAS statistical software version 9.2 (SAS Institute Inc, Cary, NC). Estimation of FFM and TBW was undertaken from bio-impedance measures using selected published prediction equations. We then validated against TBW measured by dilution. We used several measures of validation, including correlation, rank correlation, root mean squared error, and average differences (the last to estimate bias). We also used the Bland-Altman approach to determine the agreement between methods. To derive new equations for TBW and FFM we used regression techniques and inclusion of independent variables such as weight, height, age, gender, Ht^2/resistance and Ht^2/reactance. We selected the best models to be those that yield the smallest SEE, minimize loss of precision and show the best limits of agreement.

CHAPTER FOUR

RESULTS

4.1 STUDY COHORT CHARACTERISTICS

This study began in 2008. We recruited 42 black children, of whom 23 were females (55 %). Seventeen children were aged 3-4 years, 9 were aged 4-5 years and the remainder 5-6 years. We had fairly restricted racial and age distribution so we do not expect these to be a major source of confounding in the outcome of results.

All subjects met clinical eligibility criteria for commencing ARV therapy. Two children had mild HIV disease with WHO clinical stage 2, 27 had stage 3 disease and 13 had stage 4 disease. While we would have preferred to have better representation of stage 2 disease the numbers reflected here were representative of the profile of children in the study age group who were commencing ARV therapy in the treatment program at the time of the study. All children were afebrile at time of study.

We screened a total of 61 children, 18 patients were not enrolled because parents declined participation or did not arrive to sign the consent form, and one child died unexpectedly before consent or recruitment. We therefore enrolled a total of 42 children. Chronic co-morbidities were common among these children. A total of 35 samples were used for analysis of blood and 23 samples were used for saliva analysis.

Some of the characteristics of the children with inadquate samples include concurrent tuberculosis, wasting and oral pathology, oral problems such bleeding gums, dental caries, mouth sores, chronic lung disease and other chronic diseases.

These children had varying nutritional status based on anthropometry. 21% out of 42 were stunted and 14% were wasted. Average anthropometic and body composition parameters of our study cohort are displayed in (Table 4.1) below.

Table 4.1: Measured and derived body composition parameters of study patients

Physical characteristics	Mean (SD)
Weight (kg)	12.98 (2.71)
Height	93.35 (7.36)
Mid upper arm circumference	14.69 (1.72)
Weight-for-age z score	0.00 (1.00)
Height-for-age z score	0.00 (1.00)
TBW (plasma deuterium) [n = 35]	9.48 (2.12)
ECW (plasma Br) [n = 39]	4.89 (1.27)
Mean ratio of ECW(NaBr) to TBW	0.53(0.12)
Fat Free Mass (kg) [median, variance]	10.9 (3.7)

4.2 COMPARING THE RELATIONSHIP BETWEEN TBW MEASURED BY DEUTERIUM AND TBW PREDICTED BY SELECTED EQUATIONS

Objective one: To establish whether equations currently used for the determination of TBW and Fat Free Mass (FFM) in Nigerian and HIV-infected American children from bioimpedance assessment are precise enough to be applied for use on young HIV-infected children in South Africa using an isotope dilution method as the reference method. We assessed these through correlation and Bland-Altman plots of agreement.

TBW predicted by Leman (Equation 1) had the strongest correlation with measured TBW (r= 0.522). Horlick (r = 0.506) also had a good correlation. Leman (Equation 2) (r = 0.437) and Arpadi (r = 0.439) had moderate correlation with measured TBW. The use of scatter plots (Fig 4.1 to 4.4) show a fair linear relationship was present between measured and predicted values. The relationship was statistically significant but the strongest correlation was 0.52.

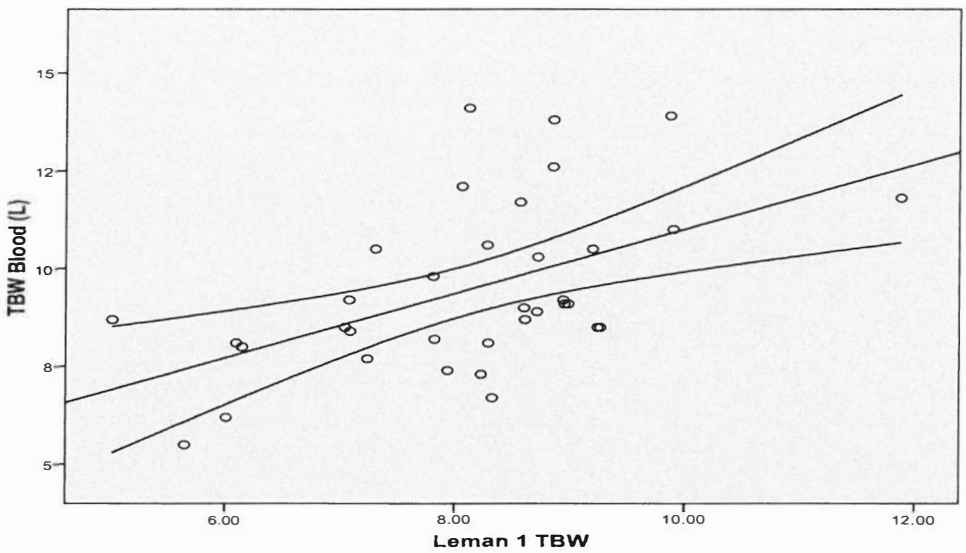

Figure 4.1: Plot of linear correlation between measured TBW and TBW predicted using Leman equation (Leman *et al.*, 2003) (Equation 1)

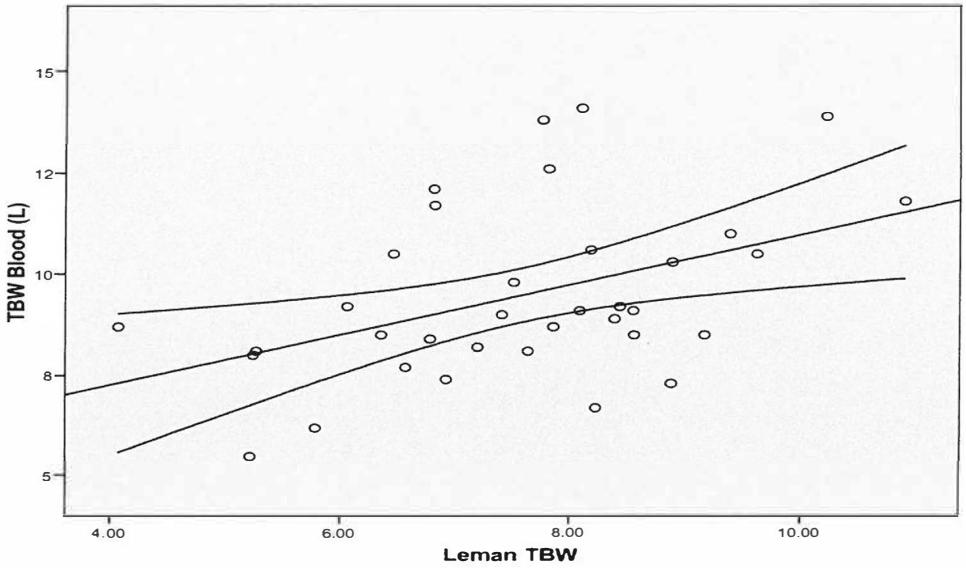

Figure 4. 2: Plot of linear correlation between measured TBW and TBW predicted using Leman equation (Leman *et al.*, 2003) (Equation 2)

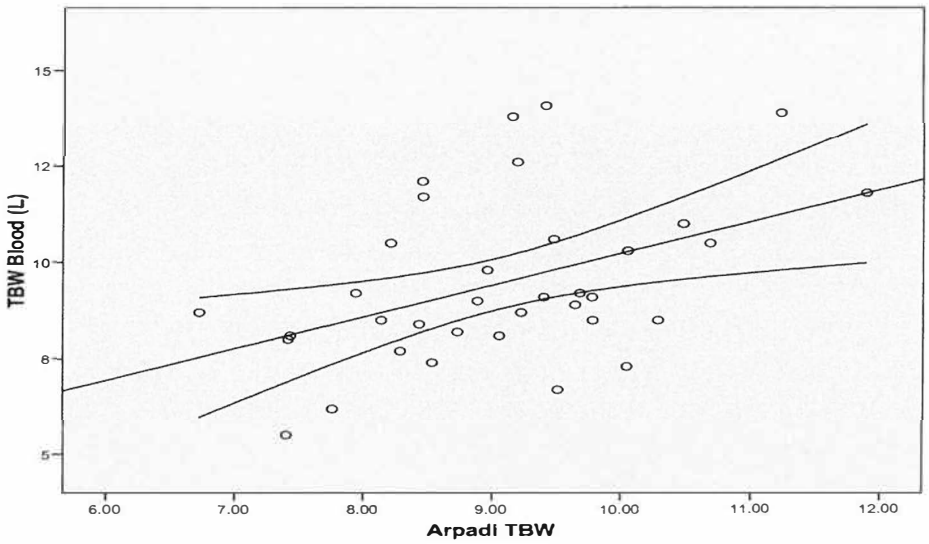

Figure 4.3: Plot of linear correlation between measured TBW and TBW predicted using Arpadi equation (Arpadi *et al.*, 1996)

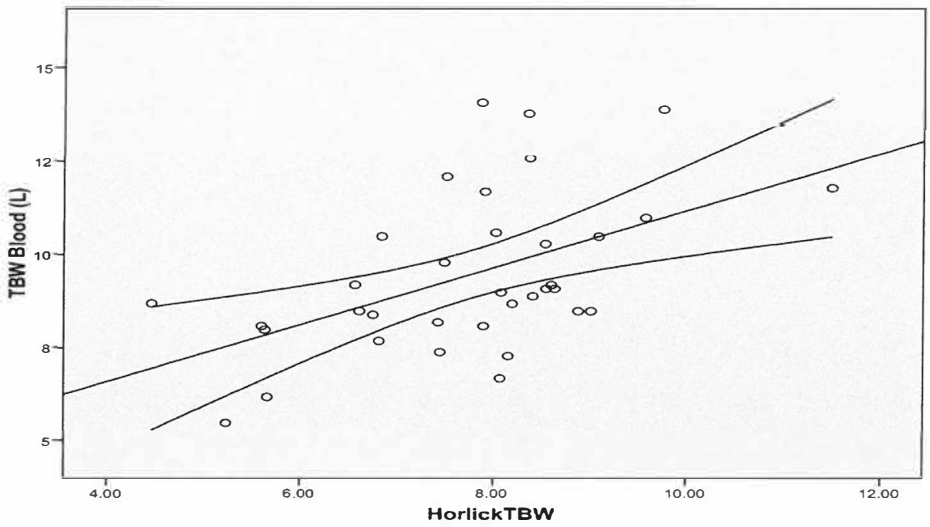

Figure 4.4: Plot of linear correlation between measured TBW and TBW predicted using Horlick equation (Horlick *et al.*, 2002)

Agreement between methods using the Bland-Altman method is summarized in (Table 4.2) and shown in (Fig 4.5 to 4.8).

Table 4.2: Agreement between predicted TBW using existing equations against measured TBW (L)

EQUATION	BIAS*	95% LIMITS OF AGREEMENT
(Leman *et al.*, 2003) (equation 1)	-1.34	-4.93 to 2.24
(Leman *et al.*, 2003) (equation 2)	-1.90	-5.81 to 2.01
(Arpadi *et al.*, 1996)	-0.40	-4.16 to 3.36
(Horlick *et al.*, 2002)	-1.72	-5.37 to 1.93

*Bias refers to the difference between TBW measured through isotope dilution and TBW estimated from the described equation

The Bland-Altman plots of agreement indicate that all equations tended to underestimate TBW. Again, examination of those children showing poor agreement on these plots revealed that those children with the most extreme underestimates on predicted equations showed more severe co-morbidities. Fifty percent of those children having bias worse than -1L with the Arpadi equations had concurrent tuberculosis.

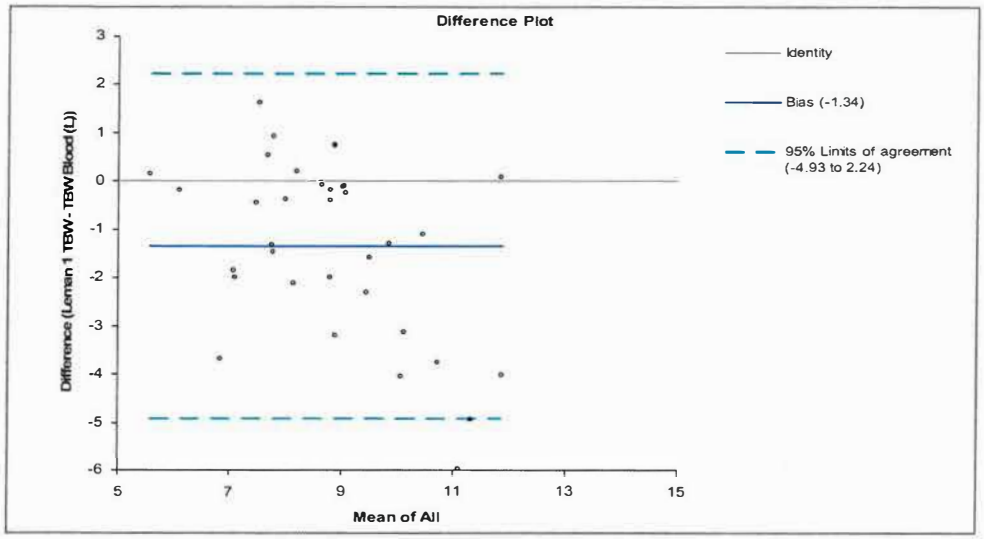

Figure 4.5: Bland-Altman Plot of agreement between TBW blood and (Leman *et al.*, 2003) (Equation 1)

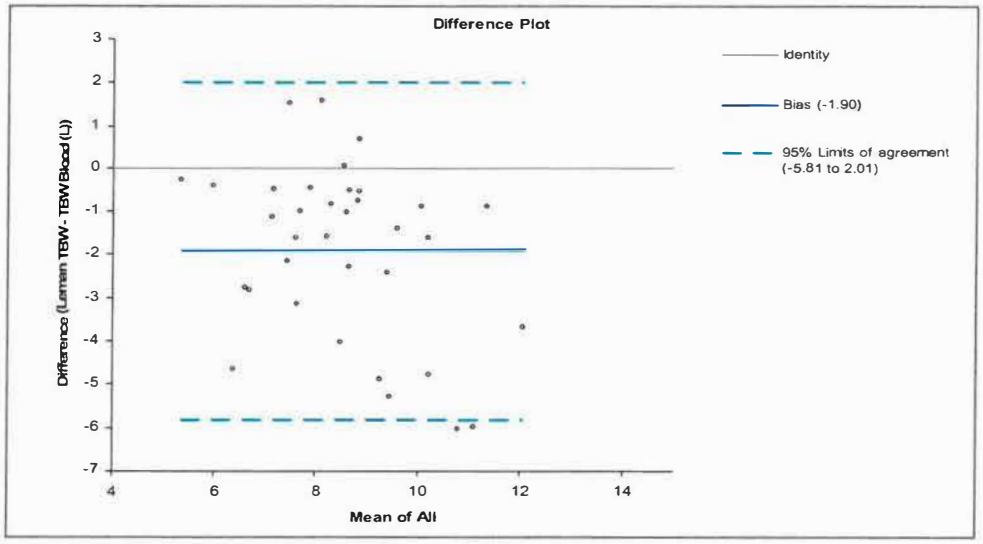

Figure 4.6: Bland-Altman Plot of agreement between TBW blood and (Leman *et al.*, 2003) (Equation 2)

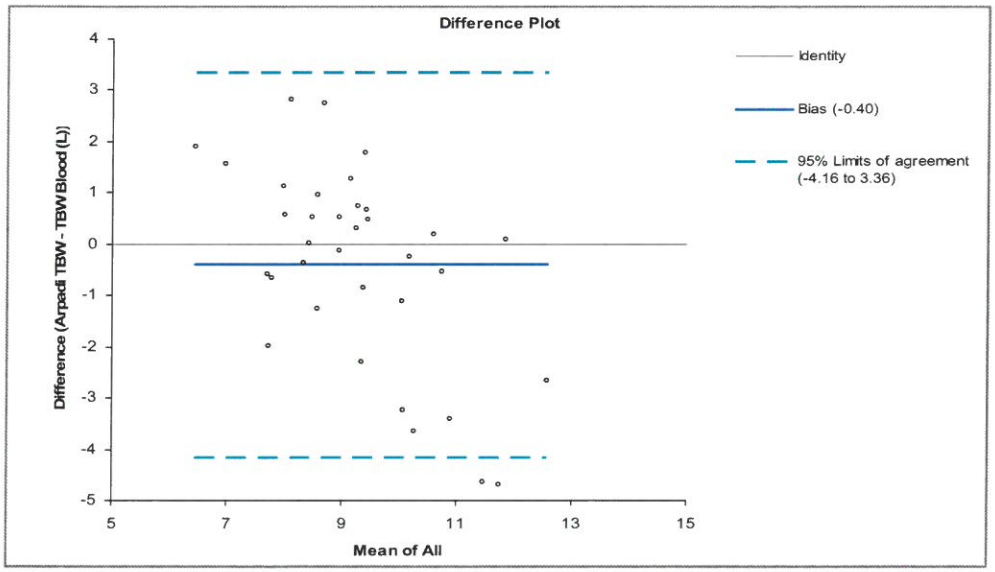

Figure 4.7: Bland-Altman Plot of agreement between TBW blood and (Arpadi *et al.*, 1996)

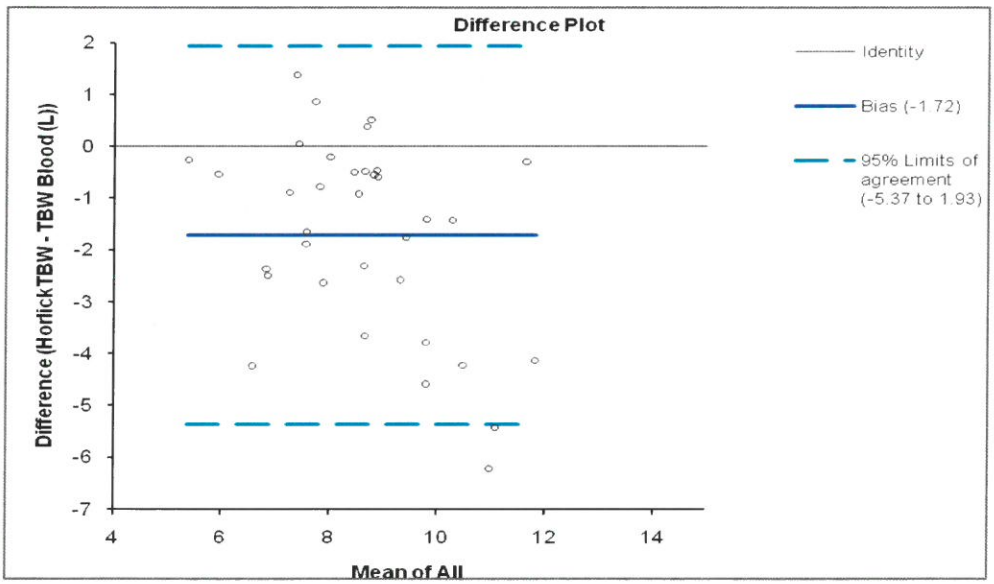

Figure 4. 8: Bland-Altman Plot of agreement between TBW blood and (Horlick *et al.*, 2002)

4.3 FAT FREE MASS ASSESSMENT

4.3.1 Relationship between FFM derived from measured TBW and FFM was predicted by use of existing equations using Bio-impedance measures

FFM derived from measured TBW showed the relationship was statistically significant with the strongest correlation 0.55. The Bland-Altman plots of agreement indicated little bias (0.22) with the Horlick *et al* equation showing better precision as shown in (Table 4.3).

Table 4.3: Agreement between predicted FFM using existing equations against FFM derived from measured TBW

EQUATION	*BIAS*	*95% LIMITS OF AGREEMENT*
(Arpadi *et al.*, 1996)	0.22	-4.19 to 4.63
(Horlick *et al.*, 2002)	0.22	-3.43 to 3.86

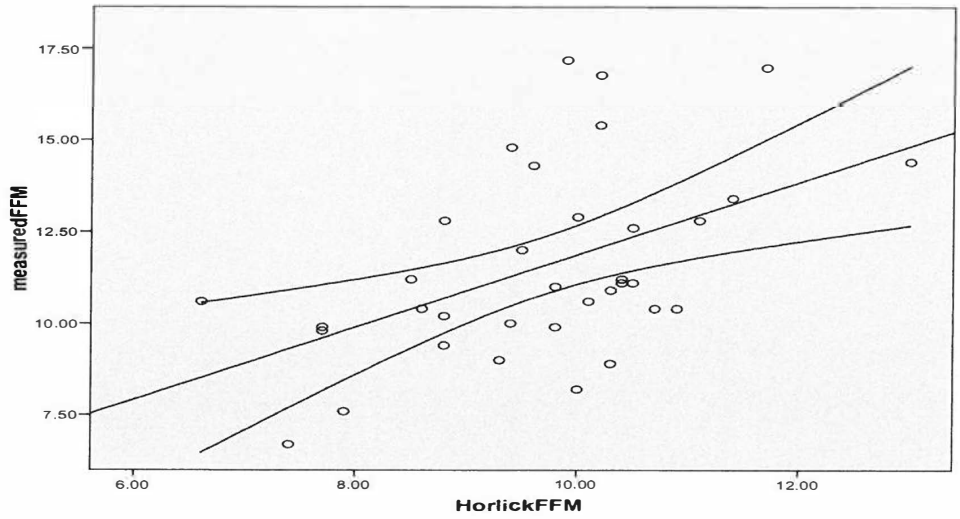

Figure 4.9: Plot of linear correlation between measured FFM and FFM predicted using (Horlick *et al.*, 2002)

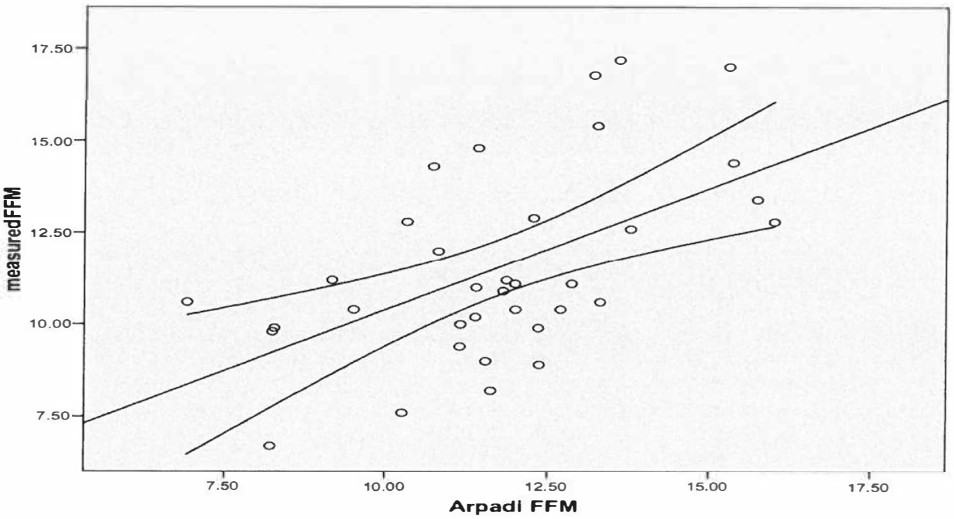

Figure 4.10: Plot of linear correlation between measured FFM and FFM predicted using (Arpadi *et al.*, 1996)

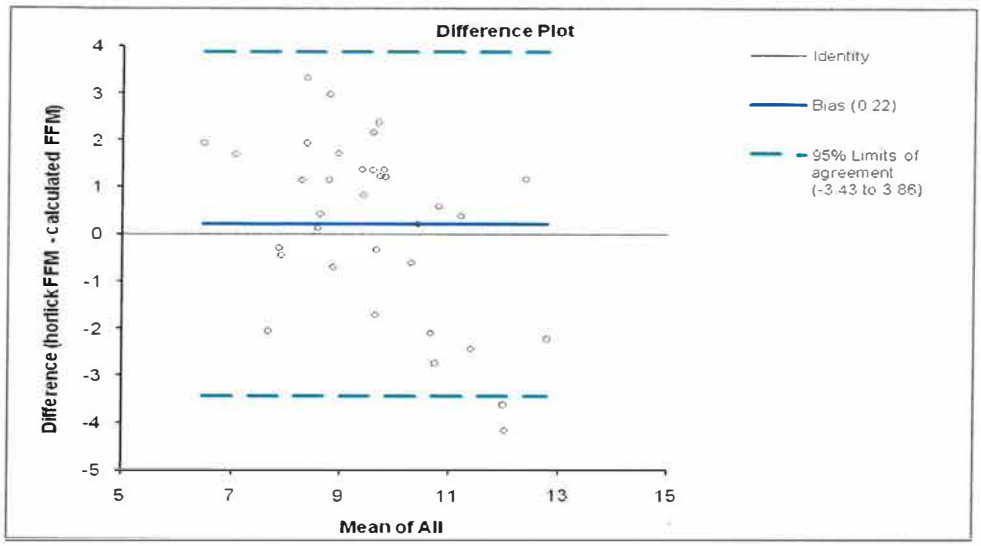

Figure 4.11: Bland-Altman Plot of agreement between measured FFM and (Horlick *et al.*, 2002)

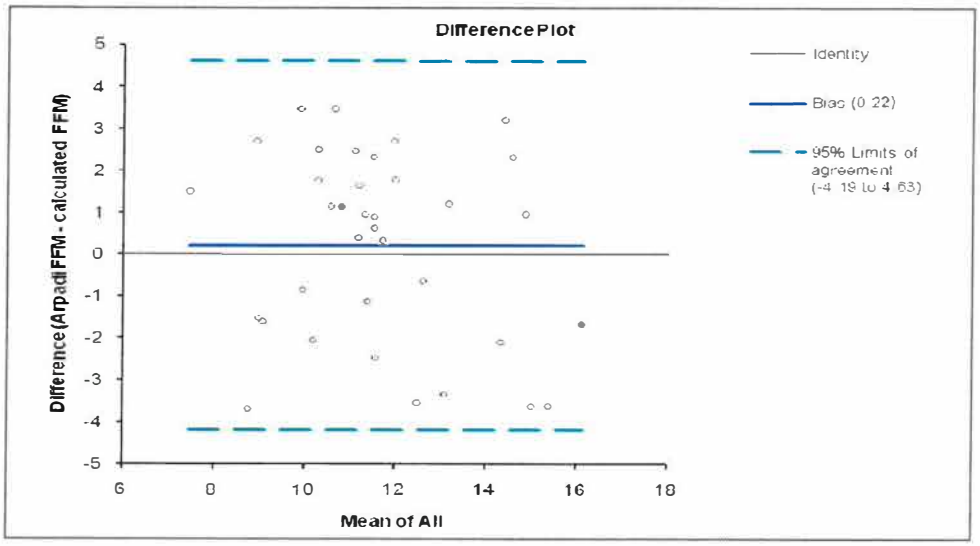

Figure 4.12: Bland-Altman Plot of agreement between measured FFM and (Arpadi *et al.*, 1996)

4.4 COMPARISON OF THE USE OF BLOOD AND SALIVA SPECIMENS FOR BODY COMPOSITION ASSAYS

Objective two: To determine the feasibility of blood and saliva specimens in isotope studies and to compare the performance of blood and saliva using the isotope dilution method in children aged 3 to 6 years. Saliva collection was difficult in some children and there were several specimens with volumes below 1ml. There were six samples that produced implausible saliva values ranging from 27 to 326L for TBW. These were eliminated from subsequent analyses.

After elimination of the six implausible salivary values, Pearson's correlation between TBW blood and saliva was very good (r = 0.78) (Fig 4.13). Mean (SD) for TBW blood was 9.48L (2.12) and for TBW saliva 10.10L (2.25).

This study found from the Bland-Altman plots (Fig 4.14) that saliva over-estimated TBW compared to blood. Agreement between the two was poor at higher range of measurement values.

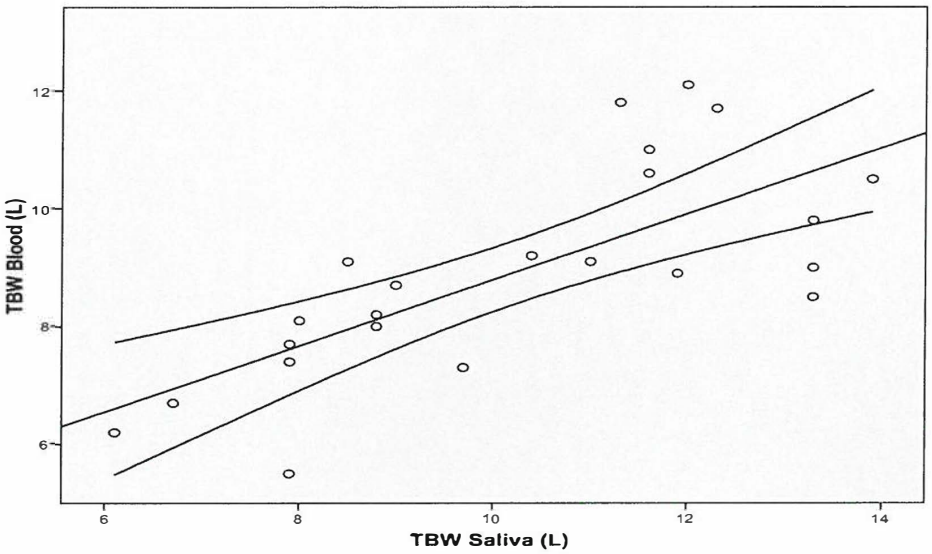

Figure 4.13: Linear correlation between TBW Blood and TBW Saliva

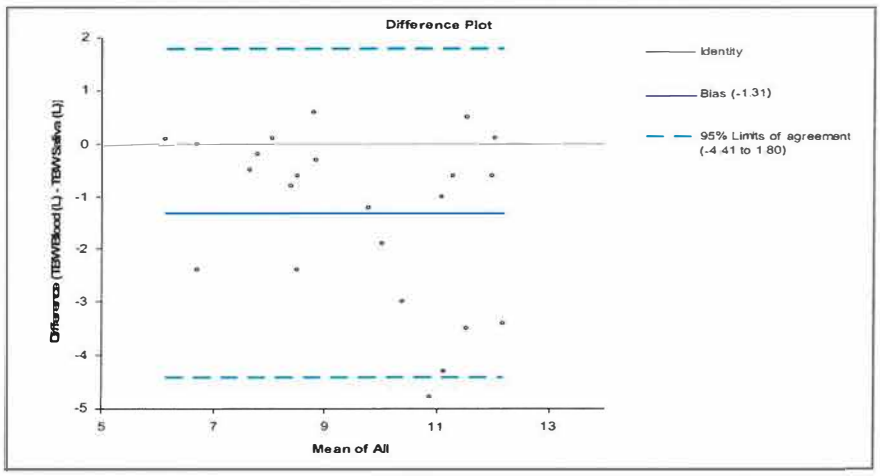

Figure 4.14: Bland-Altman Plot of agreement between TBW blood and TBW saliva measurement

4.4.1 Characteristics of children with poor correlation between blood and saliva.

Of the 42 children, nine children with poor agreement had co-morbidities. We found that five of these nine children had oral lesions, namely severe gingivitis with dental caries or blisters; and one of them also had TB meningitis. Only two of the children with good agreement, that is bias above -1.3L up to 1L, had mild oral lesions and one had chronic non-specific lung disease.

CHAPTER FIVE

DISCUSSION

The main goal of this study was to determine whether prediction equations used in Nigerian and HIV-infected American children were precise enough to be applied for use in young HIV-infected South African children using the isotope dilution method as the reference method.

The application of existing equations to our population was of interest due to the paucity of appropriately developed predictive equations in this population. Subjects from the prediction studies used had no opportunistic infections or they were relatively healthy. The data presented in this study showed predicted and measured TBW values had a poor agreement. We postulate that this may be due to altered isotope equilibration or to altered bioelectric impedance; and offer rationale for these below.

Our study cohort had a high prevalence of various chronic opportunistic infections or conditions coupled with HIV. The breakdown of intestinal mucosal barrier with microbial translocation is a major factor in HIV progression (Lackner, 2009). This maybe suggestive that an increased intestinal permeability and epithelium damage may have affected the outcome of our results by altering absorption and equilibration time of the isotope; hence leading to an overestimation of TBW values from the measured isotope. Tests such as the lactulose-mannitol test which assesses intestinal permeability have been applied in HIV studies (Lima *et al.*, 1997).

The loss of mucosal integrity leads to increased lactulose absorption and the absorption of mannitol is decreased due to loss of absorptive space (Fleming *et al.*, 1990). In a study with patients in the advanced stages of HIV, it was found that mannitol excretion was decreased (Pernet *et al.*, 1999). In another study Stockmann *et al* (1998) showed increased lactulose levels in HIV-infected patients with duodenal impaired epithelial barrier function.

Our cohort may have also had abnormalities such as altered intra- and/or extracellular hydration that affected conductivity and ultimately affected our results as compared to other predictive studies. A previous study has demonstrated altered extracellular to intracellular water ratio in longitudinal follow-up of stunted children (Hoffman *et al.*, 2006). We postulate that stunting coupled with catabolism arising from serious co-morbidities may further alter this ratio.

Previous studies have shown that HIV-infected children have a lower percentage of FFM compared to normal healthy children (Miller *et al.*, 1993) (Fontana *et al.*, 1999). We found our study cohort had a relatively low FFM even when anthropometric Z scores were not severely affected. This maybe attributed to our study cohort having preferential and inappropriate catabolism of muscle mass, as seen in cachexia rather than long-lasting semi starvation. This is further supported by a study in American HIV infected children whom had diminished FFM as compared to FM (Arpadi *et al.*, 1998).

Agreement between FFM derived from measured TBW and FFM prediction equations by (Arpadi *et al.*, 1996) and (Horlick *et al.*, 2002) showed a good correlation with Arpadi (r= 0.548). However in our Bland-Altman plots, poor agreement was observed with prediction equations used. This was seen by the Arpadi equation overestimating FFM as compared to the Horlick equation. Despite attention to standardized measurements, remaining error in measurement may be possible.

The second aim in our study was to determine feasibility of blood and saliva specimens for body composition analysis and to compare the performance of blood and saliva using the isotope dilution method in children aged 3 to 6 years. A number of studies have shown good results in using saliva for TBW analysis (Horlick *et al.*, 2002) (Arpadi *et al.,* 1996). In our study there was a good correlation between TBW blood and saliva (r = 0.78), however, Bland-Altman analysis revealed saliva overestimated TBW although an adequate equilibration time was employed. We found a large number of our study subjects with poor agreement between saliva and blood TBW measurements, had oral problems coupled with opportunistic diseases.

We discuss below possible reasons for this observation. Various substances cross the mucosal barrier either by transcellular, intracellular or pass between cells via intracellular routes (Squier and Kremer, 2001). Findings from a study by (Hoogstraate and Bodde, 1993) showed tracer penetration was limited at the boundary of granular and keratinized layers of the oral mucosa and failed to penetrate the epithelium sufficiently. This may be a mechanism at play in our study children with oral ulceration.

Findings from a study by Kaufman *et al* (2002) showed serum may reach saliva either from the gingival crevicular fuid, through the oral mucosa and from the salivary glands affecting oral mucosal integrity. In a study by (Sweet *et al.*, 1995) it was seen that *C.albains* isolates from HIV subjects adhered in vast numbers to the buccal epithelial cells causing alteration in the epithelial cell surface. Studies done related to the buccal mucosa showed that compounds pass through a thicker membrane with an increase in lag time (Nicolazzo *et al.*, 2003).

In this regard, our findings may suggest that changes in the oral mucosa due to infections, co-morbidities and breakdown of mucosal intergrity due to oral lesions, presence of blood, caries and other oral manifestations may be an eminent factor for measurement variation in salivary samples. This may be due to incomplete absorption of isotope or 'entrapment' of isotope in oral mucosa resulting in higher salivary levels of isotope, i.e. we may be measuring isotope entrapped in mucosa or salivary glands rather than that absorbed from intestine and entering saliva through equilibration.

A review of the predictive value of existing equations shows that they had excellent predictive values in the study populations that they were developed and validated in. The variables included in these equations explained 95% to 99% of the variance in TBW in the published studies. In our study however these variables explained only up to 30% of the variance in TBW using Arpadi equations, and less for the other equations. We therefore explored additional variables in multiple regression models and found reactance to be an important predictor.

The following equation: **TBW= -3.8 + 0.13(Ht) +0.07(reactance) -0.004(resistance) (Equation 5.1)** had an $\mathbf{R^2}$ of 0.54 and **root-MSE** of 1.5. Even though it still leaves much unexplained variance it has better predictive value than existing equations. The unexplained variance may be related to the nature of chronic co-morbidities in our study population. This however requires further validation.

As per description on page 52, we restricted participation to a fairly homogenous age group to overcome the variability associated with age. In terms of performance of BIA and salivary measures it is conceivable that age may have affected outcomes. Untreated HIV in young children is likely to be associated with subclinical mucosal and body composition changes that affect the performance of these measures. These children are also more likely to have more advanced HIV disease than older cohorts.

CONCLUSION

In conclusion, the present study demonstrated that predicted and measured TBW had poor agreement in our population. Co-infections coupled with HIV seen in patients in this study may have adversly affected the performance of predictive equations used. Thus far to our knowledge this is one of the first studies evaluating the performance and modification of predictive equations developed in other countries for use in South African HIV infected children. We offer additional variables for use in predictive equations specific for our population. While saliva samples are usually preferable for isotope studies in young children, we recommend caution in HIV-infected children with co-morbidities, especially oral/mucosal pathology.

RECOMMENDATIONS

1. Future Research

The development of new equations in this study was exploratory and hence provides preliminary data for subsequent research that aims to validate new equations. Findings from this study may contribute to future clinical and community-based studies to reliably document changes in body composition in HIV-infected children.

2. The use of Bioimpedance method in practice

The use of bioimpednace in non-invasive, inexpensive and portable. This technique has shown to work well in healthy persons as well as those with chronic illness. Futhermore, BIA as revealed in this study has the ability to predict TBW and intracellular and extracellular water. In practise, this is a very valuable technique that can be used in individuals with HIV, thus monitoring changes in body composition and proper nutritional interventions can be set up. BIA appears to be a useful non-invasive technique to assess body composition in HIV-infected children as young as 3 years of age in the absence of clinically apparent co-morbidities. In those with clinically apparent comorbidities its ability to predict body composition is more limited.

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APPENDICES

APPENDIX A: PREPARATION OF STANDARDS FOR TBW BY DEUTERIUM OXIDE AND QUALITY CONTROL

A) Stock standard preparation

For the stock standard (15.0mg/ml), 15000gram of D20 stock was weighed and diluted to 15mg/ml with deionised water in a 1000ml graduate flask.

B) Working standard

Six working standards were prepared with concentration ranges of 0.0, 0.15, 0.30, 0.45, 0.60 and 0.75mg/ml in water by weighing the stock standard. These were used for assay calibration.

C) Quality Control

A dose of D_2O was weighed and added to an 8 litre plastic bottle containing 6000ml of dH_2O. This mixture is called phantom The D_2O concentration in the phantom was measured before assays were done. The D_2O dilution volume in the phantom was calculated as the dose weight divided by the post equilibrium D_2O concentration in the phantom. The ratio of the calculated phantom volume to the pre set volume of 6000ml is the quality control index. If the quality control was >0.95< the assay procedure was repeated.

APPENDIX B: STOCK , STANDARD DOSE PREPARATION AND STORAGE FOR MEASUREMENT OF EXTRACELLULAR WATER (ECW) BY SODIUM BROMIDE DILUTION

Stock dose solution prepartion

A) Pre-prepration

NaBr: Purified NaBr purchased from Fisher scientific (Cat#S255-500) was dried in an oven at 50^0C prior to preparation of the stock solution.

Water: Water that was used for solution preparation and Br assay was deionized with a deionizing system (Cat# FCR of 1001, ZWDJ 02531,zwdj 02532,Ion Pure, Lowell,Ma) and filtered with a 0.2 um filter unit (Cat# 1260020,Nalgene Company, Rochester,NY)

Glassware and plastic containers: All glassware and containers that was used for making the stock colutions and Br assays were rewashed with the filtered ionic free water

B) Stock solution

NaBr was weighed to 411.6gram and dissolved in 500ml of water in a 1000ml volumetric flask. Water was added till the 1000ml mark and mixed until it was completely dissolved. This step was repeated 4 times to make up 6 litres. After mixing, a 300ml aliquot of the stock solution was transferred to a plastic bottle. Each stock solution bottle was labelled with date, concentration, date of expiration and stored in a refrigerator.

C) Quality control for stock solution

The Br concentrations of newly prepared stock solutions were measured on the HPLC system and compared with the working standards that were currently used on the same day. A paired t test was used to test the differences. If a significant difference ($p<0.05$) was observed for any standard, it was remade and if a significant difference for any two standards was observed, the stock solution was remade.

D) Preparation of working standards

A diluted stock solution (200mM/I) was made by taking 5ml of the stock solution into a 100ml volumetric flask and adding water to the 100mk mark. The solution was mixed and labelled. Working standards of 200,400,600 and 800uM/1000ml were made in a 1000ml volumetric flask with 1ml, 2ml, 3ml, and 4ml of the diluted stock solution. Each standard was capped, labelled with date and concentration and stored.

E) Quality control before sample analysis

An 8 litre bottle filled with 6 litre of water measured accurately was used as the phantom. An NaBr dose was weighed on each study day. The dose was injected into the phantom and then thoroughly mixed. The daily assay quality was tested by measuring the following three specimens prepared in a test tube (cat#14-162-10D,Fisher) prior to assay in the subject's specimens.

(1) 0.1ml pooled plasma + 0.9ml water

(2) 0.1ml pooled plasma + 0.1ml taken from a phantom prepared daily for quality control test + 0.8ml water

(3) 0.1ml pooled plasma + 0.1ml 600uM working standards + 0.8ml water

Each tube was mixed on a vortex for 10seconds. The samples were then filtered through an ultra free (10.000NMWL) Filter unit (cat#UFP1TGCBK, Millipore). The quality control samples were read the same as patient samples. The quality control score was calculated by using the peak area readings of the three samples with the following equation on a PC system:

QC % = (NaBr dose for phantom / (phantom concentration x 6)) x 100%

QC% = (((Nabr dose weight (g) for phantom x 4 M) / 1.28) /(((sample 2 – sample1) / (sample 3 – sample1)) x 600)/ 6) x 100%

*Where **4M** is the stock dose concentration, **1.28** is the specific gravity of the stock solution, and **600uM** is the working standard concentration used for the QC test.*

If the QC % was >2 Z-score of the accumulated QC %, the HPLC system was readjusted.

F) Purging the HPLC system

Purging of the HPLC system was done using the following three steps:

1) The reservoir chamber door was opened to allow helium gas to bubble through the solvents for approximately 5 minutes, therafter the chamber door was closed.

2) Increase in reservoir pressure was held between 7-9psi

3) When steady state pressure was reached, the system was ready for purging. A 10ml syringe was connected to the flush port. Water was used to purge the system and the flow rate was automatically set. The drain valve was opened and the water solvent was drained into the syringe automatically. When bubbles were expelled the purging step was complete. The steps above were repeated using NaCl. During flushing with the NaCl solution, the detector was adjusted until the baseline was stabilized at "0". The system was then ready for sample assays.

APPENDIX C: EXAMPLE OF LABORATORY ASSAY DATA

	DATA		
DATE OF ASSAY:	01/20/10		
RECOVERY DATE:	05/25/09		
REC DOSE WEIGHT (gm)	1.26633	4.45322	3.18689
REC DOSE(meq)=DOSE WT*4/1.28	3957		
HPLC READINGS:			
POOLED PLASMA:	1098184		
POOLED PLASMA + REC	1609249		
SPIKED POOLED PLASMA:	1563116		
SPIKING STD CONC:	600	meq/ml	
REC CONC (meq):	659.5351578		
RECOVERY (%):	100.0		
PATIENT NAME	TEK023 SALIVA		
BCU #:			
DATE:			
WEI SYRINGE	12.25169	gram	
SYRINGE + Br DOSE	13.86698	gram	
BR DOSE WEIGHT:	1.61529		
BR DOSE (meq) = dose wt*4/1.28	5048	meq	
HPLC READINGS:			
0' SALIVA:	36052		
120' SALIVA:	702426		
SPIKED 0' SALIVA	432244		
SPIKING STD CONC.:	800	ueq/l	
CALCULATION:			
NET 180'=	666374		
NET SPIKED STD=	396192		
180' SALIVA CON=	1346	ueq/l	
ECW = BR DOSE(meq)/pl con	3.8	l	
CORRECTED ECW = ECW * 0.9 =	3.4	l	

Figure 4.15: Assay data for the analysis of ECW by Sodium Bromide Dilution

DEFAULT REPORT

P..	Time[...	Area[µV·s]	Height[µV]	Area[%]	Norm...	BL	Area/Height[s]
4	3.292	25.72	39.51	0.00	0.00	BB	0.6764
5	3.823	10.78	31.15	0.00	0.00	BB	0.3459
6	4.058	16.53	37.73	0.00	0.00	BB	0.4383
7	4.391	2748.34	95.29	0.15	0.15	BB	28.8423
8	5.631	1127543.35	33808.68	62.04	62.04	BB	33.3507

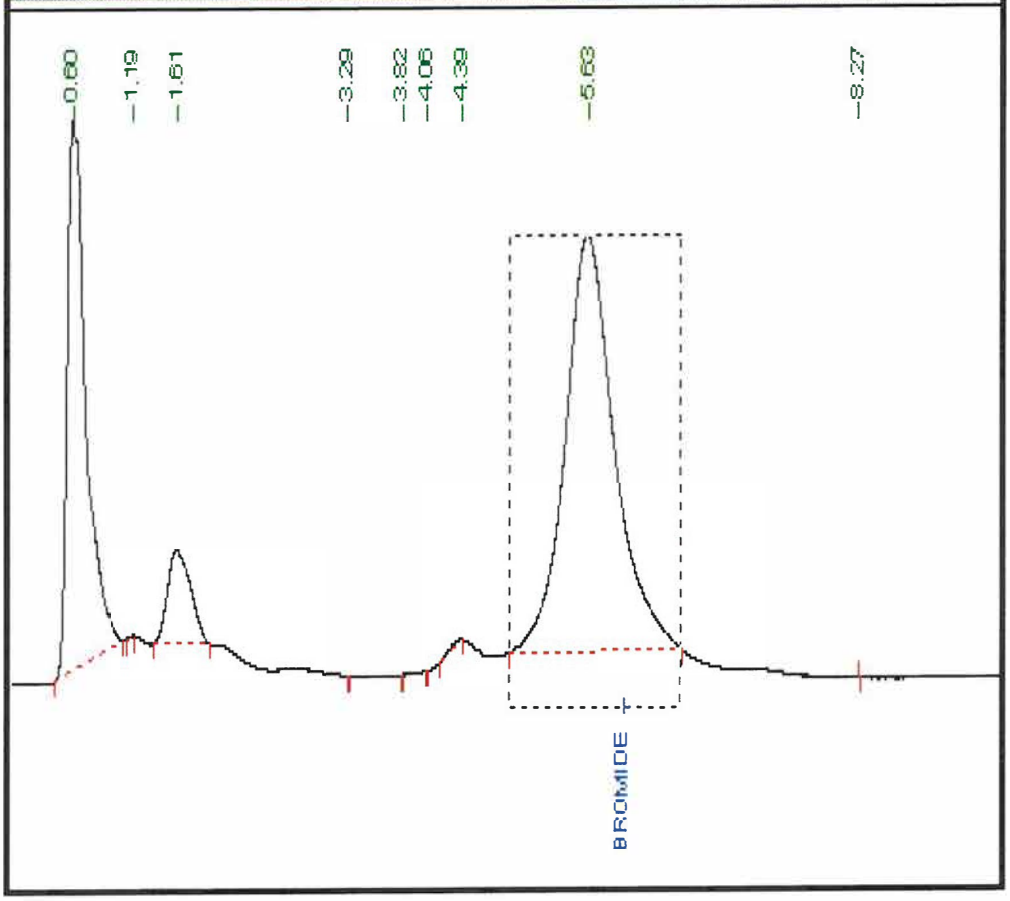


Figure 4.16: bromide peak for ECW analysis

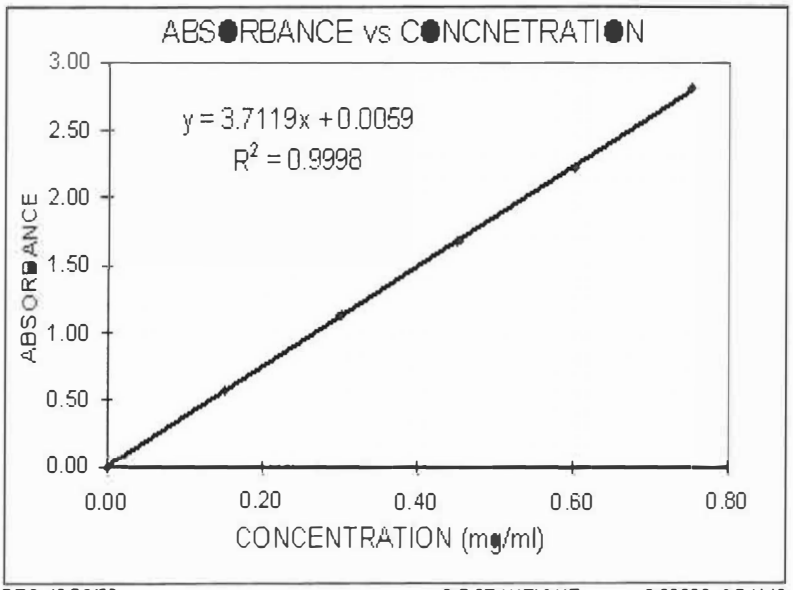
THE TBW MEASURED BY D_2O USING FT-IR

DATE: 12/11/2009 OPERATOR: wen

AREA FOR CROSS-VALIDATION

STD (mg/ml)	0.00	0.15	0.30	0.45	0.60	0.75
	-0.0390	0.4964	1.0490	1.6323	2.1774	2.7869
	0.0072	0.5658	1.1172	1.6374	2.1921	2.8473
	-0.0510	0.5962	1.1967	1.7038	2.2012	2.7925
	0.0365	0.5673	1.1011	1.6477	2.2270	2.8650
	0.0489	0.5805	1.1593	1.7447	2.2369	2.7589
Average	0.0104	0.5775	1.1436	1.6834	2.2217	2.8134
SD	0.0445	0.0141	0.0431	0.0502	0.0184	0.0508
Net	0.0000	0.5671	1.1332	1.6730	2.2113	2.8030
		0.5671	0.5661	0.5398	0.5383	0.5917

Slope: 3.712 *Intercept:* 0.00595 R^2 0.9998 *SEE:* 0.0150



REC 10/26/09 DOSE WEIGHT: 2.20208 GRAMS

						MEAN	SD
0' READING:	-0.0450	0.0511	-0.0220	0.0464	-0.0350	-0.0009	0.04496
180' READING:	1.3893	1.3664	1.3799	1.3957	1.3770	1.3817	0.01211

CONCENTRATION : 0.372 mg/ml

RECOVERY(%) 98.5 % 5.91222

Figure 4.17: FTIR standard curve and readings for TBW measurments

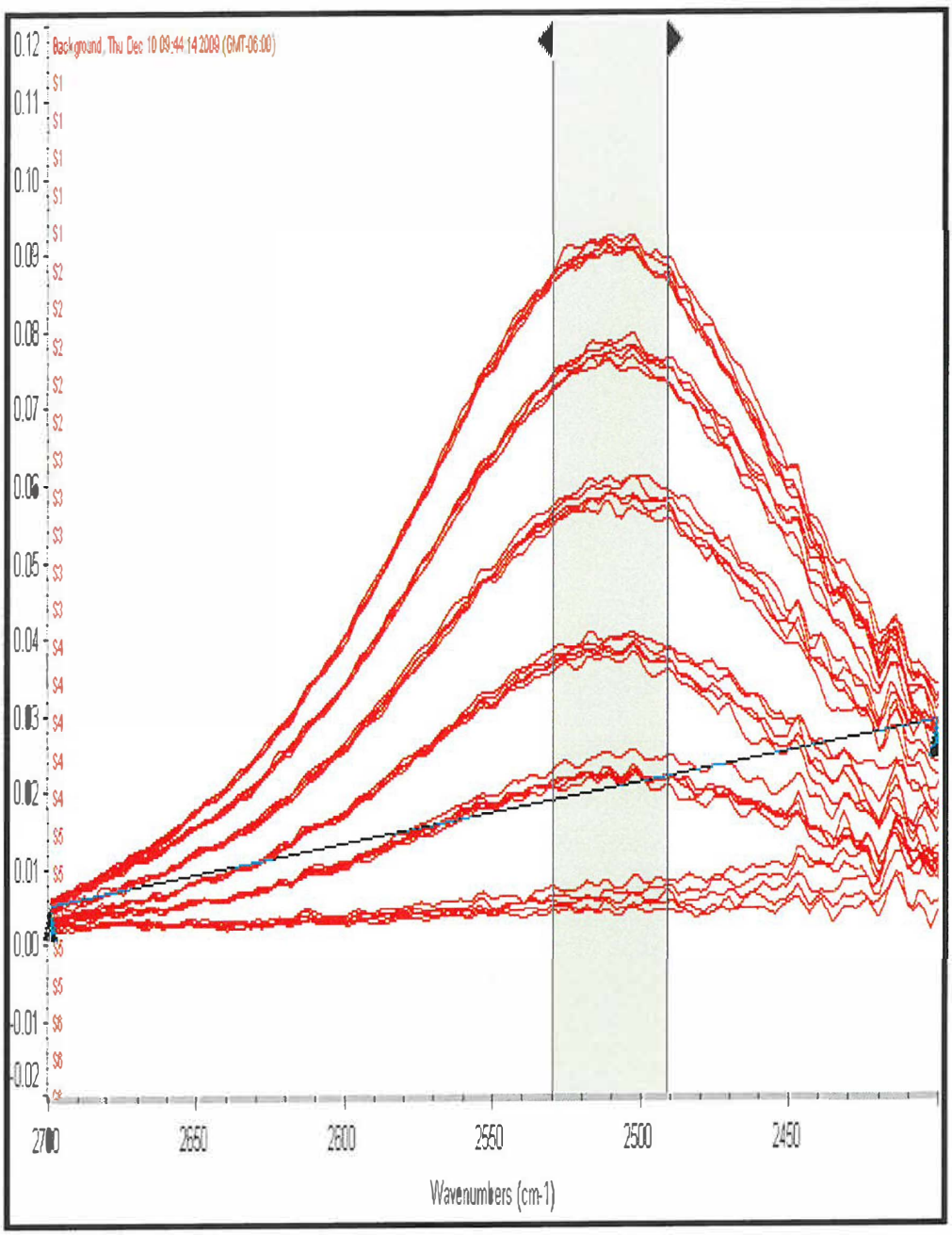


Figure 4.18: FTIR standard curve peaks

APPENDIX D: PATIENT CONSENT FORMS IN ENGLISH AND ISIZULU

CONSENT FORM

Study title: Study of bioimpedance analysis in South African children

Introduction:

A group of us (doctors and scientists) from the University of KwaZulu Natal in Durban and the St Luke's Roosevelt Hospital in New York, are doing research to see whether simple and inexpensive tests can be used in children to assess the percentage of fat and muscle in the body. These tests have already been done on adults locally. They have also been done on children in New York.

This study involves research. In research scientists or doctors try to answer some important questions that may help improve the health of other babies and of the community at large. This research may not have direct benefit for your baby, but we expect the new information to have benefits for other babies in the future.

Invitation to participate:

You are being asked to allow your child to participate in a research study entitled "Study of bioimpedance analysis in South African children" because he/she is between 3 to 5 years old.

Purpose of the Study:

The purpose of this study is to assess whether simple and inexpensive tests can be used to assess the percentage of fat and muscle in the body. Other tests are too expensive and unavailable, too difficult to perform in children, or may require blood to be drawn. In this study we will compare a test called 'Bioimpedance Assessment', which is easy to perform and not painful, to a more expensive test called 'isotope dilution', which is difficult to perform and involves taking blood. We are hoping that information from this study will enable us to use the simple, non-painful test for children rather than using other tests. The reasons for wanting to know how much muscle and fat children have in the body may be of use to doctors to see what effect different chronic illnesses have on children. It may also be used as simple way to check if children are responding to treatments.

What is involved in the study: We will enroll 30 children in total from King Edward VIII Hospital in Durban. We will need to see your child only once for this study. We will perform a few measurements that involve measurement of weight and height, a procedure called BIA or bioimpedance assessment and drawing of blood before and 2 hours after drinking a special solution. We will also collect saliva at the same times that we collect blood.

All of the procedures to evaluate your child have been used previously in research and clinical settings.

Bioimpedance assessment involves passing a very small electric current through the body. Humans are unable to feel this, and it will be similar to having an ECG done. Electrodes will be placed on the wrists and feet to obtain a recording. It will not take longer than a few minutes. Thereafter we will draw some blood and collect saliva from your child. We will then give him/her a harmless solution to drink. This solution contains

a substance that we can measure in the blood and saliva that is not normally present in the body. The substance will eventually be eliminated from the body. Two hours after drinking this we will have to take another blood and saliva sample. The total volume of blood taken, 8ml (less than 2 teaspoons), and total volume of saliva taken, 4ml will not have any harmful effect on your child.

Blood and saliva will be frozen and stored until the end of the study. Blood and saliva from all subjects will then be sent to a special laboratory in New York to be processed because there is no such facility in South Africa. The blood and saliva will only be used for the test described in this document. Any remainder will then be safely discarded. These results will not be immediately available. They will only become available after the study is completed. We will however inform you of the results by contacting you telephonically or in writing once we have interpreted these tests. The results will have no influence on the current treatment of your child. Specimens will not have your child's name on the label. They will have a code that only the researchers in this study will be able to link to your child.

Risks of being involved in the study. Some of the risks of having blood drawn include soreness and bruising at the puncture site. At the site of punture, there may be a small risk of wound infection whenever blood is drawn. We will take every precaution to avoid this from happening. If this problem arises we will help you obtain the necessary care for your child. The other procedures have no risk associated with them.

Benefits of being in the study. The study and tests will not benefit your child directly. Please do not confuse this with other tests and treatments that may be given at this clinic. You may ask if you are uncertain about what aspects are related to this study alone. We anticipate these tests to be of value in the care of other children in the future.

Participation is voluntary. You may choose not to participate in this study. It will not have any bad effect on your baby's treatment or management, or involvement in other studies.

Costs and Reimbursements: There are no direct costs to you. There are no direct financial incentives for participating in the study. We will provide you with a snack when waiting 2 hours for repeat testing. The study does not require repeated visits. We will provide you R20 as compensation for any inconvenience that the 2 hour wait may create.

Confidentiality: Efforts will be made to keep personal information confidential. All forms and computers will be protected. Personal information may be disclosed if required by law. Organizations that may inspect and/or copy your research records for quality assurance and data analysis include groups such as the Research Ethics Committee.
If results are published or presented at meetings, it may lead to group identification but the individual identity of you and your child will not be disclosed.

This study has been approved by the Ethics Committee at the University of KwaZulu Natal in Durban and St

Luke's Roosevelt Center in New York. These committees are responsible for making sure that risks (if any) to the subject will be outweighed by potential benefit to the subject and/or to the importance of the information to be gained. They want to ensure that the rights and welfare of each person is adequately protected and that informed consent will be obtained.

If you ever have questions about this study , or in the case of a research-related injury , you should contact the principal investigator Dr Meera Chhagan at (031) 260 4345, Department of Paediatrics and Child Health, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, 719 Umbilo Road, Congella, 4013 Durban.

You may contact the Biomedical Research Ethics Office at the Nelson R Mandela School of Medicine at (031) 260 4604 if you have questions about your rights as a research subject. The contact person is Ms Suraiya Buccas and the chairperson is Professor Jack Moodley. The address of this office is:

Medical Research Administration
Nelson R Mandela School of Medicine
University of KwaZulu-Natal
719 Umbilo Road
Congella, 4013

Do you wish to ask any questions at this time?

May I now ask you to sign this form consenting to your child being entered into this study and allowing us to perform these tests.

We thank you for your co-operation and time. Please feel free to approach us should you have any queries in the future. We will give you a copy of this form.

SIGNATURES

If you have read this consent form, or had it read and explained to you, and you understand the information, and you voluntarily agree for your child to participate in the study, please sign your name or make your mark below.

Participant Name (Print)	Participant Signature	Date
Study Staff Conducting Consent Discussion (print)	Study Staff Signature	Date

IMVUME EGUNYAZIWE

Isihloko socwaningo: Ucwaningo lokucubunga i"bioimpedance" kubantwana base Ningizimu Afrika

Isingeniso:

Ithimba lethu (odokotela kanye nososayensi) baseNyuvesi ya KwaZulu Natal eThekwini kanye nabase St Luke's Roosevelt Hospital e New York, senza ucwaningo ukubheka ukuthi kuhlola okulula nokungabizi kakhulu kungasetshenziswa ezinganeni ukuhlola inani lamafutha kanye nemisioha emzimbeni. Lokhu kuhlola sekuye kwenziwa vele kubantu abadala endaweni. Kwenziwe futhi kubantwana base New York.

Lolucwaningo lumbandakanya uphenyo. Ophenyweni ososayensi noma odokotela bazama ukuphendula eminye imibuzo ebalulekile engasiza ukwenza ngcono impilo yabanye abantwana kanye neyomphakathi ngokubanzi. Loluphenyo ngeke lwaba nomhlomulo oqonde ngqo enganeni yakho, kodwa silindele ulwazi olusha lokuba nemihlomulo kwabanye abantwana ngokuzayo.

Isimemo sokungenela:

Uyacelwa ukuthi uvumele ingane yakho ukuthi ingenele ucwaningo lokwelapha olubizwqa ngokuthi yi "Study of bioimpedance lokucubungula izingabe zase Ningizimu Afrika" ngoba ineminyaka ephakathi kwemi 3 kuya kwemi 5 ubudala.

Inhloso Yocwaningo:

Inhloso yalolucwaningo ukubheka ukuthi ngabe ukuhlola okulula kanye nokungabizi kakhulu kungenziwa yini ukubheka inani lamafutha kanye nemisipha emzimbeni. Okunye ukuhlola kubiza kakhulu futhi akutholakali, kunzima kakhulu ukukwenza ezinganeni, noma kungadinga ukudonswa kwegazi. Kulolucwaningo siyoqhathanisa ukuhlola okubizwa ngokuthi yi 'Bioimpedance Assessment', okulula ukukwenza futhi okungenabuhlungu, sikuqhathanisa nalokho okubiza kakhulu okubizwa ngokuthi yi 'isotope dilution', okunzima ukukwenza futhi kumbandakanya nokudonswa kwegazi.

Siyethemba ukuthi ulwazi eliluthola kulolucwaningo luyokwenza sikwazi ukwenza ukuhlola okulula, okungenabo ubuhlungu kubantwana kunokusebenzisa okunye ukuhlola. Izizathu zokufuna ukwazi ukuthi izingane zinemisipha kanye namafutha angakanani emzimbeni kungaba wusizo kodokotela ukubona yimuphi umphumela wezifo ezahlukene neziwumbelembele okubantwana. Ungasetshenziswa futhi njengendlela elula yokubheka ukuthi ngabe abantwana bayezwela yini emithini.

Yini embandakanyeka ocwaningeni: Siyobhalisa abantwana abangama 30 bebonke abaphuma Esibhedlela iKing Edward VIII eThekwini. Siyodinga ukubona ingane yakho kanye kuphela ngalolucwaningo. Siyokwenza izilinganiso ezimbalwa ezimbandakanya ukulinganiswa kwesisindo somzimba kanye nesisindo somzimba, inqubo ebizwa ngokuthi yi BIA noma i bioimpedance assessment kanye nokudonswa kwegazi ngaphambi kanye nangemuva kwamahora ama 2 emva kokuphuza ingxube elungiselelwe. Siyoqoqa futhi amathe ngezikhathi ezifanayo esiqoqa ngazo igazi. Zonke izinqubo zokuhlola ingane yakho zike zasetshenziswa phambilini ocwaningeni kanye nasekuhleleleni ukwelashwa. I "Bioimpedance assessment" imbandakanya ukudlulisa okunogesi omncane emzimbeni. Abantu abakwazi ukuzwa lokhu, futhi kuyofana nokuhlolwa kokusebenza kwenhliziyo ngomshini we ECG. Okunogesi "ama- Electrodes" ayobekwa ezihlakaleni kanye nasezinyaweni ukuthola ukuqoshwa. Ngeke kuthathe ngaphezu kwemizuzu embalwa. Emva kwalokho siyodonsa igazi elithize bese siqoqa amathe enganeni yakho. Siyobe-ke sesiyinikeza ingxube ukuthi iyiphuze. Ingxube iqukethe utho esingalulinganisa egazini kanye nasemlonyeni olungavamile ukuba khona emzimbeni. Utho ekugcineni luyoqedwa emzimbeni. Emahoreni amabili emva kokuphuza lokhu kuyomele sithathe elinye igazi kanye nesampula lamathe. Inani lomthamo wegazi elithathwayo, u 8ml (ngaphansi kothesipuni aba 2), kanye nenani lomthamo wamathe athathwayo, u 4ml ngeke kube nomphumela wokulimazeka enganeni yakho. Igazi kanye namathe kuyoqandiswa bese kulondolozwa kuze kuphele ucwaningo. Igazi kanye namathe okuthathwe kwabangenele kuyobe sekuthunyelwa elabhorethi ekhethekile ese New York ukuthi kuyosetshenzwa ngoba asikho isikhungo esinjalo eNingizimu Afrika.

Igazi kanye namathe kuyosetshenziselwa kuphela ukuhlola okuchazwe kulombhalo. Noma yikuphi okusilele kuyoshatshalaliswa ngendlela ephephile. Lemiphumela ngeke isheshe itholakale. Iyotholakal kuphela emva kokuqedwa kocwaningo. Kodwa-ke siyokwazisa ngemiphumela ngokuthi sikushayele ucingo noma sikubhalele uma nje sesihumushe

lemiphumela. Imiphumela ngeke ibe nethonya ekulashweni kwengane yakho ekuthola ngalesi sikhathi. Amasampula ngeke abe negama lengane yakho elebulini. Ayoba nekhodi leyo okunganacwaningi kuphela abakululucwaningo abayokwazi ukuluxhumanisa nengane yakho.

Izingozi zokuba socwaningeni. Ezinye izingozi zokudonswa kwegazi zimbandakanya ubuhlungu kanye nomzizima endaweni okudonswe kuyo igazi. Kulendawo okudonswe kuyo, kungaba nengozi encane yokubhibha kwesilonda noma nini uma kudonswa igazi. Siyothatha izinyathelo zokuvikela ukugwema lokhu ukuthi kwenzeke. Uma kuvela lenkinga siyokusiza ukuthola ukunakekelwa okufanele kwengane yakho. Ezinye izinqubo azinazo izingozi ezihlobene nazo.

Imihlomulo yokuba socwaningeni. Ucwaningo kanye nokuhlola ngeke kuyizuzele ingane yakho ngqo. Sicela ungaphambanisi lokhu nokunye ukuhlolwa kanye nemithi oenganikezwa yona kulomtholampilo. Ungabuza uma ungenaso isiqiniseko ngezinto ezihlobene nalolucwaningo lodwa. Silindele ukuthilokhu kuhlola kuya wusizo ekunakekelweni kwabanye abantwana ngomuso.

Ukungenela kuya ngokuzithandela komuntu. Ungakhetha ukungalungeneli lolucwaningo. Ngeke kube nomphumela omubi ekwelashweni kwengane yakho noma ekuphathweni, noma ngokungenela ezinye izincwaningo.

Izindleko kanye Nokubuyiselwa imali: Azikho izindleko eziqondene ngqo nawe. Ayikho imihlomulo yemali eqonde ngqo ngokungenela ucwaningo. Siyokuhlinzeka ngokudla okungatheni ngesikhathi usalinde amahora amabili ukuphinda ukuhlolwa. Ucwaningo aludingi ukuthi uphinde uvakashe. Siyokunikeza u R20 njengesinxephezelo sanoma yikuphi ukuxakeka okungadaleka emahore amabili usalindile.

Ubumfihlo: Kuyokwenziwa imizamo yokugcina ulwazi lwakho luyimfihlo. Wonke amafomu kanye namakhompuyutha kyovikelwa. Ulwazi lwakho lungadalulwa uma kudingwa ngumthetho. Izinhlangano ezingahlola kanye/noma zenze imifanekiso yamarekhodi ocwaningo ukuqinisekisa ngezikha kanye nokucutshungulwa kwemidanti yolwazi zimbandakanya amaqembu afana Nekomiti Locwaningo Lenkambiso enhle.

Uma imiphumela ishicilelwa noma yethulwa emihlanganweni, kungaholela ekudalulweni kwethimba kodwa ukudalulwa komuntu ngamunye kwegama lakho kanye nelengane yakho ngeke kudalulwe.

Lolucwaningo lugunyazwe Yikomiti Lenkambiso enhle eliseNyuvesi ya KwaZulu Natal eThekwini kanye nabe St Luke's Roosevelt Center e New York. Lamakomiti anesibopho sokuqinisekisa ukuthi izingozi (uma zikhona) esigulini ziyokwedlulwa ngumhlomulo ongaba khona esigulini kanye/noma ekubalulekeni kolwazi oluzozizwa. Bafuna ukuqinisekisa ukuthi amalungelo kanye nehlalakahle yomuntu ngamunye ayovikelwa ngokulinganayo futhi kuyotholwa imvume egunyaziwe.

Uma unemibuzo maqondana nalolucwaningo , noma uma kuba khona ukulimala okuhlobene nocwaningo, kumele uxhumane nomphenyi omkhulu uDkt Meera Chhagan kulenombolo yocingo (031) 260 4345 noma (031) 260 4600, ku Department of Paediatrics and Child Health, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, 719 Umbilo Road, Congella, 4013 Durban.

UNgaxhumana nabe Biomedical Research Ethics Office e Nelson R Mandela School of Medicine kulenombolo yocingo (031) 260 4604 uma unemibuzo maqondana namalungelo akho njengongenele ucwaningo. Umuntu oxhunywayo ngu Ms Suraiya Buccas kanye nosihlalo uPholofesa Jack Moodley. Ikheli lalelihhovisi lithi:

Medical Research Administration
Nelson R Mandela School of Medicine
University of KwaZulu-Natal
719 Umbilo Road
Congella, 4013

Ngabe uyafisa ukubuza eminye imibuzo ngalesi sikhathi?
Manje angikucele ukuthi usayine lelifomu lokuvumela ingane yakho ukuthi ifakwe kulolucwaningo kanye nokusivuela ukuthi sense lokhu kulohla.
Siyabonga ukusebenzisana nathi kanye nesikhathi sakho. Siza ukhululeke ukusithinta uma uba nanoma yimiphi imibuzo ngokuzayo. Siyokunikeza ikhophi yalelifomu.

AMASIGNESHA

Uma usufunde lelifomu lemvume, noma ufundelwe lona futhi wachazelwa lonau, futhi uluqonda ulwazi, futhi ngokuzithandela kwakho uvuma ukuthi ingane yakho ingenele ucwaningo, siza usayine igama lakho noma ushaye uphawu ngezansi.

Igama Longenele (Bhala wehlukanise)	Isignesha Yongenele	Usuku

Ilungu Labasebenzi Bocwaningo Eliqhuba Ingxoxo Yemvume (bhala wehlukanise)	Isignesha Yelungu Labasebenzi Bocwaningo	Usuku

APPENDIX E : DATA COLLECTION FORM

PERSONAL DETAILS

ASSIGNED STUDY NUMBER: _____

Patient Name (Surname first):_____

Patient hospital Number: _____

Clinic number: _____

Patient date of birth_____

Date of assessment: _____

Address: _____

_____

Contact number: _____

Caregiver name: _____

NUTRITIONAL ASSESSMENT

Child age: ____

Child weight: _____

Child height: _____

CLINICAL FEATURES OF MALNUTRITION

Mid upper arm circumference (triplicate):

_____mm _____mm

_____mm _____mm

_____mm _____mm

Triceps skin fold thickness (triplicate):

_____mm _____mm

_____mm _____mm

_____mm _____mm

Sub scapular skin – fold thickness (triplicate):

_____mm _____ mm

_____mm _____ mm

_____mm _____mm

Visible signs of wasting: **Y/N**_____

Visible edema: **Y/N**_____

Skin/hairmagnifications of kwashiorkor: **Y/N**_____

Thrush present: **Y/N** _____

Any other lesions in the mouth? **Y/N**_____

BIO IMPEDANCE ASSESSMENT

Reactance: _____

Resistance: _____

Impedance: _____

HYDRATION

Diarrhoea: **Y/N** _____

Signs of dehydration: **Y/N** _____

Skin temperature: _____

Record details of all fluids ingested within the last two hours:

_____

APPENDIX F : ETHICS APPROVAL

UNIVERSITY OF
KWAZULU-NATAL

Research Office
BIOMEDICAL RESEARCH ETHICS ADMINISTRATION
Nelson R Mandela School of Medicine
Private Bag 7, Congella 4013
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769
Fax: 27 31 2604609
Email: buccas@ukzn.ac.za
Website: www.ukzn.ac.za

25 January 2008

Dr M K Chhagan
Paediatrics
Nelson R Mandela School of Medicine

Dear Dr Chhagan

PROTOCOL: Study of bioimpedance analysis in South African children. M K Chhagan, Paediatrics. Ref: E049/05

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your response received on 26th March 2008 to queries raised on 25 January 2008.

We acknowledge receipt of the Export permit which expires on **31 December 2008** and a declaration that no genetic testing will be done on the saliva. These conditions have now been met and the study is given full ethics approval and may begin as at **30 April 2008.**

This approval is valid for one year from **30 April 2008.** To ensure continuous approval, an application for recertification should be submitted a couple of months before the expiry date. In addition, when consent is a requirement, the consent process will need to be repeated annually.

I take this opportunity to wish you everything of the best with your study. Please send the Biomedical Research Ethics Committee a copy of your report once completed.

Yours sincerely

Prof. D Wassenaar
Chair: Biomedical Research Ethics Committee