

**Body composition and nutritional intakes of HIV exposed  
preterm, very- and extremely-low birth weight infants in  
Tygerberg hospital, Western Cape**

**by  
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*Thesis presented in partial fulfilment of the requirements for the degree  
of Masters in Nutrition at Stellenbosch University*



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## **DECLARATION**

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## ABSTRACT

**Introduction:** The relationship between HIV exposure and body composition (and the quality thereof) of preterm infants is not well researched. Human breast milk (HBM) is considered inadequate in meeting the protein requirements of very low birth weight (VLBW) infants, which could affect the body composition.

**Objectives:** The primary objective of this study was to determine the body composition of HIV-exposed preterm VLBW and extremely low-birth weight (ELBW) infants. Secondary objectives were to evaluate the effect of the treatment duration of maternal highly active antiretroviral therapy (HAART) and the effect HBM has on the body composition of this vulnerable population. Furthermore, the effect of breast milk fortification and days infants were kept nil per os (NPO) has on body composition were also assessed.

**Methods:** A descriptive cross-sectional study was conducted between May and October 2016. HIV-exposed and -unexposed preterm infants (gestational age < 37 weeks) with a birth weight of  $\leq 1200$  g were included. Each infant's maternal medical background was recorded. Anthropometric and body composition measurements were recorded weekly during the 28-day follow-up period. Nutritional intakes and clinical progress was documented daily.

**Results:** A total of 113 preterm infants were included in this study, of which thirty infants (27%) were HIV-exposed. HIV-exposed infants had significantly ( $p = 0.01$ ) lower gestational ages than HIV-unexposed infants (25–28 weeks). HIV-exposed infants displayed significantly lower fat mass percentage (FM%) on day 21 and day 28 (0.9% vs. 1.4%,  $p = 0.02$  and 1.0% vs. 1.5%;  $p = 0.03$ ), respectively. HIV-exposed infants whose mothers received HAART for  $\geq 20$  weeks had higher weights and FM% with lower fat-free mass percentages (FFM%) at birth when compared to infants whose mothers received treatment for shorter durations ( $\geq 4$ –< 20 weeks). In this study 110 of 113 preterm infants received HBM, of which 91 infants received fortified HBM. HIV-exposed and unexposed infants receiving fortified HBM displayed differences in (FM%) (0.88% vs. 1.36%;  $p =$

0.01) compared to (0.97% vs.1.49%;  $p = 0.03$ ) and FFM% (98.98% vs. 98.68%;  $p = 0.03$ ) compared to (99.02% vs. 98.49%;  $p = 0.02$ ), on day 21 and 28 respectively. All Infants kept NPO vs. not kept NPO displayed differences in FM% on day 7, 21 and 28 of life (0.9% vs 1.3%;  $p = 0.03$ ), (0.99% vs 1.4%;  $p = 0.02$ ) and (0.9% vs 1.6%;  $p = 0.0004$ ) as well as differences in FFM% (99.1% vs 98.4%;  $p = 0.0005$ ) on day 28 of life.

**Conclusion:** Body composition differs between HIV-exposed and HIV-unexposed preterm infants and the duration of maternal HAART affects postnatal infant body composition. There were no significant differences in the body composition of HIV-exposed and HIV-unexposed infants who received HBM or fortified HBM. However, between the HIV-exposed infants significant differences in body composition measurements were found for infants who received fortified HBM and those who did not. Infants who were kept NPO were generally smaller, shorter, and had lower FM% and more FFM%.

**Key Message:** *The ward feeding protocols for preterm infants should be re-evaluated, focusing specifically on the HIV-exposed preterm infants, to improve short and long term outcomes of this vulnerable population.*

## OPSOMMING

**Inleiding:** Die verhouding tussen blootstelling aan MIV, liggaamsamestelling (en die kwaliteit daarvan) en premature babas, is nie goed nagevors nie. Menslike borsmelk (HBM) word as onvoldoende beskou om in die proteïenvereistes van babas met 'n baie lae geboortegewig (VLBW) te voorsien. Dit kan 'n effek op liggaamsamestelling hê.

**Doelwitte:** Die primêre doelwit van hierdie studie was om die liggaamsamestelling te bepaal van premature babas met baie lae geboortegewig (VLBW) en dié met uiters lae geboortegewig (ELBW), wat aan MIV blootgestel was. Sekondêre doelwitte was om te evalueer wat die effek was van die tydsduur waartydens die moeder hoogs aktiewe antiretrovirale behandeling (HAART) ontvang het, en ook om te bepaal wat die effek is van menslike borsmelk (HBM) op die liggaamsamestelling van hierdie kwesbare populasie. Hierbenewens, is die effek van borsmelkfortifisering, asook die dae waarop babas nil per os (NPO) ontvang het, op liggaamsamestelling bepaal.

**Metodes:** 'n Beskrywende deursnit-studie is tussen Mei en Oktober 2016 uitgevoer. Premature babas (gestasie-ouderdom < 37 weke) met 'n geboortegewig van  $\leq 1\ 200$  g wat aan MIV blootgestel was en nie aan MIV blootgestel was nie, is as populasie ingesluit. Die mediese agtergrond van elke baba se moeder is opgeteken. Antropometriese en liggaamsamestellingsmates is weekliks gedurende die opvolgperiode van 28 dae opgeteken. Voedings-inname en kliniese vooruitgang is daaglik gedokumenteer.

**Resultate:** 'n Totaal van 113 premature babas is by hierdie studie betrek, van wie dertig babas (27%) aan MIV blootgestel was. Babas wat aan MIV blootgestel was, het beduidend ( $p = 0.01$ ) laer gestasie-ouderdomme gehad as dié wat nie aan MIV blootgestel was nie (25–28 weke). Babas wat aan MIV blootgestel was, het 'n beduidend laer vetmassa-persentasie (FM%) (0.9% vs. 1.4%,  $p = 0.02$  en 1.0% vs. 1.5%,  $p = 0.03$ ) op Dag 21 en 28 getoon. Babas wat aan MIV blootgestel was en wie se moeders HAART vir  $\geq 20$  weke ontvang het, het by geboorte meer geweeg en hoër FM% gehad, met 'n laer vetvrye massa-persentasie (FFM%) wanneer hulle vergelyk is met babas wie se moeders behandeling vir korter periodes ( $\geq 4$ –< 20 weke) ontvang het. In hierdie studie het 110

van die 113 premature babas menslike borsmelk (HBM) ontvang en 91 gefortifiseerde menslike borsmelk (HBM). Babas wat aan MIV blootgestel was en dié wat nie aan MIV blootgestel was nie, en wat gefortifiseerde menslike borsmelk (HBM) ontvang het, het verskille getoon in FM% (0.88% vs. 1.36%;  $p = 0.01$ ) teenoor (0.97% vs. 1.49%;  $p = 0.03$ ) en FFM% (98.98% vs. 98.68%;  $p = 0.03$ ) teenoor (99.02% vs. 98.49%;  $p = 0.02$ ) op Dag 21 en 28 onderskeidelik. Die babas wat nil per os (NPO) ontvang het vs. babas wat nie NPO ontvang het nie, het geen verskille getoon in FM% (0.9% vs. 1.3%;  $p = 0.03$ ), (0.99% vs. 1.4%;  $p = 0.02$ ) en (0.9% vs. 1.6%;  $p = 0.0004$ ) op Dag 7, 21 en 28 van lewe, en ook nie in FFM% (99.1% vs. 98.4%;  $p = 0.0005$ ) op Dag 28 van lewe nie.

**Gevolgtrekking:** Die liggaamsamestelling van premature babas wat aan MIV blootgestel was en dié wat nie aan MIV blootgestel was nie, verskil. Daarbenewens beïnvloed die tydsduur waartydens die moeder HAART-behandeling ontvang, die postnatale liggaamsamestelling van 'n baba. Daar was geen beduidende verskille in die liggaamsamestelling van babas wat aan MIV blootgestel was en dié wat nie aan MIV blootgestel was nie, en wat menslike borsmelk (HBM) of gefortifiseerde menslike borsmelk (HBM) ontvang het nie. In terme van die babas wat aan MIV blootgestel was, is daar egter beduidende verskille gevind in die liggaamsamestellingsmates van dié wat gefortifiseerde menslike borsmelk (HBM) ontvang het en dié wat dit nie ontvang het nie. Babas wat nil per os (NPO) ontvang het, was oor die algemeen kleiner, korter en het 'n laer FM% en meer FFM% gehad.

**Sleutel boodskappe:** *Die saal voedingsprotokolle vir premature babas moet geherewalueer word, met spesifieke fokus op MIV blootgestelde premature babas, om kort en langtermyn-uitkomst van hierdie kwesbare populasie te verbeter.*

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## GLOSSARY OF TERMS

**2-Compartment model** – The 2-C model separates the body into two compartments: fat mass (FM) and fat-free mass (FFM), the latter consisting of heterogeneous components comprised of carbohydrates, protein, water and minerals.<sup>1 2 3 4 5</sup>

**3-Compartment model** – The 3-C model divides the human body into three compartments: fat mass, water and fat-free mass. The FFM compartment is divided into two components: body water content and remaining solids (protein and minerals), measuring water, fat and body solids in terms of body composition.<sup>3</sup>

**4-Compartment model** – The 4-C model is an expansion of the basic 3-C model as it measures not only the total body water, but also the densities of total body protein and bone minerals. Therefore, it includes measurements of total body weight, total body volume, total body water and bone mineral density.<sup>1 3 4</sup>

**5/Multi-compartment model** – The 5-C model stipulates a structural framework that divides the human body into the following compartments according to increasing complexity: atomic, cellular, molecular, tissue and whole body.<sup>2 3 6</sup>

**Adipose tissue** – Adipose tissue is anatomically defined as tissue comprised of: adipocytes/fat cells, nerves, blood vessels and extracellular fluid.<sup>2</sup>

**Air displacement plethysmography** – ADP is a highly accurate measuring technique that measures paediatric body composition in a closed chamber. ADP directly measures body mass and volume to calculate body density, thereby determining total body fat percentage.<sup>2 7</sup>

**Anthropometric measurements** – A set of measurements (weight, length and head circumference) to assess the nutritional status of an infant.<sup>8</sup>

**Antiretroviral therapy** – Specialised medication for the treatment and control of HIV infection. ART consists of a combination of three or more ARV medications used to kill or

suppress viral replication and the replication and progression of the HIV disease. Also known as HAART, it is usually given for life and comprises different classes of drugs, depending on the different stages of the HIV-cycle.<sup>9 10</sup>

**Appropriate for gestational age** – Describes the size of an infant whose birth weight against gestational age plotted on Fenton’s-premature growth chart falls between the 10<sup>th</sup> and 90<sup>th</sup> percentile.<sup>9 11</sup>

**Bioelectrical impedance** – BIA is a precise method for assessing body composition through measuring the impedance or resistance of the body by passing a weak alternating electrical current at a fixed frequency. BIA estimates total body water, fat-free mass, fat mass and body cell mass.<sup>9 12</sup>

**Biceps skinfold** – BiSFT/BS is measured in the anterior midline of the left arm over the biceps muscle at the same level of the triceps skinfold, while the participant’s arm is placed at his/her side.<sup>13 14</sup>

**Body composition** – Describes the percentage of fat, bone and muscle in human bodies.

**Catch-up growth** – A preterm infant achieving a growth rate similar to that of a healthy fetus growing in utero.<sup>15 16</sup> It can also be defined as a higher than normal growth rate after a period of growth suppression owing to an extent of illness or deprivation.<sup>9</sup>

**Donor breast milk** – DBM is when a lactating mother donates her breast milk to be used for infants whose mothers do not have an adequate supply of breast milk. DBM is safe to use as it is pasteurised and considered the next best alternative.<sup>17</sup>

**Dual-energy x-ray absorptiometry assay** – DXA is defined as a non-invasive technique commonly used to measure body composition in infants. The infant is removed from the NICU and placed in an x-ray scanning machine which measures fat mass, fat-free mass and bone mineral mass.<sup>2 18</sup>

**Extra cellular solids** – A non-metabolising part of the human body, consisting of organic

compounds: collagen, reticular and elastic fibres: i.e. muscle and inorganic compounds (bone, bicarbonate, citrate, magnesium and sodium).<sup>19</sup>

**Extracellular water** – A non-metabolising fluid made up of 94% water that surrounds body cells. Extra-cellular water provides a medium for gas exchange, transferring of nutrients and excretion of metabolic end products. It can also be used to describe fluid shifts and balances to determine hydration status.<sup>19 20</sup>

**Extremely low birth weight** – An infant weighing < 1 000 g.<sup>9 11</sup>

**Fat-free mass** – FFM is a heterogeneous component comprised of carbohydrates, protein, water and minerals.<sup>12</sup>

**Fat mass** – The part of the human body that is comprised of fat from all of the bodily sources found in the brain, skeleton and adipose tissue.<sup>9</sup>

**Fat** – A sub-component of adipose tissue/lipid, predominantly found at a molecular level.<sup>2</sup>

**Fixed-dose combination** – Multiple antiretroviral drugs combined into one pill. FDC is made up of three drugs used in the first-line regimen for treating HIV. It is comprised of Tenofovir (TDF, 300 mg) + Emtricitabine (FTC, 200 g) + Efavirenz (EFV, 600 mg) to improve adherence and retention.<sup>10 21</sup>

**Feeding intolerance** – vomit or an aspirate.

**Fortified breast milk** – Supplements of protein, carbohydrates, fat, minerals, and vitamins added to human breast milk to meet the increased nutrient needs of preterm infants.<sup>9</sup>

**Gestational age** – Gestational age (GA) is calculated on the basis of the date of the mother's last menstrual cycle or according to the first trimester ultrasound prior to 12 weeks.<sup>22</sup>

**Growth failure** – Poor growth in infants resulting in infants not reaching weight-for-age,

height-for-age or both.<sup>9 23 24</sup>

**Highly active antiretroviral therapy** – HAART is a triple drug combination for the treatment of HIV infection.<sup>25</sup>

**Human immunodeficiency virus** – HIV is the retrovirus isolated and recognised in the etiology of AIDS.

**HIV-positive** – Infected with the human immunodeficiency virus.

**HIV-negative** – Not infected with the human immunodeficiency virus.

**HIV-exposed** – An infant born to a woman who is HIV-positive or who becomes HIV-positive any time during pregnancy, labour and delivery, or breastfeeding. The infant is at risk of acquiring the HIV infection.<sup>10</sup>

**HIV-exposed uninfected** – HIV-EU is an internationally used term to describe an infant born to a mother who tested positive any time during pregnancy, labour and delivery, or breastfeeding but who has not had a positive PCR test.<sup>26</sup>

**HIV-unexposed** – An infant born to a woman who is HIV negative or who does not become HIV positive any time during pregnancy, labour and delivery, or breastfeeding. The infant is not at risk of acquiring HIV. This is also known as HIV-unexposed uninfected (HIV-UU).

**Intrauterine growth restriction** – A decrease in fetal growth rate as determined by the obstetrician during pregnancy.<sup>9</sup>

**Large for gestational age** – Describing the size of an infant whose birth weight against gestational age, plotted on Fenton's-premature growth chart, falls above the 90<sup>th</sup> percentile.<sup>9 11</sup>

**Lean body mass** – LBM is the part of the body free that is from adipose tissue. It is comprised of skeletal muscle, water, bone and a small amount of essential fat found in

the organs, bone marrow and nerves.<sup>9 19 27</sup>

**Lipodistrophy** – A change in the way the body produces, uses and distributes fat mass. Patients have a significant loss of fat from the extremities (arms and legs) with an increase in visceral fat deposits.<sup>39</sup>

**Low birth weight** – LBW is the birth weight of an infant that is < 2500 g.<sup>9</sup>

**Metabolic syndrome** – A medical condition or representation of a variety of syndromes associated with glucose intolerance, insulin resistance (often associated with type-2 diabetes), hyperlipidemia, and hypertension; and strongly linked to abdominal obesity.<sup>9 28</sup>

**Nutritional intervention** – The nutritional related activities or actions which can have an impact on nutrition and health outcomes.<sup>29</sup>

**Pre-eclampsia** – A common complication that arises during pregnancy, characterised by high blood pressure.<sup>30</sup>

**Premature birth** – defined as the birth of an infant before 37+0 week's gestation.<sup>9 31 32</sup>

**Prevention of mother-to-child transmission** – PMTCT is a set of guidelines derived from the World Health Organization (WHO) and South African National guidelines, which stipulates that all pregnant and breastfeeding HIV-infected women qualify for lifelong ART. HIV and appropriate preparation counselling must be done on the day of the HIV diagnosis. HIV-infected women should initiate treatment on the same day as diagnosis. Patients should receive ongoing counselling from trained counsellors throughout and after pregnancy.<sup>21</sup>

**Protease inhibitor** – An antiretroviral drug that prevents the functioning of the viral protease enzyme and prevents viral replication.<sup>9</sup>

**Skinfold thickness** – A double fold of subcutaneous adipose tissue excluding any underlying tissue is precisely measured by callipers at several standardised points to determine body fat percentage.<sup>33 34</sup>

**Small for gestational age** – Describing the size of an infant whose birth weight against gestational age, plotted on Fenton’s-premature growth chart, falls below the 10<sup>th</sup> percentile.<sup>9 11</sup>

**Subcutaneous fat** – Fat that is stored directly under the skin that insulates the body, preserving body heat and maintaining body temperature.<sup>9</sup>

**Subscapular skinfold** – SSSF/SBS is measured on the left side, below the tip of the inferior angle of the scapula. The SSSF/SBS is picked up on the diagonal, inclined infero-laterally at a 45° angle versus the horizontal plane in the natural cleavage line of the skin.<sup>13</sup>  
14

**Suprailiac skinfold** – SiSF/SPS is measured right above the iliac crest on the left side 1 cm medial from the edge of the bone.<sup>13 14</sup>

**Triceps skinfold** – TSF/TS is measured in the posterior midline of the left arm, over the triceps muscle, at the midway point between the lateral projection of the acromion process of the scapula and the inferior margin of the olecranon process of the ulna, while the participant’s arm is placed at his/her side.<sup>13 14</sup>

**Very low birth weight** – An infant who weighs < 1 500 g.<sup>9 11</sup>

**Viral load** – The measurement of the amount of HIV virus in the blood, stated as copies per millimetre.<sup>9</sup>

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## LIST OF ABBREVIATIONS AND ACRONYMS

<b>2-C:</b>	Two-compartment model
<b>3-C:</b>	Three-compartment model
<b>4-C:</b>	Four-compartment model
<b>5-C:</b>	Five-compartment model/multi-compartment model
<b>ADP:</b>	Air displacement plethysmography
<b>AGA:</b>	Appropriate for gestational age
<b>ART:</b>	Antiretroviral therapy
<b>ANOVA:</b>	Analysis of variance
<b>AZT:</b>	Zidovudine
<b>BIA:</b>	Bioelectrical impedance
<b>BM:</b>	Body mass
<b>BCM:</b>	Body cell mass
<b>BF:</b>	Body fat
<b>BFHI:</b>	Baby Friendly Hospital Initiative
<b>CVD:</b>	Cardiovascular disease
<b>DBM:</b>	Donor breast milk
<b>EFV:</b>	Efavirenz
<b>ELBW:</b>	Extremely low birth weight (< 1 000 g)
<b>EN:</b>	Enteral nutrition
<b>ESPGHAN:</b>	The European Society for Paediatric Gastroenterology, Hepatology and Nutrition

<b>FFM:</b>	Fat-free mass
<b>FM:</b>	Fat mass
<b>FTC:</b>	Emtricitabine
<b>GA:</b>	Gestational age
<b>HAART:</b>	Highly active antiretroviral therapy
<b>HBM:</b>	Human breast milk
<b>HIV:</b>	Human immunodeficiency virus
<b>HIV-EU:</b>	HIV-exposed uninfected
<b>HIV-UU:</b>	HIV-unexposed uninfected
<b>IUGR:</b>	Intra-uterine growth restrictions
<b>IL-2:</b>	Interleukin-2
<b>LBW:</b>	Low birth weight (< 2 500 g)
<b>LGA:</b>	Large for gestational age
<b>MOM:</b>	Mother's own milk
<b>MTCT:</b>	Mother-to-child transmission
<b>MUAC:</b>	Mid-upper arm circumference
<b>NCPAP:</b>	Nasal continuous positive airway pressure
<b>NEC:</b>	Necrotising enterocolitis
<b>NHANES:</b>	National Health and Nutrition Examination Survey
<b>NICU:</b>	Neonatal intensive care unit
<b>NPO:</b>	Nil per os
<b>NVP:</b>	Nevirapine
<b>PCA:</b>	Post-conception age

<b>PCR:</b>	Polymerase chain reaction
<b>PBM:</b>	Pasteurised breast milk
<b>PMTCT:</b>	Prevention of mother-to-child transmission
<b>TSD:</b>	Standard deviation
<b>SFT:</b>	Skinfold thickness
<b>SGA:</b>	Small for gestational age
<b>SSSF:</b>	Subscapular skinfold
<b>TBF:</b>	Total body fat
<b>TAH:</b>	Tygerberg Academic Hospital
<b>TBCH:</b>	Tygerberg Children's Hospital
<b>TDF:</b>	Tenofovir
<b>TSF:</b>	Triceps skinfold
<b>TPN:</b>	Total parenteral nutrition
<b>VLBW:</b>	Very low birth weight (< 1 500 g)
<b>WHO:</b>	World Health Organization
<b>%BF:</b>	Body fat percentage
<b>%TBF:</b>	Total body fat percentage

## MOTIVATION FOR THE STUDY

HIV is highly prevalent in South Africa and HIV-infected pregnant mothers have a considerably higher risk (13–34%) of delivering an infant preterm.<sup>1 2</sup> The literature concludes that there is an evident gap in documented research in developed and developing countries regarding the relationship between HIV, nutritional intakes, pharmacological interventions, body composition and preterm infants.<sup>3 4 5 6 7 8 9</sup> Preterm infants do not necessarily achieve the extra-utero growth rates or have body compositions similar to those of their term counterparts. Preterm infants, who experience difficulty achieving these growth rates could suffer from growth failure. These infants display increased intra-abdominal adiposity and abnormal body composition when they achieve catch-up growth. These factors affect the quality of weight gain, as these infants are not only shorter and lighter than their term counterparts, but they also have more fat mass and less fat-free mass, resulting in a higher total body fat percentage. This could cause metabolic syndrome and cardiovascular problems to develop later in a preterm infant's life.

The methods used to determine body composition in preterm infants should be simple, quick, and non-invasive. Available literature was reviewed and the Dauncey anthropometric model, which includes skinfold thickness at two primary sites and measures nine body dimensions, was considered in this review to be the best method to accurately determine body composition in preterm infants, especially in resource-poor countries. It is imperative to accurately assess the quality of growth and body composition of this fragile population to determine whether currently prescribed nutritional interventions are beneficial to the overall nutritional status and quality of life in the short and long term of the preterm infant, and to enable timely implementation of appropriate interventions, if required.

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## CONTRIBUTORS

Klara Strydom (principal researcher), Dr Evette van Niekerk (supervisor) and Prof. Muhammad Ali Dhansay (co-supervisor) contributed equally to the design and conceptualisation of the research study.

Klara Strydom and Dr Evette van Niekerk reviewed the data.

Klara Strydom performed data collection, contributed to the acquisition and analysis of the data and interpreted the data.

Prof Daniel G Nel conducted the statistical analysis.

Klara Strydom wrote all included papers and critically appraised all the content.

Dr Evette van Niekerk and Prof. Muhammed Ali Dhansay critically reviewed the papers.

All authors critically revised and approved the final version of this thesis and agreed to be fully accountable for ensuring the integrity and accuracy of the work.

## BRIEF OUTLINE OF THE THESIS

This thesis is divided into four chapters. A brief overview of each chapter will now be given.

**Chapter 1** covers the literature review and includes the following review article:

Factors affecting body composition in preterm infants: Assessment techniques and nutritional interventions. The article focuses on the body composition (in terms of fat mass and fat-free mass) of preterm, very- and extremely-low birth weight infants and concentrates on the broad assessment techniques available to accurately assess and determine body composition in preterm infants, especially in a resource-poor setting. This review also focuses on the relationship between nutritional interventions and pharmacological interventions and body composition of preterm infants.

This review article was published in the journal *Paediatrics and Neonatology* in November 2017.

*Strydom K, et al. Factors affecting body composition in preterm infants: Assessment techniques and nutritional interventions, Pediatrics and Neonatology (2017), <https://doi.org/10.1016/j.pedneo.2017.10.007>*

A literature review was conducted on different breast milk fortification strategies and the body composition of preterm, very- and extremely-low birth weight infants. The nutritional requirements are much higher in preterm infants when compared with their term counterparts. This review assessed which fortification strategy was most suitable for implementation in a resource-limited hospital to ensure preterm infants achieved their high nutritional requirements to ensure adequate growth and body composition.

A literature review was conducted on the body composition of HIV-exposed and HIV-unexposed, preterm, very- and extremely-low birth weight infants. HIV is highly prevalent in South Africa and HIV-infected pregnant mothers have a considerably higher risk of delivering an infant prematurely. However, the body composition of HIV-exposed infants was unclear. This review focuses on the effect that the treatment duration of maternal

highly active antiretroviral therapy (HAART) had on the body composition of preterm infants, HIV-exposed and HIV-unexposed infant outcomes and HIV-exposed infants and their body composition.

**Chapter 2** consists of the methodology of this cross-sectional descriptive study.

**Chapter 3** presents the study results in article format. Two articles are included in Chapter 3:

**Article 1: The effect of maternal HIV status and treatment duration on body composition of HIV-exposed and HIV-unexposed preterm, very- and extremely-low birth weight infants.** This article addresses the primary and secondary objectives of the study. The primary aim of this study was to determine the body composition of HIV-exposed and HIV-unexposed preterm, VLBW and ELBW infants. The secondary objective was to assess the effect of the treatment duration of HAART of HIV-infected mothers on the body composition of these infants.

This article was published in the journal *Paediatrics and International Child Health (YPCH)* in April 2018.

*Klara Strydom, Daniel Gerhardus Nel, Muhammad Ali Dhansay & Evette van Niekerk (2018): The effect of maternal HIV status and treatment duration on body composition of HIV-exposed and HIV-unexposed preterm, very and extremely low-birthweight infants, Paediatrics and International Child Health, DOI: 10.1080/20469047.2018.1466481*

**Article 2: How does nutritional intake affect the body composition of HIV-exposed and HIV-unexposed preterm, very- and extremely-low birth weight infants?** This article addresses the final objective of this study, which was to determine the effect nutritional intakes had on the body composition of HIV-exposed and HIV-unexposed preterm, VLBW and ELBW infants.

This article is under review by the journal *of Breastfeeding Medicine*.

**Chapter 4** concludes the study objectives and findings. This chapter briefly accepts or

rejects the set of null hypotheses of this cross-sectional descriptive study. Limitations are discussed and recommendations for further research are made.

The Vancouver referencing style has been used throughout the dissertation, unless otherwise stipulated by a journal's specification.

In this thesis, the terms HIV-exposed and HIV-exposed uninfected (HIV-EU) and HIV-unexposed and HIV-unexposed uninfected (HIV-UU) may be used interchangeably. This was done in accordance with the selected journals' specifications and standards. Refer to the glossary of terms on page xvi for the definitions of terms.

# CHAPTER 1

## LITERATURE REVIEW

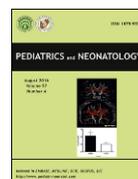
## **REVIEW ARTICLE**

### **1.1 FACTORS AFFECTING BODY COMPOSITION IN PRETERM INFANTS: ASSESSMENT TECHNIQUES AND NUTRITIONAL INTERVENTIONS**

Strydom K, et al. Factors affecting body composition in preterm infants: Assessment techniques and nutritional interventions, *Pediatrics and Neonatology* (2017), <https://doi.org/10.1016/j.pedneo.2017.10.007>

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journal homepage: <http://www.pediatr-neonatology.com>

## Review Article

# Factors affecting body composition in preterm infants: Assessment techniques and nutritional interventions

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Available online ■ ■ ■

## Key Words

body composition;  
developing country;  
nutritional  
interventions;  
preterm infant;  
skinfold thickness

**Abstract** Limited research has been conducted that elucidates the growth and body composition of preterm infants. It is known that these infants do not necessarily achieve extra-uterine growth rates and body composition similar to those of their term counterparts. Preterm infants, who have difficulty in achieving these growth rates, could suffer from growth failure. These infants display an increased intra-abdominal adiposity and abnormal body composition when they achieve catch-up growth. These factors affect the quality of weight gain, as these infants are not only shorter and lighter than term infants, they also have more fat mass (FM) and less fat-free mass (FFM), resulting in a higher total fat percentage. This could cause metabolic syndrome and cardiovascular problems to develop later in a preterm infant's life. The methods used to determine body composition in preterm infants should be simple, quick, non-invasive and inexpensive. Available literature was reviewed and the Dauncey anthropometric model, which includes skinfold thickness at two primary sites and nine body dimensions, is considered in this review the best method to accurately determine body composition in preterm infants, especially in resource-poor countries. It is imperative to accurately assess the quality of growth and body composition of this fragile population in order to determine whether currently prescribed nutritional interventions are beneficial to the overall

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nutritional status and quality of life—in the short- and long-term—of the preterm infant, and to enable timely implementation of appropriate interventions, if required.

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## 1. Bibliographic search

The bibliographic search was performed electronically using PubMed and SUNLearn databases as search engines. The following keywords were used: "Air Displacement Plethysmography (ADP) preterm infant", "anthropometry preterm infant", "assessment of body composition preterm infant", "bioelectrical impedance preterm infant", "body composition preterm infant", "compartment models body composition", "Dauncey anthropometric model preterm infant", "developing country body composition preterm infant", "DXA preterm infant", "equipment body composition", "formula feeds body composition preterm infant", "fortification of human breast milk", "growth preterm infant", "Holtain calipers skinfolds preterm infant", "human breast milk body composition preterm infant", "measuring methods body composition", "medication preterm infant body composition", "nutritional interventions preterm infant", "nutritional status preterm infant", "resource-poor setting body composition preterm infant", "skinfold thickness preterm infant", "South Africa body composition preterm infant", "total parenteral nutrition body composition preterm infant", "zinc preterm infant body composition", and "zinc preterm infant growth".

## 2. Introduction

According to an article in a 2012 edition of *The Lancet*, it was estimated that in 2010 14.9 million babies worldwide were born preterm, with this number rising. Fifty-two percent of global livebirths occur in Sub-Saharan Africa and South Asia, and of these 60% are born preterm. On average, 12% and 9% of preterm infants are born in low-income and higher-income countries respectively.<sup>1</sup>

There is an increasing interest in the relationship between nutritional status during early infancy and childhood, and the increased risk for adverse health effects as adults. Nutritional care of preterm infants attempts to achieve growth rates similar to the term infant in relation to size, anthropometry and body composition at term equivalent age (TEA).<sup>2–5</sup>

However, it has been found that these infants generally do not meet the required growth rates, resulting in growth failure and adverse outcomes. Infants who experience rapid catch-up growth develop an increased intra-abdominal adiposity, resulting in an abnormal body composition.<sup>6</sup> Preterm infants are not only shorter and lighter than term infants, but have more FM and less FFM, resulting in a higher total body fat percentage.<sup>7</sup> These factors can increase their risk of developing metabolic syndrome and cardiovascular diseases in later life.<sup>6,8–12</sup>

A number of methods exist to determine body composition in the preterm infant. These methods range from

highly sophisticated, invasive and expensive to simple, quick, non-invasive and inexpensive. They are used to determine accurate measurements of anthropometric status and body composition. Assessing the quality (fat and muscle mass) of weight gain and growth in the preterm infant is more important than assessing weight gain only, even in a developing country where resources are limited.

Published research on nutritional interventions and the effect thereof on preterm body composition is scarce.<sup>9,13</sup> The purpose of this article is to review existing publications on body composition in the preterm infant with special focus on different assessment methods and different nutritional interventions. In addition, the effect of various interventions on the short- and long-term overall growth and body composition of these fragile infants will also be considered.

## 3. Body composition in preterm infants

Limited literature exists on the assessment of preterm body composition, with only a few studies focusing on the growth and development of preterm infants.<sup>8,14</sup> Research shows that preterm infants should achieve extra-utero growth rates in relation to size, anthropometry and body composition similar to the term infant still growing in utero.<sup>2–5</sup> However, preterm infants generally do not meet these growth rates resulting in growth failure and debilitating outcomes.<sup>6,15</sup> A systematic review and meta-analysis by Johnson et al.<sup>13</sup> confirmed this by comparing the FM, FFM and total body fat percentage (TBF%) of preterm infants to term equivalent age (TEA). The review concluded that infants born preterm did not achieve the reference data for either growth nor body composition at TEA.

The meta-analysis comprised eight studies, which compared the body composition of appropriate for gestational age (AGA) preterm infants with a mean gestational age of 30 weeks, to term infants. The study concluded that the body composition of TEA preterm infants displayed more similar FM and less FFM, resulting in a higher percentage TBF than infants born at term.<sup>5,13</sup> These results were supported by Gianni et al.<sup>8</sup> and showed that preterm infants at TEA are not only shorter and lighter; they also displayed more FM and less FFM when compared to reference data of term infants.<sup>2,12,15–17</sup>

### 3.1. The relation of body composition to growth in preterm infants

Little research has been conducted on the changes in body composition in preterm infants during the first few months after birth. It is imperative to determine neonatal adiposity as it can be used to predict morbidity during the first few

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months of life and thereafter.<sup>8,14</sup> Preterm small for gestational age (SGA) infants who experience rapid catch-up growth throughout infancy have shown to be at risk to develop increased intra-abdominal adiposity, abnormal body composition and an altered isolated decline in insulin sensitivity. These factors increase their risk of developing metabolic syndrome in later life.<sup>8,10–12</sup> A recent study by Simesk et al.<sup>18</sup> concluded that preterm infants tend to have a higher percentage TBF at TEA when compared to term newborn infants. This may increase the chances of preterm infants to develop cardiovascular problems in the future.<sup>6</sup>

Research confirms that weight loss in term and preterm infants during the first week of life is common.<sup>4</sup> However, rather than focusing on weight gain only, neonatologists and dietitians in the clinical setting need to know the body composition of preterm infants to evaluate the treatment progress and special nutritional care of these fragile infants.<sup>19</sup> Non-invasive and accurate measurements of the preterm infant are valuable to assess the quality of anthropometry and body composition, allowing the monitoring of nutritional requirements and the possible effect of nutritional interventions on these components.<sup>7</sup>

### 3.2. Compartment models of body composition

Compartment models divide the body into different compartments, each containing distinctive components. These models allow for the opportunity to develop new methods of assessing unidentified components by using an identified component.<sup>20</sup> According to current literature, four main compartment models are identified: 2-compartment (2-C), 3-compartment (3-C), 4-compartment (4-C) and 5-compartment or multi-compartment models. Please refer to Fig. 1. Each compartment model is an expansion on the

more basic model. For example, the 3-C model is an expansion on the 2-C model, the 4-C model is an expansion on the 3-C model, and so on.

The most recent literature states that the 3-C model was developed to decrease limitations when used on healthy adults and children. Therefore, it is not suitable for use on newborns, infants and subjects experiencing muscle wastage or depleted bone mineral mass (BMM).<sup>20–23</sup> The generally accepted 5-C or multi-compartment model, which requires a structural framework, was reviewed by Ellis,<sup>24</sup> who concluded that this method had become the standard for body composition research.<sup>21,25</sup> According to a review by Lee and Gallagher,<sup>26</sup> the 4-C model is the most applicable and commonly used criteria method to assess body composition in children. Their conclusion was based on the fact that the 4-C model includes the Extra Cellular Water (ECW) component that measures hydration status. However, they do not disclose whether this method is suitable for newborns and infants.

Compartment models are useful when one body compartment has not or cannot be measured, but the values of other compartments within the model are known. Using simple arithmetic, the value of the unknown compartment can be determined.

The compartment models are dependent on the different body components for assessment or research, and different types of measuring methods to assess body composition are dependent on the number of compartments assessed or researched.

### 3.3. Assessment of body composition

Basic non-invasive anthropometric measurements such as weight, length and head circumference assess basic

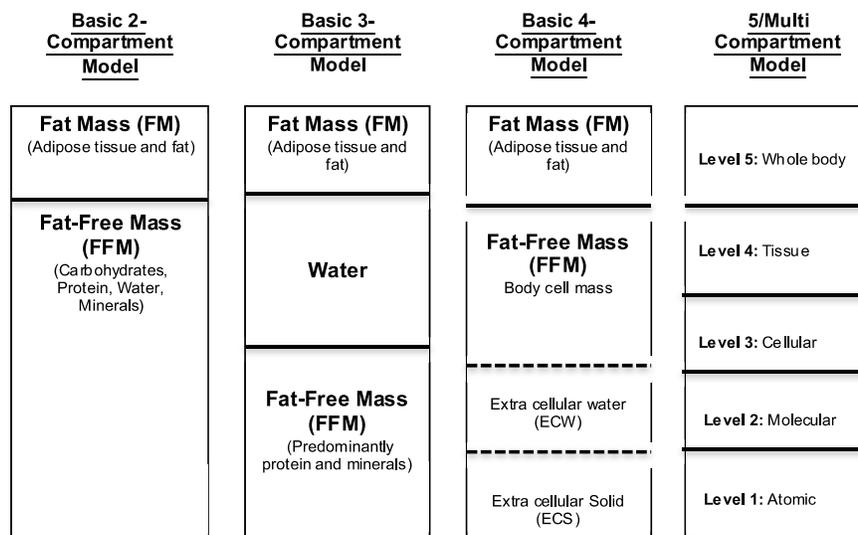


Figure 1 Basic compartment model figure adapted from Ellis,<sup>24</sup> "Human body composition: In Vivo methods".

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growth of the preterm infant. A need exists to accurately assess relative components such as FM, FFM and the quality of weight gain to predict body composition.<sup>9</sup> Body composition can predict short- and long-term adverse outcomes, which are associated with altered fat deposition and a high TBF%.<sup>27</sup> There are many different types of equipment and measuring methods available to assess body composition in the preterm infant. The equipment and measuring methods range from simple, quick, non-invasive, safe and inexpensive to complex, sophisticated and expensive.

### 3.3.1. Skinfolds

Even though standard anthropometric assessment of preterm infants such as weight, length and head circumference is easy to record in the clinical setting, it is not appropriate for a full assessment of the nutritional status and body composition of this population.<sup>13,18,19</sup> Therefore, more sophisticated methods to assess and measure the body composition of these infants are suggested as a priority in early life.<sup>13</sup>

Performing Skinfold Thickness (SFT) measurements on fragile preterm infants could perhaps be considered controversial and invasive due to the size of the instrument in comparison to the infant. In addition, the possibility exists of injuring the skin of the premature infant when SFT measurements are taken, especially in the case of an extremely low birth weight (ELBW) infant. However, these measurements are useful, simple, inexpensive and non-invasive to determine body composition in these infants.<sup>18,28,29</sup> Determining neonatal subcutaneous fat with caliper SFT measurements allows for the evaluation of the distribution of FM in specific areas of the body.<sup>30</sup> Caution is advised when measuring SFT in infants with immature, friable skin, as in the case of ELBW infants. In such cases, conducting SFT measurements may not be indicated.

Holtain calipers provide a common method to assess and measure SFT in order to determine subcutaneous fat stores in preterm infants, their term counterparts and children.<sup>18,30–35</sup> However, SFT is notoriously influenced by the hydration status of the preterm infant. In order to obtain more precise body composition measurements it is recommended to use additional sophisticated methods with specialized technology.<sup>26</sup>

Dauncey et al.<sup>36</sup> conducted a study to assess the TBF in infants by using SFT at two sites (subscapular and triceps) in conjunction with nine other body dimensions. The formula has been tentatively applied to infants up to the GA of 40 weeks and for preterm infants. The measurements needed for the dimensions include circumferences (head, chest, the abdomen at the umbilicus, mid-upper arm, mid-thigh and mid-calf) and lengths (upper arm, lower arm and crown-rump length). The triceps and subscapular SFT measurements are used to determine the amount of fat covering the limbs and trunk respectively.<sup>36,37</sup>

### 3.3.2. Bioelectric impedance

Bioelectric Impedance Analysis (BIA) is described as a method for assessing body composition by measuring the impedance or resistance of the body. This is done by

passing a weak alternating electrical current at a fixed frequency, usually 50 kHz, through the body.<sup>9</sup> The measure of impedance is directly and inversely proportional to the volume of the conductor through which the current flows. In the human body the conductor is the total body water (TBW), as it is almost entirely found in the lean body mass (LBM) and made up of water and electrolytes, which are both excellent conductors. Therefore, it can be used as an estimate to calculate FFM. FM and bone are resistant to electrical current. In conclusion, BIA measures the TBW, which provides for the calculation of FM and FFM.<sup>38</sup>

At present, BIA has been identified as a method with great potential value to assess body composition in preterm infants.<sup>19</sup> This method is inexpensive, simple, quick, safe, portable and minimally invasive, as it does not involve the removal of supportive ventilation and monitoring cords of the preterm infant, or the removal of the infant from the Neonatal Intensive Care Unit (NICU).<sup>5,9,19,26,38,39,29,40–42</sup> However, conflicting results from Dung et al.<sup>19</sup> suggested that no research evidence confirmed the use of FFM prediction equations in neonates using BIA.

### 3.3.3. Dual-energy X-ray Absorptiometry (DEXA) and Air Displacement Plethysmography (ADP)

Specialized equipment, such as the common Dual-energy X-ray Absorptiometry (DEXA)<sup>19</sup> and gold standard Air Displacement Plethysmography (ADP),<sup>14</sup> is expensive to use to assess body composition in preterm infants. These assessments can be challenging to perform on preterm infants as the DEXA method requires the infant to be removed from the NICU and minimal movement from the infant is required for accurate results.<sup>16,18,22,43</sup> The ADP method, on the other hand, requires the infant to be removed from the incubator and placed in the PEA POD<sup>®</sup> machine for an average of 2 min.<sup>18,44</sup> Both apparatus are quick, safe and non-invasive when used to determine body composition in preterm infants, but do require immobilization of the infant.<sup>19,45</sup>

Continuous assessment and monitoring of body composition by means of simple as well as sophisticated methods are imperative to obtain accurate results in preterm infants. According to the literature available, the best method with which preterm body composition can be assessed is the anthropometric model of Dauncey.<sup>29,36,37,46</sup> This method is safe, inexpensive, quick, non-invasive and can be conducted at the patient's bedside.<sup>46</sup> Little well-documented research regarding preterm infants and body composition exists, especially in the South African context. With the above-mentioned measuring methods available, valuable research can be done to investigate these fragile populations. Please refer to [Table 1](#): Summarizing the advantages and disadvantages of different techniques for measuring body composition in preterm infants.

## 3.4. Interventions affecting body composition in preterm infants

### 3.4.1. Nutritional interventions

Preterm infants require high recommended daily allowances to help them achieve in utero growth rates.<sup>6,39</sup> Due

**Table 1** Summarizing the advantages and disadvantages of different techniques for measuring body composition in preterm infants.

Technique	Advantages	Disadvantages
SFT and the Dauncey anthropometric model	Evaluation of the distribution of FM in specific areas of the body. Simple, inexpensive and non-invasive and can be done at the bedside. Assess the quality of weight gain and indicates a pattern of change over a time period.	Can be considered controversial and invasive due to the size of the instrument in comparison to the infant. Influenced by the hydration status.
BIA	Measures the TBW, which provides for the calculation of FM and FFM. Simple, inexpensive, quick, safe, portable and minimally invasive. Does not require removal of supportive ventilation and monitoring cords.	Dung et al. <sup>19</sup> suggested that no research evidence confirmed the use of FFM prediction equations in neonates using BIA.
DEXA	Quick, safe and non-invasive.	Expensive. Infant needs to be removed from the NICU. Minimal movement from preterm infant to ensure accurate readings.
ADP	Golden standard. Quick, safe and non-invasive.	Expensive. Preterm infant is removed from the incubator and placed in the PEA POD <sup>®</sup> .

ADP, Air Displacement Plethysmography; BIA, Bioelectrical Impedance; DEXA; Dual-energy X-ray Absorptiometry; FM, Fat Mass; FFM Fat-Free Mass; NICU, Neonatal Intensive Care Unit; SFT, Skinfold Thickness; TBW, Total Body Water.

Conclusion: In a resource poor setting, the use of the Dauncey anthropometric model is one of the best methods to determine body composition in the preterm infant in terms of FM and FFM as it is accurate, safe, inexpensive, non-invasive and can be performed at the bedside.

to the underdeveloped and immature systems of the preterm infant, growth failure is common. However, high postnatal weight gain can also result in adverse outcomes in the long term.<sup>6,9,10,15</sup>

Preterm infants, especially those born with a very low birth weight (<1500 g), are challenging to feed due to the high recommended daily allowance (RDA) of energy, protein and nutrients required to achieve in utero growth rates. The European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) recommends enteral and parenteral energy intakes of 110–135 kcal/kg/day and 110–120 kcal/kg/day respectively and a protein intake of 3.5–4 g/kg/day. Growth failure in the preterm infant is common, which can put these fragile infants at risk of poor neurodevelopment, complications, detrimental effects and adverse outcomes.<sup>2,4,9,10,47,48</sup>

It is suggested that rapid weight gain in the preterm infant (which is associated with aggressive nutritional management) can increase the risk of developing metabolic disease and cardiovascular problems in later life.<sup>6,9,10,15</sup>

Limited literature exists on nutritional interventions and the effect thereof on the quality of growth and body composition of the preterm infant.<sup>9</sup> This is supported by a meta-analysis of the detailed relationship between nutritional interventions and body composition in preterm infants.<sup>12</sup>

The primary nutritional interventions prescribed for preterm infants include: human breast milk, supplemented or fortified human breast milk, formula feed and total

parenteral nutrition. These are explained in more detail below.

**3.4.1.1. Human breast milk.** Human breast milk is globally accepted as the optimal nutritional intervention for infants and preterm infants. Intake is associated with better neurodevelopmental outcomes.<sup>49</sup>

Despite its benefits, human breast milk alone is considered inadequate to meet the preterm nutritional requirements during early neonatal life, especially in infants born with very low birth weight (VLBW), as it only provides about 60% of the protein requirements when fed in adequate amounts to obtain energy requirements in preterm infants. This is of concern as a low protein-energy ratio can result in fat retention during growth.<sup>2,49</sup> However, Atkinson and Randall-Simpson<sup>16</sup> were of the opinion that this needed further investigation.

**3.4.1.2. Fortification of human breast milk.** Supplementation of human breast milk with a fortifier (protein, nutrients, vitamins and minerals) is common practice in many NICUs to achieve nutritional requirements and successive intrauterine growth rates in preterm infants, while the benefits of human breast milk are continually derived.<sup>49</sup> A study comparing post-breastfeeding practices and supplementation of breast milk with formula-feeding practices after hospital discharge found that the fortification of breast milk showed improvements in short-term growth. However, there appears to be no benefit for

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long-term outcomes with regard to growth and body composition.<sup>49</sup> Furthermore, VLBW infants who received fortified breast milk or formula feed, achieved recommended intrauterine growth rates, but showed a significant increase in adiposity, when assessed at TEA.<sup>17</sup>

**3.4.1.3. Formula feeds.** Often formula feeds are given to infants when human breast milk is unavailable. Roggero et al.<sup>7</sup> studied the changes in body composition of 48 preterm infants with a birth weight of  $\leq 1800$  g and a GA of  $\leq 34$  weeks. The infants received exclusive formula feed on demand, from birth to discharge, and the changes in body composition were studied in relation to protein and energy intakes from term until 3 months corrected age (CA). According to the results from this study the high-protein intake was indirectly proportional to the percentage FM and the high-energy intake had no effect on the percentage FM. The study concluded that a high-protein intake with an adequate energy supply resulted in a lower weight gain but a higher percentage LBM during the first month of CA.

**3.4.1.4. Total parenteral nutrition.** An evident gap in literature exists regarding the effect of Total Parenteral Nutrition (TPN) on the body composition of preterm infants.<sup>47</sup> For this reason, this area requires more research.

The various nutritional interventions all strive towards similar outcomes for preterm infants. The most important factor to consider when nutritional intervention is necessary, is the energy-protein ratio of the preterm infant. Research showed that a high protein nutritional intervention resulted in preterm infants having a lower overall weight gain but a higher LBM. This is beneficial to the health of the preterm infant in the long term.<sup>2</sup> Breast milk is universally known as the most optimal feed for preterm infants. However, it is also a known fact that the protein-energy ratio is not achieved when preterm infants receive solely breast milk.<sup>2</sup> Therefore, fortification of breast milk is necessary to achieve optimal growth rates. The type of sector, such as hospitals in a developing country or resource-poor setting, could have an influence on the type of nutritional interventions prescribed for preterm infants. This could in turn have an effect on the nutritional status, body composition and future outcomes of preterm infants.

#### 3.4.2. Preterm infants: pharmacological interventions

A gap in investigatory literature exists regarding the effects of certain medications commonly prescribed to preterm infants on their body composition. These medications include antenatal corticosteroids,<sup>50</sup> surfactant,<sup>51</sup> dopamine, antibiotics,<sup>52</sup> diuretics and micronutrient supplementation. The TBF% in extremely preterm infants can be as little as 1%. Infants and neonates have higher TBF% and intracellular and extracellular water volumes when compared to adults.<sup>53</sup>

Several nutritional intervention studies indicate that zinc supplementation has a positive effect on linear growth in preterm infants.<sup>54,55</sup> A longitudinal, double-blind, randomized clinical trial found higher mean values in total body weight in the group that received supplementation.

However, no significant difference in FM was noted, which could indicate a positive effect of zinc supplementation on FFM.<sup>56</sup> It is known that antenatal corticosteroids can influence an infant's gestational size, increase postnatal growth, impair muscle mass accretion and result in growth failure, all of which are associated with changes in adult body composition.<sup>50,57</sup> A recent study by Simon et al.<sup>50</sup> found that antenatal corticosteroid treatment had a significant influence on the preterm infant's body composition at hospital discharge, resulting in preterm infants having a greater FM and impaired FFM deposition in the neonatal period. Postnatal glucocorticoid treatment has also been known to increase protein breakdown in the preterm infant.

## 4. Conclusion

A gap in published research regarding the relationship between preterm infants, nutritional and pharmacological interventions and body composition exists,<sup>8,13,14</sup> particularly in a resource-poor setting as there is a definitive nonentity regarding this.

There are many different types of factors with regards to measuring techniques that can affect the assessment of body composition in preterm infants. These factors include equipment and techniques which range from simple, quick, non-invasive, safe and inexpensive to complex, sophisticated and expensive. The effect of nutritional interventions on the body composition of preterm infants is determined by and dependent on the protein-energy ratio of the feed. Fortification of breast milk allows for ideal achievement of protein-energy ratio as well as optimal growth rates in the preterm infant, which could have an effect on the body composition and nutritional status of these infants.

Available literature confirms the use of the Dauncey anthropometric model, which includes SFT at two primary sites and nine body dimensions, as one of the best methods to determine body composition in the preterm infant in terms of FM and FFM. The use of this method to determine body composition is accurate, safe, inexpensive, non-invasive and can be performed at the bedside,<sup>29,36,37,46</sup> which therefore does not excessively disturb these fragile infants.<sup>36</sup> Nonetheless, great care should still be taken during SFT measurements on the preterm infant due to the infant's delicate skin and general fragility.

Accurate assessment of this population is imperative when appropriate nutritional interventions are prescribed to increase the quality of life for these fragile infants. Such research could be important to researchers, neonatologists and dietitians in a resource-poor setting, as it could allow experts in the field to better understand the nutritional status, body composition and growth of the preterm infant, future outcomes and how to implement timely and appropriate interventions.

## Conflict of interest

The author declares no conflict of interest with respect to the research, authorship, and/or publication of this article.

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For the purposes of this thesis, it was necessary to include the following subject areas in the literature review as they did not form part of the published review article titled ***Factors affecting body composition in preterm infants: Assessment techniques and nutritional interventions***. They include i) fortification strategies and how they affect infant body composition and ii) the effects of HIV infection on body composition.

## **1.2 LITERATURE OVERVIEW OF FORTIFICATION STRATEGIES AND BODY COMPOSITION**

In 2012, the World Health Organization (WHO) estimated that eight out of every 100 babies were born prematurely in South Africa.<sup>1</sup> The third trimester of pregnancy is an imperative period in a neonate's life as this is when the infant undergoes rapid growth in-utero. Compared with their term counterparts, an infant born prematurely misses out on this fundamental growth period and therefore their nutritional requirements are much higher than an infant carried to term.<sup>2</sup>

The composition of breast milk from mothers who delivered preterm infants is initially higher in protein and fat, when compared with term breast milk. The composition of human breast milk (HBM) changes over time.<sup>2 3 4 5</sup> A study by Hsu et al.<sup>6</sup> examined the change in macronutrient composition of preterm breast milk over a four-week post-partum period. The results concluded that the composition of fat increased gradually and the composition of protein decreased slowly over the first weeks of lactation. The European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) recommends that very low birth weight (< 1.5 kg; VLBW) infants receive enteral and parenteral energy intakes of 110–135 kcal/kg/day and 110–120 kcal/kg/day, respectively and a protein intake of 3.5–4 g/kg/day.<sup>3 4 27</sup> These infants are particularly challenging to feed because of the high recommended daily allowance of energy, protein and nutrients required to achieve in-utero growth rates, in conjunction with the slow feeding progressions and small feeding volumes tolerated, as well as the gastrointestinal immaturity of these very small and immature infants.<sup>1 6 8 9 10 11</sup>

When fed in amounts that satisfy energy requirements, preterm milk only provides about

60% of an infant's protein requirements.<sup>1 12 13</sup> Therefore, HBM from mothers with preterm infants is not nutritionally adequate and must be fortified to ensure adequate growth and body composition in terms of fat mass (FM) and fat-free mass (FFM). Supplementation of HBM with a fortifier is common practice in many neonatal intensive care units (NICUs) to achieve nutritional requirements and improve intrauterine growth rates in preterm infants, while the many benefits of HBM, such as better neurodevelopmental outcomes, improved gastrointestinal function and a decrease in late-onset sepsis and necrotising enterocolitis (NEC) continue to be recorded.<sup>1 6 9 14</sup>

Please refer to Chapter 3, Article 2: *How does nutritional intake affect the body composition of HIV-exposed and HIV-unexposed preterm, very- and extremely-low birth weight infants?*; table 1 and 2 in this thesis. Table 1 and 2 compares the breast milk composition, in terms of volume (ml/kg/day), energy density (kcal/kg/day) and macronutrient concentration, for HBM alone and fortified HBM and the nutritional intake of HIV-exposed and HIV-unexposed preterm infants.

South Africa has only one commercially available fortifier, namely FM85.<sup>15</sup> There are three main types of fortification strategies namely, standard, super and individualised fortification.

- i. Standard fortification is the method most commonly used in NICUs, and entails the addition of a fortifier to a volume of HBM according to the manufacturer's specifications. This fortification strategy is quick and simple, but has been found to rarely meet the protein requirements of preterm infants.<sup>1 12 15 16</sup>
- ii. Super fortification is the addition of a dose of fortifier to a lower volume of HBM than that recommended by the manufacturer. This fortification strategy ensures that a higher protein intake is achieved. However, because the energy and micronutrient intake is also greater, it does not change the protein to energy ratio which could increase the lean body mass of these infants. Super fortification and standard fortification are the most commonly implemented fortifiers in NICUs across South Africa.

- iii. Individualised fortification can include adjustable or targeted fortification. Adjustable fortification is a modified fortification method based on the preterm infant's metabolic response. Periodic blood urea nitrogen values determine the amount of additional protein that can be supplemented by adding modular protein to a standard amount of fortifier.<sup>1 12 17</sup>
- iv. Targeted fortification analyses HBM by means of infrared spectrometry. This is a method of fortification tailored according to the macronutrient composition of HBM after it has been analysed and according to the individual needs of the preterm infant.<sup>1 12 15</sup>

A recent review by Kemp<sup>1</sup> critically analysed the various fortification strategies. This review concluded that, if implemented correctly, standard fortification was a safe and well-tolerated method to improve in-hospital growth of preterm infants. If adequate growth of the preterm infant was not achieved, alternative strategies such as the super, adjustable or targeted fortification should be explored. A study by Natale<sup>15</sup> concluded that targeted fortification is the most accurate and suitable strategy, leading to higher protein intakes and adequate growth. However, this strategy is expensive, labour-intensive and requires specialised technologies.<sup>17</sup>

Fortification strategies can range from simple, quick, safe and inexpensive (standard fortification and super fortification) to complex, sophisticated and expensive (adjustable and targeted fortification). The standard fortification strategy is the most suitable for implementation in a resource-limited hospital, as it is imperative for preterm infants to receive their recommended nutritional requirements, to ensure the adequate growth, body composition and development of this fragile population.

*This study was conducted in a resource-limited area of the Western Cape, South Africa. For the purposes of this study, ward protocol was followed by the researcher for the fortification of HBM in Tygerberg Children's Hospital (TBCH). FM85 was the fortifier used and the standard fortification strategy was implemented.*<sup>15 18</sup>

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### **1.3 LITERATURE REVIEW OF THE BODY COMPOSITION OF HIV-EXPOSED AND HIV-UNEXPOSED PRETERM INFANTS**

Almost no literature exists within the South African context regarding preterm infants, HIV, medication and the effects these have on growth and body composition.<sup>1</sup> The relationship between HIV, prematurity and medication is an on-going global debate.<sup>2</sup> According to the literature, different countries, even different health care systems provide different regimes, drug combinations and drug lines of HIV medication based on individual circumstances. HIV treatment regimes, lines and drugs are complex and specialised as they are constantly improved owing to increasingly sophisticated technology.

HIV is highly prevalent in South Africa and HIV-infected pregnant mothers have a considerably higher risk (13–34%) of delivering an infant preterm.<sup>3</sup> <sup>2</sup> The Western Cape province of South Africa's health department follows a set of guidelines to prevent the transmission of HIV from the mother to her unborn or born infant, which has produced favourable results.<sup>4</sup> The most recent national consolidated guidelines, for the prevention of mother-to-child transmission of HIV (PMTCT) and the management of HIV in children, adolescents and adults released in April 2015 presented the following statistics:<sup>5</sup>

- The coverage of HIV testing of pregnant women is close to 100%.
- The PMTCT programme is now offered in 98% of health facilities around South Africa.
- The percentage of HIV-positive pregnant women receiving antiretroviral therapy (ART) to reduce mother-to-child transmission (MTCT) increased from 83% in 2009 to 87.1% 2012.
- The percentage of MTCT had decreased to 2.7% in 2011.

Research conducted in Africa provides evidence that HIV-exposed children have lower birth weights, poorer early growth and poorer health and survival rates compared with HIV-unexposed children. However, there is limited literature on the actual causes of poor growth and health of HIV-exposed infants in African countries.<sup>6</sup> <sup>7</sup> A combination of factors may contribute to the increase in morbidity among HIV-exposed infants, including a

reduction in maternal transfer of antibodies, increased exposure to infectious pathogens from the mother and an altered immunologic development.<sup>8</sup>

Owing to the absence of literature regarding preterm infants and the effects of HIV and HIV medication on body composition, the literature based on children who were HIV-positive or HIV-exposed was used for this study.<sup>1</sup> According to this research, HIV-positive children who received treatment had an altered body composition when compared with their HIV-negative counterparts.<sup>9</sup> <sup>1</sup> For this reason, anthropometric measurements and body composition techniques are vital to assess the nutritional status of infants.

### **1.3.1 Preterm HIV medication**

In a systematic review, Alemu, Yalew, Fantahun and Ashu<sup>2</sup> compared perinatal outcomes of HIV-infected pregnant women who were or were not receiving ART. The study found that the prevalence of HIV-infected pregnant women in parts of southern Africa was  $\geq$  30%. This systematic review acknowledged that premature birth, low birth weight (LBW) and intra-uterine growth restrictions were universally seen as causes of perinatal morbidity and mortality. However, there is an on-going global debate regarding the role of maternal highly active antiretroviral therapy (HAART) in adverse pregnancy outcomes. In developing countries, the risk of MTCT is still very high owing to the low standards of health care, poor antenatal care, late diagnosis of pregnancy, shortage of ART from primary health care clinics and unsystematic interventions for PMTCT.

In June 2014, a set of PMTCT guidelines were adapted from the World Health Organization (WHO) and South African National guidelines to include international and national standards, which met the needs and recommendations of the Western Cape Province. The 2014, PMTCT guidelines stipulated that all HIV-negative pregnant women needed to have routine HIV tests at: the first antenatal visit, 32 weeks of pregnancy, during labour, six weeks after delivery and every three months while breastfeeding.<sup>4</sup> The recent 2015 PMTCT guidelines differed slightly and stipulated that all pregnant women needed to have repeated HIV testing at: three-monthly intervals during pregnancy, during labour/delivery, at the six-week Expanded Programme on Immunisation (EPI) visit and

three-monthly during breastfeeding. The HIV-testing would be done during routine antenatal care, postnatal care and EPI/ follow up visits. <sup>5</sup>

The 2014 PMTCT guidelines required all pregnant and breastfeeding HIV-positive women, regardless of their CD4 lymphocyte count or viral load (VL), to qualify for lifelong ART.<sup>4</sup> The most recent national consolidated guidelines for PMTCT, released in April 2015, corresponded with this statement. However, the 2015 guidelines also included immediate initiation of lifelong ART for all HIV-positive women within one-year postpartum. This guideline was validated as of January 2015.<sup>5</sup> All patients on first-line ART should receive the fixed-dose combination ART, including Tenofovir (TDF, 300 mg) + Emtricitabine (FTC, 200 g) + Efavirenz (EFV, 600 mg) unless they were contraindicated. Pregnant women with contraindications to FDC should be classified as high-risk pregnancies. However, patients who were on the second-line ART did not change to the FDC ART regime. The regime combinations are complex, specific and prescribed to HIV-positive patients depending on their individual circumstances.<sup>5 4 10</sup>

Infants whose mothers are HIV-positive should undergo a birth polymerase chain reaction (PCR) test to assess whether the infant had been infected with HIV during pregnancy or in the postnatal period. The 2014 and 2015 guidelines stipulated that if the birth PCR test was negative, a prophylaxis such as Nevirapene (NVP) should be administered. The duration of prophylaxis treatment was dependent on whether or not the mother was breastfeeding. If the PCR test of the HIV-exposed infant was negative and the mother was breastfeeding, the administration of extended NVP should be continued up until 12 weeks. If the PCR test of the HIV exposed infant was negative and breastfeeding was discontinued, NVP should be administered up until 6 weeks. All HIV-exposed infants would repeat a HIV PCR test at 10 weeks and infants receiving extended NVP would have the repeated PCR test at 18 weeks. If an infant at any time presents to a health care facility with symptoms of poor health, the PCR test is repeated, even if previous PCR tests had a negative result. <sup>4 5</sup>

HIV-infected pregnant mothers have a considerably increased risk (13–34%) of delivering

an infant preterm. Studies have shown that women who take HAART during pregnancy could face such possible adverse effects.<sup>3</sup> Grosh-Woerner, Puch, Maier, Niehues, Notheis and Patel et al.<sup>11</sup> demonstrated coherent evidence from European studies, which confirmed an increased risk of preterm delivery when HIV-infected pregnant mothers took an antenatal combination of ART and HAART. The study further showed that this was particularly the case when a regimen containing a protease inhibitor was followed. The findings from a German/Austrian cohort study were confirmed by a noticeable occurrence of infants being born preterm for the group of HIV-infected women using HAART with a PI before or during pregnancy.<sup>9</sup>

Castetbonet al.<sup>12</sup> and Dreyfuss, et al.<sup>13</sup> conducted studies in Africa and found that HIV-positive mothers were at risk of delivering preterm infants. A retrospective observational cohort study conducted in Johannesburg, South Africa<sup>14</sup> stated that HIV-infected pregnant women receiving HAART, consisting of a triple-drug combination, prevented MTCT. In addition, it also decreased the incidence of maternal morbidity and mortality. However, this cohort study found that HIV-positive pregnant mothers, to whom HAART had been administered, were at increased risk of PTB in comparison with HIV-positive pregnant women to whom HAART had not been administered.<sup>2</sup>

### **1.3.2 HIV-exposed vs. HIV-unexposed infant outcomes**

HIV-exposed infants seem to have poorer growth and greater early mortality and morbidity rates than their HIV-unexposed counterparts, although HIV-exposed uninfected infants do not grow as poorly as HIV-infected infants.<sup>6 7</sup> Research conducted in Africa reveals that HIV-exposed uninfected children have lower birth weight, poorer early growth and poorer health and survival rates compared with HIV-unexposed children.<sup>15</sup> A study conducted by Bailey et al.<sup>16</sup> agreed and stated that HIV-infected and HIV-exposed infants are smaller and have a lower birth weight than HIV-unexposed infants. There was limited literature on the causes of poor growth and health of HIV-exposed uninfected infants in African countries.<sup>6</sup>

A retrospective cohort study conducted by Slyker et al.<sup>8</sup> stated that a combination of

factors may contribute to an increase in morbidity among HIV-exposed uninfected infants including: a reduction in maternal transfer of antibodies, increased exposure to infectious pathogens from the mother and an altered immunologic development.

It has been comprehensively documented that neonates experience a functional deficiency of neonatal T-cells. The immature neonatal immune system is more susceptible to numerous types of infections that have been associated with increased morbidity and mortality rates. The independent stimulation of T-cell receptors promotes corresponding interleukin-2 (IL-2) production and proliferation in cord blood mononuclear cells and adult peripheral blood mononuclear cells (PMBC).<sup>17</sup> A study by Chougnet et al.<sup>17</sup> concluded that the deficit in cytokine IL-2 production in infants born to HIV-positive mothers might be related to the maternal environment and/or to in-utero exposure to viral products which are potentially immunosuppressant. This study also reported that PMBC from HIV-exposed infants have different production levels of IL-2 and a higher level of immune activation than PMBC from HIV-unexposed infants. Therefore, both HIV-infected and HIV-exposed infants might be at a greater risk of developing infections as a result of intracellular pathogens. These infants may also be less responsive to conventional immunisation procedures. Considering the literature available on HIV, body composition and preterm infants, these factors could have a profound effect on the infants' weight, body composition and overall development.

### **1.3.3 Preterm infants, HIV and body composition**

Published literature addressing HIV-exposed preterm infants and their body composition is limited. As a result, this literature review incorporates studies focused on infants and young children.<sup>1</sup>

It is universally known that children who are HIV-positive experience growth and metabolic abnormalities, which has a profound effect on their short-term survival.<sup>1 9 18</sup> Changes in body fat distribution, as well as glucose and bone metabolism in HIV positive children, can increase their risk of future morbidities.<sup>9</sup>

Chantry et al.<sup>1</sup> reported that HIV-positive adults and children who received ART, displayed an altered fat distribution, especially an increase in central adiposity. This is of concern, as it is generally known to be a risk factor for morbidities such as cardiovascular diseases (CVD) and metabolic syndrome. This study also reported that children in the pre-pubertal stages, who received a protease inhibitor, were associated with developing lipodystrophy. However, limited information is available on this. Assessing body composition in the preterm infant population is, therefore, vital for appropriate treatment and interventions, to improve the future and long-term outcomes of these infants.

A technical review for the World Health Organization (WHO)<sup>9</sup> measured the variations in body composition in HIV-positive paediatric subjects. Measurements of body composition were conducted by means of simple anthropometric measurements such as skin fold thickness (SFT), as well as more sophisticated methods such as dual-energy x-absorptiometry (DXA) techniques and indirect measurements such as bioelectrical impedance (BIA). This review concluded that anthropometric measurements resulted in HIV-positive paediatric subjects displaying a lower lean body mass when compared with HIV-negative controls. The use of more sophisticated methods such as DXA and BIA techniques yielded lower fat-free mass (FFM) and lean body mass measures in HIV-positive paediatric subjects. This population also experienced HIV associated growth failure when compared with the HIV-negative control group.

Neri et al.<sup>19</sup> conducted a longitudinal observation study with a cross-sectional evaluation to compare the growth and body composition of HIV-exposed uninfected children less than 2 years old. This longitudinal observation study compared results to a control group of HIV-unexposed children less than 2 years old and to American (US) references. This study did not find any significant difference in the growth of HIV-exposed uninfected children when compared with HIV-unexposed children. However, when comparing the HIV-exposed uninfected group to US references, they had lower birth weights. This longitudinal observation study also compared body composition measurements of HIV-exposed uninfected children with US standards. Subscapular and triceps SFT were used to measure the subcutaneous fat tissue of both groups. The results concluded that HIV-

exposed uninfected children had significantly lower subcutaneous fat stores when compared with National Health and Nutrition Examination Survey (NHANES) standards.

HIV is increasingly prevalent in many countries including South Africa.<sup>2 20</sup> According to the latest HIV impact assessment summary (July 2018), although the prevalence of HIV is high in South Africa the incidence has decreased from the 2012 estimates.<sup>21</sup> HIV incidence Adverse birth outcomes such as small for gestational age (SGA) and preterm infants are common to HIV-positive mothers. However, a specific relationship between maternal HIV, SGA and prematurity remain unclear.<sup>20</sup> A non-randomised interventional cohort study<sup>20</sup> evaluated the effects of maternal HIV on prevalent risk factors of unborn infants to be born SGA or preterm in rural areas of South Africa. This study comprised 1 189 HIV-positive and 1 179 HIV-negative pregnant women in antenatal clinics in KwaZulu-Natal, South Africa, who were recruited from 2001 to 2004. The results concluded that the antenatal clinics in the province had a very high antenatal HIV prevalence rate of 39.5% and a very high preterm delivery rate of 21.4%. The study also found that 16.6% of those infants, who were born preterm, were SGA. Maternal HIV was correlated with a 1.2-fold-increased risk for delivering SGA infants. There is, however, very little information and literature available on HIV infection and its effect on body composition, LBW, preterm infants and intrauterine growth restriction – all of which are risk factors for neonatal mortality and morbidity.<sup>12</sup>

The prevalence of HIV in South Africa is increasing, however the incidence is decreasing.<sup>2</sup>  
<sup>21</sup> The on-going debate as to whether or not HIV increases the incidence of infants being born preterm is still undetermined. However, research suggested that prescribing a protease inhibitor to HIV-positive mothers before or during pregnancy correlates positively with preterm births of infants.<sup>9 11</sup> Currently, the PMTCT guidelines are functioning well in the Western Cape province of South Africa as well as nationally, indicating a decrease in MTCT.<sup>5 4</sup> The relationship between body fat distribution and the body composition of HIV-infected or HIV-exposed children receiving ART prophylaxis medication has not yet been determined. However, it remains vital to conduct accurate and continuous anthropometric and body composition measurements on preterm infants to assess their nutritional status

in order to intervene timely and appropriately so as to prevent long-term adverse outcomes.

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# CHAPTER 2

## METHODOLOGY

## **This study formed part of a larger study, titled:**

***“Body composition, Growth and nutritional intake of HIV exposed preterm, very- and extremely-low birth weight infants in Tygerberg Children’s Hospital, Western Cape- The role of the Gut Microbiome and Human Milk Oligosaccharide composition. BIG HMO study.***

Two Masters in Nutrition (MSc Nutrition) students, Division of Human Nutrition, Stellenbosch University, conducted this large study. Each focussed on their own research question. Other components evaluated included: the infant gut microbiome and maternal Human Milk Oligosaccharide (HMO) composition.

Ethics reference #N15/10/102.

### **2.1 RESEARCH QUESTION**

Is there a difference in the body composition and nutritional intake of HIV exposed and HIV-unexposed preterm, very-low birth weight (VLBW) and extremely-low birth weight (ELBW) infants in Tygerberg Children’s Hospital, Western Cape?

### **2.2 AIM OF THE INVESTIGATION**

The aim of the study was to determine the body composition and nutritional intake of HIV-exposed and HIV-unexposed preterm, VLBW and ELBW infants in Tygerberg Children’s Hospital, Western Cape.

### **2.3 OBJECTIVES FOR THE BODY COMPOSITION STUDY**

#### **2.3.1 Primary objective**

- a. To evaluate and compare the body composition of HIV-exposed and HIV-unexposed, preterm VLBW and ELBW infants.
  - i. To evaluate the body composition in terms of fat mass and fat-free mass (muscle mass, bone mass and water mass).

### 2.3.2 Secondary objectives

- b. To evaluate the effects of the treatment duration of HAART of HIV-infected mothers on the body composition of HIV-exposed, preterm, VLBW and ELBW infants.
- c. To evaluate the effect of nutritional intake on the body composition of HIV-exposed and HIV-unexposed, preterm, VLBW and ELBW infants.
  - i. To evaluate the effect of breast milk fortification on body composition.
  - ii. To evaluate the effect of feeding intolerances and days that infants are nil per os on the body composition.

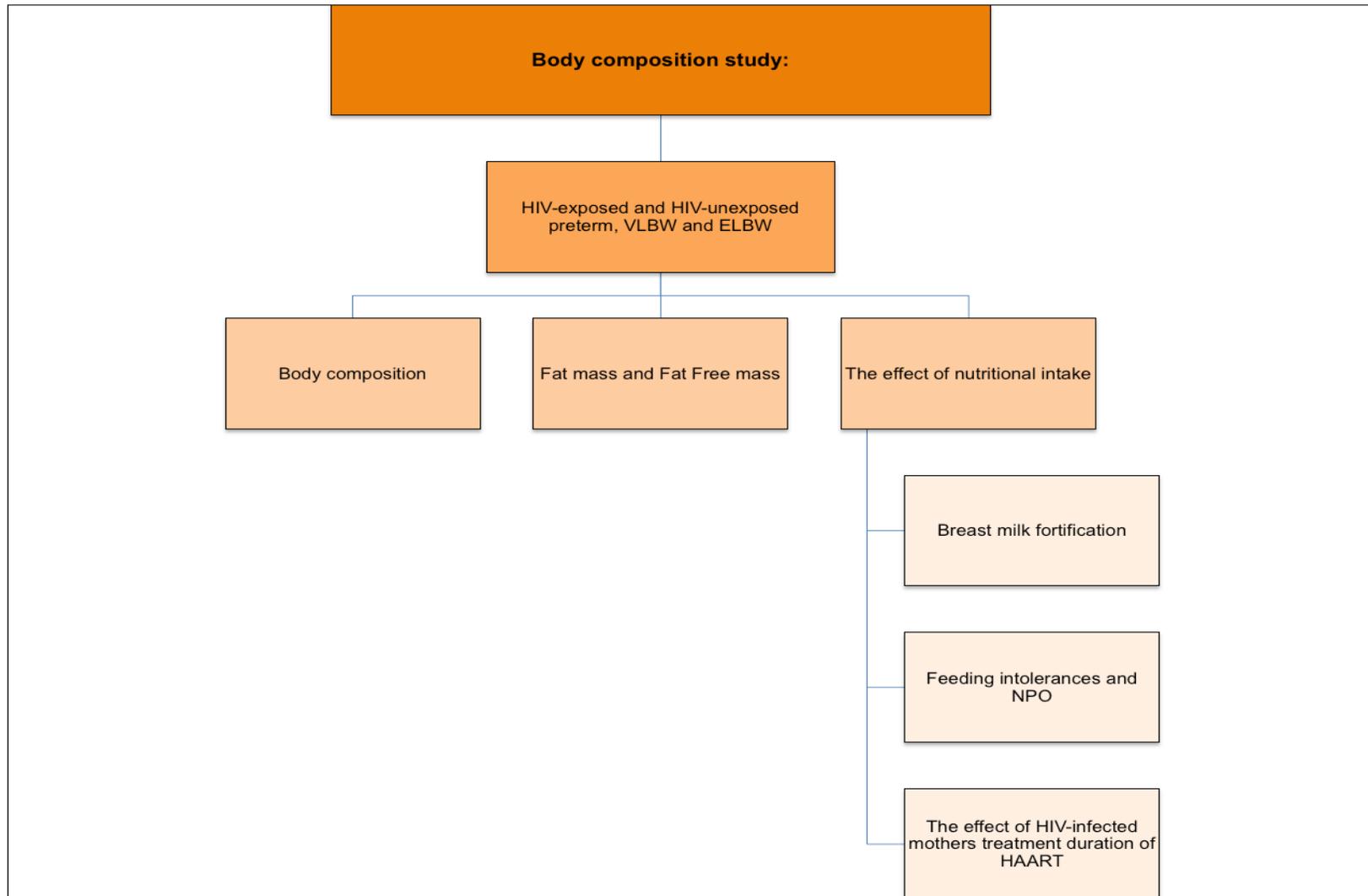
### 2.3.3 Null hypotheses

The following null hypotheses will guide the body composition study:

- a.  $H_0$ : There will be no difference in the body composition of HIV-exposed and HIV-unexposed, preterm, VLBW and ELBW infants.
- b.  $H_0$ : The treatment duration of HAART of HIV-infected mothers will have no effect on the growth and body composition of HIV-exposed, preterm, VLBW and ELBW infants.
- c.  $H_0$ : Nutritional intake has no effect on the body composition of HIV exposed and – unexposed, preterm, VLBW and ELBW infants.

## 2.4 CONCEPTUALISATION

The conceptual framework of the study is outlined in Figure 2.1. As shown in this diagram, the aim of the body composition study was to assess the body composition and nutritional intake of HIV-exposed and HIV-Unexposed, preterm, VLBW and ELBW infants. Paediatric anthropometrical measurements included the weight, length and skinfold thickness at two sites (triceps and subscapular) in conjunction with nine other body dimensions which included: circumferences (head, chest, mid-upper arm, mid-thigh and mid-calf) and lengths (upper arm, lower arm, supine length and crown-rump length).



**Figure 2.1:** Conceptual framework of the study

## **2.5 SETTING**

Wards G1, G2, J3 and G8 at Tygerberg Children's Hospital (TBCH) situated in Tygerberg Academic Hospital (TAH) were selected for data collection.

## **2.6 STUDY DESIGN**

The study was a descriptive cross-sectional study with an analytical component.

## **2.7 STUDY POPULATION**

The study population consisted of male and female preterm and very-low birth weight infants that were HIV-exposed and HIV-unexposed. These patients were born at TBCH and admitted or transferred to wards G1, G2, J3 and G8.

### **2.7.1 Sample size**

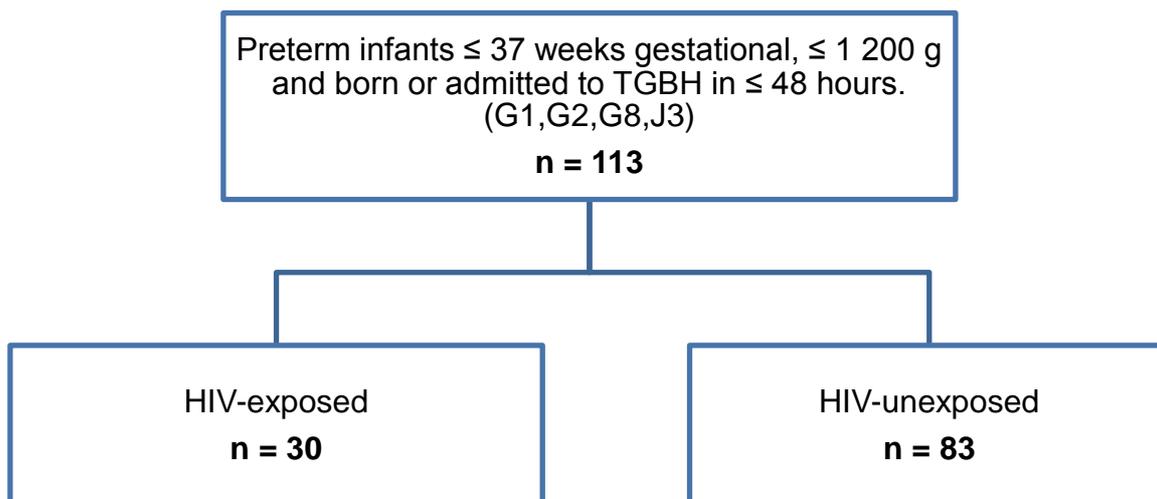
According to the 2013 and 2014 live birth statistics of TBCH, 801 and 815 infants were born with a birth weight of < 1 500 g respectively. Furthermore 283 ELBW infants (<1 000 g) were born in 2013 and 2014. According to the 2013 and 2014 live birth statistics from the Prevention of Mother to Child Transmission (PMTCT) clinic at TAH, 116 and 136 infants, respectively, were born with a birth weight of < 1 500 g. The ELBW infants (< 1 000 g) statistics show 86 live births in 2013 and 77 in 2014.

Infants with a birth weight of < 1 200 g were included in this study. The sample size was estimated according to the statistics mentioned above, the inclusion and exclusion criteria (refer to 2.7.2) and the recommendations of a statistician. The study population consisted of 113 participants in total, with 83 HIV-unexposed and 30 HIV-exposed preterm VLBW and ELBW infants.

A power analysis for two-way analysis of variance (ANOVA) with two levels for each factor with a power of 90% to detect interaction effect sizes of  $\delta = 0.40$  yielded samples of size  $n = 67$  in each of the two groups. An additional 20% was added to each group to accommodate patient loss due to early discharge.

After three months of data collection the researchers and primary investigators realised that only 20% of the study infants already included were HIV-exposed infants. Owing to the limited allocated time frame for data collection and financial constraints, the

researchers decided to consult with the statistician and review the sample size for the HIV-exposed infants. A power analysis for one-way analysis of variance (ANOVA) with a one-levelled factor with a power of 90% to detect interaction sizes of  $\delta = 0.49$  yielded a sample size  $n=23$  in the HIV-exposed group. An additional 30% was added to this group to accommodate patient loss due to early discharge. Refer to Figure 2.2 for a description of the sample sizes.



**Figure 2.2:** Description of sample size

### 2.7.2 Sample selection

The sample was selected from a population of consecutive preterm infants (< 37 weeks gestation) with a birth weight of < 1 200 g who were admitted or transferred to wards G1, G2, J3 and G8 at TBCH.

The inclusion and exclusion criteria that was used is outlined below.

#### 2.7.2.1 Inclusion criteria

The following inclusion criteria were used for preterm or low birth weight infants:

- i. All male and female HIV-exposed and HIV-unexposed preterm infants with a gestational age of 25–37 weeks,
- ii. All preterm infants with a very-low birth weight and/or extremely-low birth weight ( $\geq 500$  g– $\leq 1\ 200$  g).

- iii. All preterm infants receiving nutritional support in the form of enteral nutrition [EN: breast milk and supplementation thereof (FM85), formula feed) and parenteral nutrition (TPN)].
- iv. Infants from multiple gestations (twins, triplets, etc.) were included but they were noted as being from multiple births.
- v. Written parental consent to the study.

#### **2.7.2.2 Exclusion criteria**

The following exclusion criteria were used for preterm or low birth weight infants:

- i. Birth weight < 500 g.
- ii. Preterm infants with a birth age of < 25 weeks' gestation.
- iii. Infants with major congenital malformations and/ or developmental disabilities.
- iv. Preterm infants who had other disorders/conditions that could have affected body composition (oedema, myopathy)
- v. Preterm infants with major gastro-intestinal abnormalities.
- vi. Preterm infants who had a positive polymerase chain reaction (PCR) test at birth.
- vii. Unavailability of mother of preterm infant within 48 hours post birth (due to illness, adoption, etc.)

#### **2.7.2.3 Exit criteria**

Participants were exited under the following circumstances:

- i. Day 28 of the study completed.
- ii. Transferred to neonatal or paediatric intensive care unit (NICU/PICU).
- iii. Discharged within the 28 days of recruitment.
- iv. Confirmed necrotising enterocolitis (NEC): Bells stage II or III.

- v. Mothers admitted to ICU or those who had given their infant/s up for adoption.
- vi. If the mother of an eligible infant could still not be found after 48 hours.
- vii. Death.

## **2.8 SUBJECT RECRUITMENT**

- i. The researchers visited ward G2 (recruitment ward) every morning and looked at the admissions book for new admissions or births.
- ii. New births were screened to determine whether they had adhered to the inclusion criteria.
- iii. If the preterm infant met the criteria, their mothers were visited by the researcher in the labour wards.
- iv. The researchers explained the consent form to the mothers in detail (Addendum A).
- v. A mother had 48 hours to consider partaking in the study and to either provide consent or not.
- vi. As mentioned in the study methodology, preterm infants were included in the study during their first 48 hours of life. If, for some reason, the mother was not present at or during the first visit to the ward, the researchers returned to the ward later that day or the next morning so as to include all eligible preterm infants.

### **2.8.1 Establishment of the HIV-exposed and HIV-unexposed groups**

- i. All admitted preterm VLBW infants in ward G2 in TBCH who conformed to the inclusion and exclusion criteria with informed parental consent formed part of the total study population. Assessments continued for those patients who were transferred to wards G1, G8 and J3 unit during the study period.
- ii. The HIV-exposed group consisted of infants of consenting HIV-infected mothers who had a confirmed HIV-infected rapid-test result, as obtained from the PMTCT-programme during the prenatal period.

- iii. The HIV-unexposed group consisted of infants of all consenting HIV-uninfected mothers.

The researchers were Masters of Nutrition/Dietetics students. The nature of the study and procedure of the data collection was explained to the parent/primary caregiver of the potential participant by the researchers. Each potential participant was requested to complete a consent form. Thereafter, the researchers using the data collection forms obtained the relevant information.

The time frame allocated for data collection was approximately five months. This was based on the live birth statistics received from Tygerberg Hospital and the effect of the follow-up period. However, data was collected for a period of six months, from May 2016 to October 2016.

## **2.9 METHODOLOGY: MEASUREMENTS, METHODS AND INSTRUMENTS**

For this study, infants were weighed on a daily basis and length, head circumference and skinfolds were measured on the infants on five occasions during the 28-day follow-up period. Furthermore, infants were assessed on a daily basis and clinical, medical and nutritional data was collected. The specific measurements, methods and instruments used to collect the data will be discussed in further detail below.

The researchers underwent training pertaining to study procedures at the University of Stellenbosch by the department of Human Nutrition, therefore ensuring accuracy of measurements and methods.

### **2.9.1 Anthropometric measurements**

All measurements were done on preterm infants on day 1 of life (or no later than the first 48 hours). No anthropometric measurements were done for the time period that infants received nasal continuous positive airway pressure (NCPAP). The following anthropometric measurements were done:

#### **2.9.1.1 Weight**

All study infants, irrespective of whether or not these infants were incubated, were

weighed daily by the nursing staff. Daily weights were recorded from each participant's medical file by the researcher. All scales were calibrated on Mondays with the use of 500 g weights. Any significant weight changes were queried and the measurements were repeated under the supervision of the researcher

The weight of each infant was determined as follows:<sup>1</sup>

- i. An electronic scale (SECA 354) was used, with an accuracy of 0.001 kg.
- ii. All instruments were calibrated before beginning the measurements.
- iii. Infants were weighed without any clothing or a nappy. If a nappy was worn, the weight was corrected by subtracting the weight of the nappy.
- iv. Infants were placed in the middle of the scale and the baby had to lie still before the reading was taken.
- v. The measurements were read to the nearest 0.001 kg.

### **2.9.1.2 Length**

Infants were measured on day 1 of life; thereafter, the length was measured weekly. Upon exiting the study (day 28), the length was measured again. Therefore, five measurements, on days 1, 7, 14, 21 and 28, were taken. The researcher, with the help of the infant's mother, if needed, performed the length measurements. If there were any weekly discrepancies of more than 1 cm growth, the length was recorded for a second time.

If weekly lengths were taken by nursing staff, those values were also recorded and if there were discrepancies of  $> 0.5$  cm against the researcher's recording, a second measurement was done. Using the nursing staff's recordings was viewed as a quality control measure for this study.<sup>1</sup>

The length of each infant was measured according to the following criteria: <sup>2</sup>

- i. A SECA 207 measuring rod was used, with an accuracy of 0.1 cm.
- ii. Crown-heel length was obtained by placing the SECA 207 measuring rod over the infant who was lying on a smooth flat surface.

- iii. The participant's head was placed in the Frankfurt plane (a line between the lowest point on the margin of the orbit, the bony socket of the eye, and the trignon, the notch above the tragus, of the ear) in the centre of the SECA 207 measuring rod, and the shoulders were held down on the smooth flat surface in a straight line by the researcher or mother. This ensured that the infant's body lay parallel to the long axis of the SECA 207 measuring rod.
- iv. The measuring rod was positioned with the slide at the infant's head.
- v. The researcher, using her right hand, slid the measuring rod in the direction of the infant's feet and held the infant steady using her left hand.
- vi. The researcher then fully extended the infant's right leg and held the infant's foot at a right angle to the smooth flat surface.
- vii. The measuring slides on the SECA 207 measuring rod were then moved towards the infant's foot by applying constant pressure to the measuring slides until they met the infant's right heel at a right angle.
- viii. The measurement was then read from the number counter (inside of measuring rod) to the nearest 0.1 cm.

### **2.9.2 Body composition measurements**

Body composition was assessed through skinfold thickness at two primary sites: triceps and subscapular. The measurements were taken in the respective wards by the researchers or research assistants. Each infant's skinfold thicknesses were measured on day 1 of the study, and subsequently measured on a weekly basis. Upon exiting the study (day 28), the skinfold thickness was measured again. Five measurements, on days 1, 7, 14, 21 and 28, were thus recorded.

No skin fold measurements were done for the time period that infants had a weight of  $\leq 700$  g. Two skinfold measurements were taken at each skinfold site, weekly. If the results differed by more than  $> 0.5$  mm a third measurement was taken.<sup>3</sup>

The Dauncey anthropometric method was used to determine total fat mass and fat-free mass by assessing skinfold thickness at two sites (triceps and subscapular) in

conjunction with nine other body dimensions. The body dimensions included: circumferences (head, chest, mid-upper arm, mid-thigh and mid-calf) and lengths (upper arm, lower arm, supine length and crown-rump length).<sup>4 5</sup>

The following skinfold thickness measurements were performed:

### **2.9.2.1 Subcutaneous adipose tissue fat mass**

Fat mass was determined by the use of Holtain skinfold callipers, with a calibration sensitivity of 0.1 mm and divisions of 0.2 mm. The same pair of callipers was used for all of the measurements and was calibrated before each measurement period. Measurements were repeated on the right hand side of the body. Only two measurements at each body site were assessed owing to the fragility of the study population and to cause minimal invasion.<sup>a 6 7 8</sup>

The researcher or research assistant, with the help of the infant's mother/caregiver, if needed, performed skinfold thickness measurements.

The skinfold thickness of each infant was measured according to the following criteria:

#### **A) Triceps skinfold (TSF/TS)<sup>5 6 7 9 10</sup>**

- i. A Holtain calliper determined SSSF/SBS to the nearest 0.1mm.
- ii. TSF/TS was measured in the posterior midline of the left arm, over the triceps muscle, at the midway point between the lateral projection of the acromion process of the scapula and the inferior margin of the olecranon process of the ulna, while the participants' arm was placed at his/hers side.
- iii. Once located, the measurement site was marked with a pen.

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<sup>a</sup> After examining the study population the researchers decided not to conduct SFT measurements on infants' with a birth weight of  $\leq$  700 g, owing to their high vulnerability. Even though other research stipulated that these measurements are safe to conduct.

## **B) Subscapular skinfold (SSSF/SBS)<sup>5 6 7 9 10</sup>**

- i. A Holtain calliper determined SSSF/SBS to the nearest 0.1mm.
- ii. SSSF/SBS was measured on the right side, below the tip of the inferior angle of the scapula. The SSSF/SBS was picked up on a diagonal, inclined infero-laterally at a 45° angle versus the horizontal plane in the natural cleavage line of the skin.
- iii. Once located, the measurement site was marked with a pen.

The researcher picked up skin from the marked areas of measurement with her thumb and index finger, making sure to pick up the full thickness of the skin and taking care to exclude any underlying tissue<sup>9 10</sup>. This elevated a double fold of subcutaneous adipose tissue that was approximately 1 cm proximal to the site at which the skinfold was measured. The calliper was placed at this site, while the researcher still elevated the double fold of subcutaneous adipose tissue, to measure the subcutaneous adipose tissue for three seconds<sup>8 9 11</sup>. The average of the two skinfold thickness measurements were calculated to the nearest 0.1 mm and used as the actual skin fold thickness for that site in the study<sup>7 8 10</sup>.

The nine body dimensions: circumferences (head, chest, mid-upper arm, mid-thigh and mid-calf) and lengths (upper arm, lower arm, supine length and crown-rump length) of each infant were measured on day 1 of the study, and subsequently measured on a weekly basis. Upon exiting the study (day 28), the nine body dimensions were measured again. Five measurements, on days 1, 7, 14, 21 and 28, were thus recorded.

The following circumference measurements were performed:

## **C) Circumferences<sup>4 5</sup>**

The circumferences of each infant were measured according to the following criteria:

### **Head circumference**

If weekly head circumferences were taken by nursing staff, those values were also recorded and in the case of discrepancies of > 0.5 cm against the researcher's

recording; a second measurement was done. Using the nursing staff's recordings was viewed as a quality control measure for this study.

The head circumference of each infant was measured according to the following criteria:

- i. A standard non-stretchable measuring tape with 0.5 cm and 1 cm dimensions was used.
- ii. All head coverings were removed.
- iii. The measuring tape was placed on the supraorbital ridge and on the occiput.
- iv. The tape was on the same plane on both sides of the head.
- v. Measurements were read to the nearest millimetre.

#### **Chest circumference<sup>12 13</sup>**

- i. A standard non-stretchable measuring tape with 0.5 cm and 1 cm dimensions was used.
- ii. All clothes covering the chest area were removed.
- iii. The patient was held in a supine position with arms parallel to the body.
- iv. The measuring tape was placed at the level of the xiphoid nipples during the mid-stage of expiration of breath.
- v. The measuring tape was fitted snugly around the chest without compressing the skin.
- vi. Measurements were read to the nearest 0.1 cm.

#### **Mid-point of the arm (recumbent position)<sup>14 15</sup>**

- i. A standard non-stretchable measuring tape with 0.5 cm and 1 cm dimensions was used.
- ii. All clothes covering the arm area were removed.

- iii. The patient was held in a supine position with the right arm bent at a 90 ° angle at the elbow and the right palm facing up.
- iv. The lateral projection of the acromion process of the scapula was located and marked.
- v. The zero end of the measuring tape was held at this mark and extended down to the posterior surface of the arm to the inferior margin of the olecranon process of the ulna (bony part of the mid-elbow).
- vi. The tape was kept in position and located half the distance from the acromion to the olecranon process. (mid-point of the upper arm)
- vii. Measurements were read to the nearest 0.1 cm.
- viii. The measurement was marked with a cosmetic pen.

#### **Mid-upper arm circumference (MUAC)<sup>14 15</sup>**

- i. A standard non-stretchable measuring tape with 0.5 cm and 1 cm dimensions was used.
- ii. All clothes covering the arm area were removed.
- iii. The patient was held in a supine position with the arms loosely parallel to the body.
- iv. The measuring tape was wrapped around the right arm at the level of the upper arm mid-point mark.
- v. The tape was positioned perpendicular to the long axis of the upper arm.
- vi. The measuring tape was fitted snugly around the arm without compressing the skin.
- vii. Measurements were read to the nearest 0.1 cm.

#### **Mid-thigh circumference<sup>13 16</sup>**

- i. A standard non-stretchable measuring tape with 0.5 cm and 1 cm dimensions was used.
- ii. All clothes and diapers covering the right thigh area were removed.
- iii. The patient was held in a supine position with the arms parallel to the body and legs fully extended in a straight line.
- iv. The measuring tape was placed at the inguinal fold anteriorly, to lie at the level of the lowest crease in the gluteal region posteriorly, with the tape lying perpendicular to the to the long axis of the lower limb.
- v. The measuring tape was fitted snugly around the thigh without compressing the skin.
- vi. Measurements were read to the nearest 0.1 cm.

#### **Mid-calf circumference<sup>13 16</sup>**

- i. A standard non-stretchable measuring tape with 0.5 cm and 1 cm dimensions was used.
- ii. All clothes covering the right lower leg area were removed.
- iii. The patient was held in a supine position with the arms parallel to the body and legs fully extended in a straight line.
- iv. The measuring tape was placed at the level of the largest circumference of the calf.
- v. The measuring tape was fitted snugly around the calf without compressing the skin.
- vi. Measurements were read to the nearest 0.1 cm.

The following length measurements were performed:

#### **D) Length measurements<sup>4 5</sup>**

##### **Crown-rump length (Cl)**

- i. A standard non-stretchable measuring tape with 0.5 cm and 1 cm dimensions was used.
- ii. All clothes and diapers covering the crown rump area were removed.
- iii. The patient was in a prone position with the arms loosely parallel to the body.
- iv. The measuring tape was placed at the vertex of the cranium to the mid-point between the apices of the buttocks.
- v. Measurements were read to the nearest 0.1 cm.

#### **Upper arm length<sup>14 15</sup>**

- i. A standard non-stretchable measuring tape with 0.5 cm and 1 cm dimensions was used.
- ii. All clothes covering the right arm area were removed.
- iii. The patient was held in a supine position with the right arm bent at a 90° angle at the elbow and the right palm was facing up.
- iv. The lateral projection of the acromion process of the scapula was located and marked.
- v. The zero end of the measuring tape was held at this mark and extended down the posterior surface of the arm to the inferior margin of the olecranon process of the ulna (bony part of the mid elbow).
- vi. Measurements were read to the nearest 0.1 cm.

#### **Lower arm length<sup>4</sup>**

- i. A standard non-stretchable measuring tape with 0.5 cm and 1 cm dimensions was used.
- ii. All clothes covering the right arm area were removed.
- iii. The patient was held in a supine position with the arms loosely parallel to the

body.

- vii. The measuring tape was placed at the tip of the olecranon process (bony part of the mid elbow) to the tip of the lateral ulna styloid process.
- viii. Measurements were read to the nearest 0.1 cm.

### **Thigh and lower leg length <sup>4</sup>**

= Supine length–crown rump length

= S–Cl

## **2.10 DATA COLLECTION FORMS**

The data collection forms consisted of the following forms: the patient medical background, daily monitoring and anthropometric data. Refer to Addendum B for the data collection forms. All forms had been pre-coded. The coding list was supplemented as new possibilities or options occurred.

### **2.10.1 Patient medical background data collection form**

This form was used to collect medical background information of the infants as well as the mothers. The researchers documented this information upon the infants' entry into the study. The maternal information was divided into three sections and included general information, medical information and medication received. The infant information also consisted of these three sections. The investigator or research assistants documented this information upon the infant's entry into the study.

**Table 2.1: Maternal medical background information**

<b>Maternal information</b>	
General information	<ul style="list-style-type: none"> <li>• Age.</li> <li>• Race.</li> <li>• Lifestyle information (Smoking and the use of ethanol and narcotics).</li> <li>• Gravida/parity.</li> <li>• Mode of delivery.</li> <li>• Maternal HIV status.</li> <li>• Maternal CD4 cell count.</li> <li>• Whether the mother was be enrolled on the PMTCT scheme and the duration of enrolment.</li> <li>• Maternal tuberculosis history and treatment.</li> <li>• Maternal syphilis (Venereal Disease Research Laboratory [VDRL] test) history and treatment.</li> </ul>
Medical information	<p>Whether the following conditions were applicable to the mother:</p> <ul style="list-style-type: none"> <li>• Hypertension/preeclampsia.</li> <li>• Diabetes.</li> <li>• Antepartum and post-partum haemorrhage.</li> <li>• Abruptio placentae.</li> </ul>
Maternal medication	Maternal antibiotics and steroids given prior to/during labour.

**Table 2.2: Infant medical background information**

<b>Infant information</b>	
General information	<ul style="list-style-type: none"> <li>• Gender.</li> <li>• Date of birth.</li> <li>• Apgar counts.</li> <li>• Birth weight, length and head circumference.</li> <li>• Gestational ages – dates.</li> <li>• Foot length.</li> <li>• Infant syphilis (VDRL test) status and whether being treated.</li> </ul>
Medical information	<ul style="list-style-type: none"> <li>• Respiratory complications.</li> </ul> <p>Whether the infant underwent any of the following:</p> <ul style="list-style-type: none"> <li>• Cranial ultrasound.</li> <li>• Phototherapy.</li> </ul>
Infant medication	<p>The following medications were noted:</p> <ul style="list-style-type: none"> <li>• Nevirapine.</li> <li>• AZT.</li> <li>• Surfactant.</li> <li>• Antibiotics.</li> <li>• Corticosteroid.</li> <li>• Other.</li> </ul>
Blood sample collections	<p>Whether the following blood samples had been collected:</p> <ul style="list-style-type: none"> <li>• CRP.</li> <li>• Full blood count.</li> <li>• Blood culture.</li> <li>• HIV PCR.</li> </ul>

### 2.10.2 Daily monitoring form

The daily monitoring forms assessed the nutritional intake and output of patients. Furthermore, some treatments such as phototherapy and blood products received

were noted. All medications, intravenous fluids and blood transfusions administered to the study infants were documented on the medical form by the investigator or research assistants. Only medical information that was directly linked to body composition was recorded. Antiretroviral treatment was documented for the HIV-exposed infants. The daily clinical assessments of the study infants were done by the attending doctors and were documented by the investigator or research assistants.

### **2.10.3 Nutritional intake**

The standard feeding protocol of the ward was applied during the study. The time of initial feeding was at the discretion of the attending physician. The daily volume of milk feeds was determined according to the feeding protocol of the institution. According to ward protocol, feeds were increased in daily increments of 25 ml/kg/d. Furthermore, per ward protocol, infants of HIV-infected mothers received pasteurised expressed breast milk. Infants of HIV-uninfected mothers received unpasteurised breast milk (expressed or breastfed). The breast milk of HIV-infected mothers was pasteurised using the Pretoria flash method. Pasteurisation is standard protocol in the ward; therefore it did not form part of the methodology of this study.

Breast milk fortifier was introduced when infants reached daily feeds of above 100 ml/kg. Fortification of feeds was, however, dependent on feeding tolerance and may have differed from one individual to another. The dietary intake of all study infants was documented on a feed monitoring form. Any feeding intolerance was documented.

The following data was captured on the daily monitoring form:

- Feeding route of the infant.
- Volume/amount of breast milk, formula, TPN, Neonatelite (glucose-electrolyte-containing solution) and saline provided to the infant.
- Supplementation with FM85 (a breast milk fortifier) and medium-chain triglyceride) oil, multivitamin and iron supplementation.
- Urinary output.
- Stool frequency and colour/consistency.

- Phototherapy and blood products received.
- Medication administered. All the medications that the infants received were noted.
- Any feeding intolerance was documented. The attending doctor noted the presence of a feeding intolerance in the patient folder. Furthermore, for the purposes of this study, a feeding intolerance was identified when vomiting and aspirates occurred. The colour and frequency of vomits and aspirates was noted. Also, abdominal distension, stool volume and consistency were reported on.

## **2.11 DATA CAPTURING**

Data capturing was done using Microsoft Excel 2007<sup>®</sup>.

## **2.12 DATA ANALYSIS**

The data analysis for the different aspects of data collection is discussed in detail below:

### **2.12.1 Data analysis: Body composition measurements**

#### **2.12.1.1 Skinfold thickness measurement**

For the analyses of skinfolds, the average of the two measurements at each site was used, unless a third measurement was taken. In that case, if two of the three measurements differed by less than the prescribed amount ( $< 0.5$  mm), the average of those two measurements was used; otherwise, the average of all three measurements was used.<sup>3</sup>

## VOLUME OF SUBCUTANEOUS FAT <sup>4 5</sup>

**Crown rump length** – Cl

**Diameter of head** – d

**Supine length** – l

**Length of upper arm and lower arm (Arm length)** – a

**Skinfold calliper measurement** – S

**Thickness of subcutaneous fat layer** - $\phi = (S - 0.2 \text{ cm})$

**Trunk** = (Cl – d)

$\phi$ **Trunk** = (Subscapular skin fold – 0.2 cm)

$\phi$ **Arm** =  $\phi$ **Leg** = (Triceps skin fold – 0.2 cm)

**0.9** = The density factor of human fat

**Trunk circumference** = Cc (Chest circumference)

The following were measured to determine FM and FFM in the preterm infant:

### A) Volume of fat covering trunk (ml)

A = (Crown rump length minus diameter of head) × (Circumference of trunk) × (Subscapular skin fold – 0.2 cm)

**A** = (trunk) × (Cc) × ( $\phi$ trunk)

### B) Volume of fat covering upper limbs (ml)

B = (Length of upper arm + length of lower arm) × (Circumference of upper arm × (Tricep skinfold – 0.2 cm)

**B** = (a) × (MUAC) ×  $\phi$ arm

### C) Volume of fat covering lower limbs (ml)

C = (Supine length – crown rump length) ×  $\frac{1}{2}$  (circumference of mid-thigh + circumference of calf) × (Triceps skinfold – 0.2 cm)

**C** = (l-Cl) ×  $\frac{1}{2}$  (Circumference of mid-thigh + Circumference of calf) ×  $\phi$ Leg

#### **D) Total body fat (FM)**

$$D = (A + 2B + 2C) \times 0.9$$

#### **E) Total fat-free mass (FFM)**

$$E = \text{Body weight} - \text{FM}$$

#### **F) Percentage of FM**

$$F = (\text{FM}/\text{Total body weight}) \times 100$$

### **2.13 STATISTICAL ANALYSIS**

Microsoft Excel<sup>®</sup> was used to capture the data and STATISTICA version 11 (StatSoft Inc. (2013) STATISTICA (data analysis software system), [www.statsoft.com](http://www.statsoft.com).) was used to analyse the data. Summary statistics were used to describe variables such as the daily intake data of infants, routine examinations, medication, intravenous fluids, blood transfusions and mode of delivery.

Distributions of variables were presented with histograms and/or frequency tables. Medians or means were used as the measures of central location for ordinal and continuous responses and standard deviations and quartiles as indicators of spread. The relation between nominal variables was investigated with contingency tables and appropriate chi-square tests, such as the likelihood ratio chi-square test. A  $p$ -value of  $p < 0.05$  represents statistical significance in hypothesis testing and 95% confidence intervals were used to describe the estimation of unknown parameters.

### **2.14 PILOT STUDY**

Local ethical consent was obtained and a pilot study was performed in May 2016 to streamline all data collection procedures. Pilot study participants were recruited for a period of one week.

### **2.15 FINANCIAL DISCLOSURE**

There were no financial disclosures for this study.

## **2.16 ETHICAL CONSIDERATIONS**

The protocol was submitted to the Human Research Ethics Committee of the Faculty of Health Sciences, Stellenbosch University and TAH for approval. All the investigators adhered to Good Clinical Practice (GCP) guidelines during the study period. All participating mothers were informed about the study in their first language (Afrikaans, English or Xhosa). Each participant (the study infant's mother) received a copy of the consent form once recruited and written consent was obtained for participation in the study. In some instances, participants were given 48 hours to consider partaking in the study before the written consent form was signed. Each participant received a copy of the consent form. Participating in the study was voluntary. Participants were free to leave the study at any time and would not be penalised or prejudiced in any way for doing so.

Confidentiality and anonymity were maintained during this study. All infants received an allocated participant number. The HIV status of the mother and her infant were treated with confidentiality and were not revealed to any persons other than the patient's doctor, the research assistant and the investigator.

Participation in this study did not affect the quality of care that infants and mothers received during or after the study. Appropriate medical care provided to HIV-infected mothers as determined by the provincial protocol for the treatment of HIV/AIDS was adhered to. Refer to Addendum C for the ethics approval notification.

## **2.17 RISK TO BENEFIT RATIO**

Available literature stipulated that one of the best methods for determining body composition of the preterm infant in terms of fat mass and fat-free mass is the use of the Dauncey anthropometric model, which includes skinfold thickness at two primary sites and nine body dimensions. This body composition method is accurate, safe, inexpensive, non-invasive and can be performed at the bedside, avoiding excessive disturbance to these fragile infants.

## **2.18 VULNERABLE STUDY POPULATION**

Limited literature exists on the assessment of neonatal body composition and only a

few studies have focused on the growth and development of preterm infants.<sup>2 3</sup> Research shows that preterm infants should achieve growth rates similar to the term infant. In other words, the preterm infant should grow at the same rate in size, anthropometrically and in body composition extra-utero as infants who are still growing in utero.<sup>5 6 7 8</sup> However, generally infants do not meet these growth rates, resulting in growth failure and debilitating outcomes. After examining the study population, the researchers decided not to conduct SFT measurements on infants with a birth weight of  $\leq 700$  g, because of their high vulnerability, even though other research stipulated that these measurements were safe to conduct.

## **2.19 CONFIDENTIALITY AND QUALITY OF CARE**

Confidentiality and anonymity were maintained during this study. All infants received an allocated participant number that was used for data capturing. The HIV status of the mother and her infant were treated with confidentiality and were not revealed to any persons other than the nursing staff, the patient's doctor, the researchers and the investigator.

Participation in this study did not affect the quality of care that infants and mothers received during or after the study.

## **2.20 INSURANCE**

The protocol provided insurance for research-related adverse events (Addendum D).

## 2.21 BUDGET

Table 2.3 indicates the study budget.

**Table 2.3: Study budget**

Item	Description	Unit cost	Total cost
Printing	Printing of consent forms and data collection forms		R 700.00
Research equipment	1×SECA 207 model measuring rod.	R2 365	R2 365.00
Human resources	Statistician	R400/hr × 10 hours	R 4 000.00
	Research assistant	R6 000/month – 3 hours per day	R 6 000.00
<b>Total cost</b>			<b>R 13 065.00</b>

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# CHAPTER 3

## RESULTS

## ORIGINAL ARTICLE 1

### **The effect of maternal HIV status and treatment duration on body composition of HIV-exposed and HIV-unexposed preterm, very- and extremely-low birth weight infants**

Klara Strydom, Daniel Gerhardus Nel, Muhammad Ali Dhansay & Evette van Niekerk (2018): The effect of maternal HIV status and treatment duration on body composition of HIV-exposed and HIV-unexposed preterm, very and extremely low-birthweight infants, *Paediatrics and International Child Health*, DOI: 10.1080/20469047.2018.1466481



## The effect of maternal HIV status and treatment duration on body composition of HIV-exposed and HIV-unexposed preterm, very and extremely low-birthweight infants

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### ABSTRACT

**Background:** There is an evidence gap regarding the relationship between HIV exposure, body composition (and the quality thereof) and preterm infants.

**Aim:** This study determined the body composition of HIV-exposed, preterm very low-birthweight (VLBW) and extremely low-birthweight (ELBW) infants and to assess the effect of maternal HAART duration on the body composition of this vulnerable population.

**Methods:** A descriptive cross-sectional study was conducted. HIV-exposed and -unexposed preterm infants (<37 weeks) with a birthweight of  $\leq 1200$ g were included. Maternal medical background was recorded. Infant body composition measurements were recorded weekly during the 28-day follow-up period.

**Results:** Thirty preterm infants (27%) were HIV-exposed. HIV-exposed infants had significantly ( $=0.01$ ) lower gestational ages than HIV-unexposed infants (25–28 weeks). HIV-exposed infants had significantly lower measurements on day 21 and day 28 for triceps skinfold (TSF) (2.5 mm vs 2.7 mm,  $=0.02$  and 2.6 mm vs 2.9 mm,  $<0.01$ ), subscapular skinfold (SSSF) (2.3 mm vs 2.6 mm,  $=0.02$  and 2.4 mm vs 2.7 mm,  $=<0.01$ ) and fat mass percentage (FM%) (0.9% vs 1.4%,  $=0.02$  and 1.0% vs 1.5%,  $=0.03$ ). HIV-exposed infants whose mothers received HAART for  $\geq 20$  weeks were heavier and had a higher FM% and lower fat-free mass percentage (FFM%) at birth than HIV-exposed preterm infants whose mothers received highly active antiretroviral therapy for  $\geq 4$ – $<20$  weeks.

**Conclusion:** Mothers receiving HAART could have increased risk of preterm delivery, and the duration of maternal HAART affects postnatal body composition of their infants. Body composition differs between HIV-exposed and HIV-unexposed preterm infants.

**Abbreviations:** ANOVA, analysis of variance; ART, antiretroviral therapy; CVD, cardiovascular diseases; DRC, Democratic Republic of Congo; ELBW, extremely low birthweight; FFM, fat-free mass; FFM%, fat-free mass percentage; FM, fat mass; FM%, fat mass percentage; HAART, highly active antiretroviral therapy; HIC, high-income countries; GA, gestational age; IUGR, intrauterine growth restriction; LGA, large for gestational age; LMIC, low- and middle-income countries; LBW, low birthweight; MCT, medium-chain triglycerides; MTCT, mother-to-child transmission; NCPAP, nasal continuous positive airway pressure; NHANES, National Health and Nutrition Examination Survey; NVP, nevirapine; NICU, neonatal Intensive Care Unit; PMTCT, prevention of mother-to-child transmission; PCR, polymerase chain reaction; SFT, skinfold thickness; SGA, small for gestational age; SAPMTCTE, South African prevention of mother-to-child transmission evaluation; SSSF, subscapular skinfold; TEA, term equivalent age; TSF, triceps skinfold; TAH, Tygerberg Academic Hospital; TBCH, Tygerberg Children's Hospital; USA, United States of America; VLBW, very low birthweight; AZT, zidovudine

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Body composition; fat mass; fat-free mass; preterm infant; HIV-exposed; HIV-unexposed; HAART duration

### Introduction

There is a gap in documented research in high-income countries (HIC) and in low- and middle-income countries (LMIC) regarding the relationship between HIV exposure, pharmacological interventions, duration of maternal highly active antiretroviral therapy (HAART) and body composition in preterm infants [1–7].

There is increasing interest in the association between nutritional status during early infancy and childhood, and the increased risk of adverse health effects in adult life. Nutritional recommendations for preterm infants attempt to achieve growth rates similar to the term infant in relation to size, anthropometry and body composition at term equivalent age (TEA) [8–12]. However, it has

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been found that these infants generally do not achieve the required growth rates, resulting in growth failure and adverse outcomes in later life. Preterm infants who experience rapid post-natal catch-up growth develop increased intra-abdominal adiposity which causes an abnormal body composition [13].

According to gestational age (GA) at birth, preterm birth can be defined as moderate-to-late preterm ( $\geq 32$ – $< 37$  weeks), very preterm ( $\geq 28$ – $< 31$  weeks) and extreme preterm ( $< 28$  weeks) [14,15]. Prematurity, low birthweight (LBW) and intrauterine growth restriction (IUGR) show strong correlations with mortality and morbidity in the neonatal period, as well as in the first years of life. LBW is often owing to IUGR, prematurity or a combination of both [16–18]. By definition, very low-birthweight (VLBW) infants or extremely low-birthweight (ELBW) infants weight, respectively,  $\geq 1000$ – $< 1500$  g or  $< 1000$  g [15,19]. Contributors to very low birthweight include maternal, infant and environmental factors often associated with LMIC and correlate with and influence the nutritional status of the infant [16,17,20].

According to the 2017 South African mid-year population estimates report [21], 21.2% of women aged 15–49 years are HIV-infected. HIV is highly prevalent in South Africa and HIV-infected pregnant women on HAART could have a considerably greater risk (13–34%) of delivering an infant preterm [22,23]. HIV-exposed infants have poorer growth and greater early mortality and morbidity rates than their HIV-unexposed counterparts, although HIV-exposed/uninfected infants do not grow as poorly as HIV-infected infants [24–28]. It is widely known that HIV-infected children who are receiving antiretroviral therapy (ART) experience growth and metabolic abnormalities which have a profound effect on their short-term survival [4,29,30]. Changes in body fat distribution, as well as glucose and bone metabolism in HIV-infected children receiving ART can increase the risk of future morbidities [29].

As far as we are aware, the relationship between body fat distribution and body composition in HIV-infected or -exposed preterm infants receiving ART or prophylactic medication such as nevirapine (NVP) or zidovudine (AZT) [31,32] has not yet been determined. It is vital to consistently and accurately measure anthropometric and body composition continuously in preterm infants in order to monitor their nutritional status and intervene promptly and appropriately to prevent long-term adverse outcomes.

Body composition in terms of fat mass (FM) and fat-free mass (FFM) should be assessed and monitored continuously as they are more significant than weight gain only, even in an LMIC with limited resources. The use of simple as well as sophisticated methods is imperative for neonatologists and dietitians to obtain accurate results in preterm infants. According to the available literature, the most appropriate method of assessing preterm body composition in an LMIC is Dauncey's anthropometric model [33–36].

Measuring skinfold thickness (SFT) on fragile preterm infants might be considered invasive and controversial because of the size of the instrument relative to the infant. However, the measurements are useful, simple, inexpensive and non-invasive for determining body composition in these infants [33,37,38]. Determining neonatal subcutaneous fat with calliper SFT measurements allows evaluation of the distribution of FM in specific parts of the body [39].

Published research on maternal HIV status, duration of treatment and its effect on preterm infant body composition is scarce [1,4]. The aim of this study was to determine the body composition of HIV-exposed preterm VLBW and ELBW infants and to assess whether the duration of maternal HAART has an effect on body composition in this vulnerable population.

## Subjects and methods

### *Study design and setting*

This cross-sectional descriptive study is part of a larger study undertaken in the Neonatal Unit of Tygerberg Children's Hospital (TBCH) in Tygerberg Academic Hospital (TAH), Cape Town, South Africa. The study was conducted from 16 May to 22 October 2016.

### *Sample size*

The sample sizes needed for the study population was determined according to the live birth statistics for infants born with a birthweight of  $< 1500$  g at the institution. A power analysis for two-way analysis of variance (ANOVA) with a power of 90% to detect interaction effects of  $\delta = 0.49$  (regarded as a medium effect size) yielded a required sample size of 23 in each of the exposure groups.

### *Participants*

Participants were recruited by consecutive sampling. The study population was male and female HIV-exposed and -unexposed preterm infants who met the inclusion criteria and whose mothers gave written informed consent. All preterm infants with a gestational age of  $\geq 25$ – $< 37$  weeks and a birthweight of  $\geq 500$ – $\leq 1200$  g born at TBCH were included.

### *Outcomes*

The primary outcome of the study was determination of the body composition of HIV-exposed and -unexposed preterm, VLBW and ELBW infants. HIV-infected mothers received HAART treatment for  $\geq 4$  weeks at delivery. Therefore, the effect of the duration of that treatment on the body composition of their infants was a secondary outcome.

## Methods

Participants were included in the study for a 28-day follow-up period, day 1 being the first day of life. Each infant's maternal medical background was recorded. Infant clinical and medical data were collected daily and weekly anthropometrical and skinfold measurements were undertaken by the researchers. The researchers documented medical information, medications, supplementation and all fluid input and output. Maternal ARV treatment was documented for the HIV-exposed preterm infants. Daily clinical assessments were undertaken by the attending doctors and the results recorded. The following data were assessed and recorded daily: supplementation with medium-chain triglycerides (MCT) oil, multivitamin supplementation, phototherapy and blood products received, medication administered and urinary output, stool frequency and colour/consistency, and feeding intolerance frequency and colour.

The Dauncey anthropometric model was used to determine total FM and FFM by assessing skinfold thickness at two sites (triceps and subscapular) in conjunction with nine other body dimensions. These included: circumferences (head, chest, mid-upper arm, mid-thigh and mid-calf) and lengths (upper arm, lower arm, supine and crown-rump length) [34,35]. Each infant's skinfold thicknesses were measured on day 1 of the study and then weekly. Upon exiting the study (day 28), the skinfold thicknesses were measured again. Five measurements, on days 1, 7, 14, 21 and 28, were thus recorded. Holtain skinfold calipers with a calibration sensitivity of 0.1 mm and divisions of 0.2 mm were used and calibrated before each measurement period. Measurements were undertaken on the right-hand side of the body. Because of the fragility of the study population and to cause minimal invasion, measurements were assessed only twice at each body site [37,40–42]. No skinfold measurements were done when infants weighed  $\leq 700$  g. If the results differed by more than  $> 0.5$  mm, a third measurement was taken [40].

## Statistical analysis

Data were analysed using STATISTICA version 11 (StatSoft Inc., 2013, Stellenbosch, South Africa). Statistical significance was  $p < 0.05$ .

## Ethics

Ethics approval was granted by the Human Research Ethics Committee of the Faculty of Health Sciences, Stellenbosch University and Tygerberg Academic Hospital (Ethics reference #N15/10/102).

## Results

The final study population was 113 preterm infants after parental informed consent was obtained. Thirty infants

**Table 1.** Clinical and demographic characteristics of study infants.

	HIV-exposed infants <i>n</i> = 30 (27%)	HIV-unexposed infants <i>n</i> = 83 (73%)	<i>p</i> -value
Male, <i>n</i> (%)	17 (57)	44 (53)	0.73
Female, <i>n</i> (%)	13 (43)	39 (47)	
Race, <i>n</i> (%)			0.23
Black	19 (63)	42 (51)	
Mixed race	11 (37)	41 (49)	
White	–	–	
Other	–	–	
Gestational age, wks, <i>n</i> (%)			
32–37	–	4 (5)	<b>0.11</b>
28–32	7 (23)	39 (47)	<b>0.02</b>
25–28	23 (77)	40 (48)	<b>0.01</b>
Birthweight, <i>n</i> (%)			
VLBW	13 (43)	44 (53)	0.36
ELBW	17 (56)	39 (47)	
APGAR, <i>n</i> (%)			
<4	1 (3)	4 (5)	0.72
4–7	3 (10)	7 (84)	0.80
>7	25 (83)	70 (84)	0.90
NCPAP			
No. of days, mean (SD)	7.23 (6.8)	6.5 (6.24)	0.60
Phototherapy			
No. of days, mean (SD)	4.2 (4)	4.9 (3.4)	0.06

Notes: APGAR, activity, pulse, grimace, appearance, respiration; NCPAP, nasal continuous positive airway pressure. Values are means and standard deviations (SD) unless otherwise indicated. Figures in bold are statistically significant.

Contingency tables with a  $\chi^2$  was used.

(27%) were HIV-exposed, of whom 13 (43%) and 17 (57%) were VLBW and ELBW, respectively. Eighty-three of the infants (73%) were HIV-unexposed, 44 (53%) of whom were VLBW and 39 (47%) ELBW. Seventy infants (62%) completed the 28-day follow-up period and 43 (38%) did not. There were no withdrawals from the study (Appendix 1).

Using contingency tables, the  $\chi^2$  statistical test indicated no significant difference in gender, racial distribution, birthweight category (VLBW or ELBW), APGAR scores and number of days receiving nasal continuous positive airway pressure (NCPAP) and phototherapy in HIV-exposed and -unexposed preterm infants (Table 1). Only four (4%) HIV-unexposed infants were classified as moderate-to-late preterm (32–37 weeks). Post hoc analyses showed a significant difference in the GA categories of the two groups, given that 23 of the HIV-exposed infants (77%) were extremely premature (25–28 weeks) ( $p = 0.01$ ). Post hoc analyses also indicated significant differences in the GA categories for HIV exposures for infants born very preterm (GA 28–32 weeks) ( $p = 0.02$ ; with a power = 0.999).

Using one-way ANOVA, the variables discussed in this paragraph were compared with HIV exposure. The mean (SD) birthweight of HIV-exposed preterm infants was 929 g (155) and the mean (SD) birth length of the same infants was 35.1 cm (2.8). In HIV-unexposed preterm infants, mean (SD) birthweight was 986 g (163) and the mean (SD) birth length 36.6 cm (2.8). There was a significant difference

**Table 2.** Weekly anthropometric and body composition measurements.

	HIV-exposed infants	HIV-unexposed infants	p-value
	n = 30 (26.5%)	n = 83 (73.5%)	
<i>Day 1 of life, mean (SD)</i>			
Weight, g	929 (155)	986 (163)	<b>0.05</b>
Length, cm	35.1 (2.8)	36.6 (2.8)	<b>0.01</b>
Head circumference, cm	25.1 (1.6)	26 (1.8)	<b>&lt;0.01</b>
TSF, mm	2.6 (0.5)	2.8 (0.5)	0.08
SSSF, mm	2.4 (0.5)	2.4 (0.4)	0.50
FM, %	1.1 (1.0)	1.3 (0.85)	0.28
FFM, %	98.8 (1.0)	98.7 (0.85)	0.28
<i>Day 7 of life, mean (SD)</i>			
Weight, g	899 (137)	941 (164)	0.19
Length, cm	35.5 (1.7)	36.4 (2)	0.12
Head circumference, cm	25.1 (1.3)	25.9 (1.3)	0.09
TSF, mm	2.4 (0.4)	2.6 (0.4)	<b>0.03</b>
SSSF, mm	2.3 (0.5)	2.4 (0.4)	0.19
FM, %	0.9 (0.65)	1.2 (0.7)	0.20
FFM, %	99.1 (0.65)	98.9 (1.2)	0.28
<i>Day 14 of life, mean (SD)</i>			
Weight, g	919 (170)	1009 (161)	<b>0.02</b>
Length, cm	35.8 (1.8)	36.7 (2)	0.06
Head circumference, cm	25 (1.5)	26 (1.4)	<b>&lt;0.01</b>
TSF, mm	2.5 (0.3)	2.6 (0.4)	0.27
SSSF, mm	2.3 (0.3)	2.4 (0.4)	0.51
FM, %	1.2 (0.6)	1.1 (0.7)	0.90
FFM, %	99.0 (1.4)	99.1 (1.6)	0.81
<i>Day 21 of life, mean (SD)</i>			
Weight, g	1013 (198)	1121 (191)	<b>0.01</b>
Length, cm	36.7 (2)	37.5 (1.7)	0.09
Head circumference, cm	25.9 (1.8)	27 (1.5)	<b>&lt;0.01</b>
TSF, mm	2.5 (0.4)	2.7 (0.4)	<b>0.02</b>
SSSF, mm	2.3 (0.45)	2.6 (0.4)	<b>&lt;0.01</b>
FM, %	0.9 (0.65)	1.4 (0.7)	<b>0.01</b>
FFM, %	99 (1.0)	98.7 (0.8)	<b>0.03</b>
<i>Day 28 of life, mean (SD)</i>			
Weight, g	1026 (144)	1216 (222)	<b>&lt;0.01</b>
Length, cm	36.6 (1.6)	38.0 (1.9)	<b>&lt;0.01</b>
Head circumference, cm	26.0 (1.6)	27.6 (1.5)	<b>&lt;0.01</b>
TSF, mm	2.6 (0.4)	2.9 (0.6)	<b>&lt;0.01</b>
SSSF, mm	2.4 (0.4)	2.7 (0.5)	<b>&lt;0.01</b>
FM, %	1.0 (0.7)	1.5 (0.82)	<b>0.03</b>
FFM, %	99.0 (0.7)	98.5 (0.8)	<b>0.03</b>

Notes: TSF, triceps skinfold thickness; SSSF, subscapular skinfold thickness; FM%, fat mass percentage; FFM%, fat-free mass percentage. Values are mean (SD) unless otherwise indicated. Figures in bold are statistically significant. One-way ANOVA test was used.

between mean birthweight ( $p = 0.05$ ) and mean birth length ( $p = 0.01$ ) in the two groups (Table 2). The HIV-exposed infants were generally smaller, shorter and leaner at birth than the HIV-unexposed infants. There were significant differences between HIV exposures for triceps skinfold (TSF) and subscapular skinfold (SSSF) thicknesses [on days 7, 21, 28 ( $p = 0.03$ ,  $p = 0.02$  and  $p < 0.01$ ) and days 21 and 28 ( $p \leq 0.01$  and  $p \leq 0.01$ ) of life, respectively] (Figure 1). There were also significant differences in fat mass percentages (FM%) and fat-free mass percentages (FFM%) on days 21 ( $p = 0.01$  and  $p = 0.03$ ) and 28 ( $p = 0.03$  and  $p = 0.03$ ) of life (Figure 2).

Using one-way ANOVA, an almost equal mean (SD) weight loss was noted in the first week of life in both the HIV-exposed and -unexposed groups [ $-58.5$  g (60.6) and  $-58.2$  g (60.15) ( $p = 0.32$ ), respectively]. Generally, HIV-exposed preterm infants had smaller weight (g) increases throughout the 28-day study period. Statistically significant differences in weight (g) occurred between days 21–28 ( $p = 0.03$ ) and days 1–28 ( $p = 0.04$ ) in the HIV-exposed and -unexposed groups, respectively. The TSF

and SSSF thickness measurements concurred with the FM% infants lost or gained during the study period. SSSF thickness was significant between days 1 and 28 ( $p = 0.01$ ) (Supplementary Table 1).

There were significant differences relating to HIV exposure and FM% between days 1 and 7 ( $p = 0.01$ ) and days 7 to 14 ( $p = 0.05$ ) of life. During the first week of life, HIV-exposed preterm infants had a mean (SD)  $-0.47\%$  (0.63) lower FM% than their HIV-unexposed counterparts with an FM% of  $0.03\%$  (0.8). Between days 7 and 14 of life, HIV-exposed infants had a mean (SD) increased FM% of  $0.3\%$  (0.53) and HIV-unexposed infants had an FM% decrease of  $-0.06\%$  (0.7%) (Figure 3).

Comparison of the weekly anthropometric and body composition measurements in HIV-exposed and -unexposed per birthweight category (VLBW and ELBW) showed no significant differences in weight, length, skinfold thickness, FM% and FFM% for the duration of the study period.

There were 10 pairs of twins in the study, of which three pairs were born to HIV-infected mothers and seven

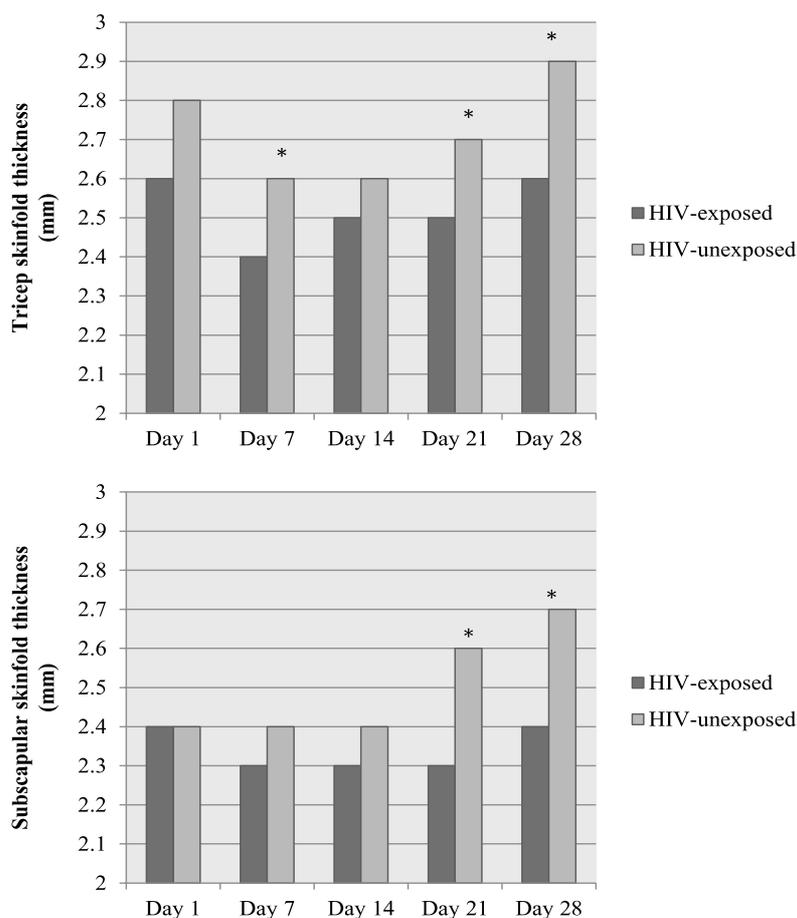


Figure 1. Comparison of skinfold thickness in HIV-exposed and -unexposed preterm infants.

\*Statistically significant when  $p < 0.05$ . One-way ANOVA test was used.

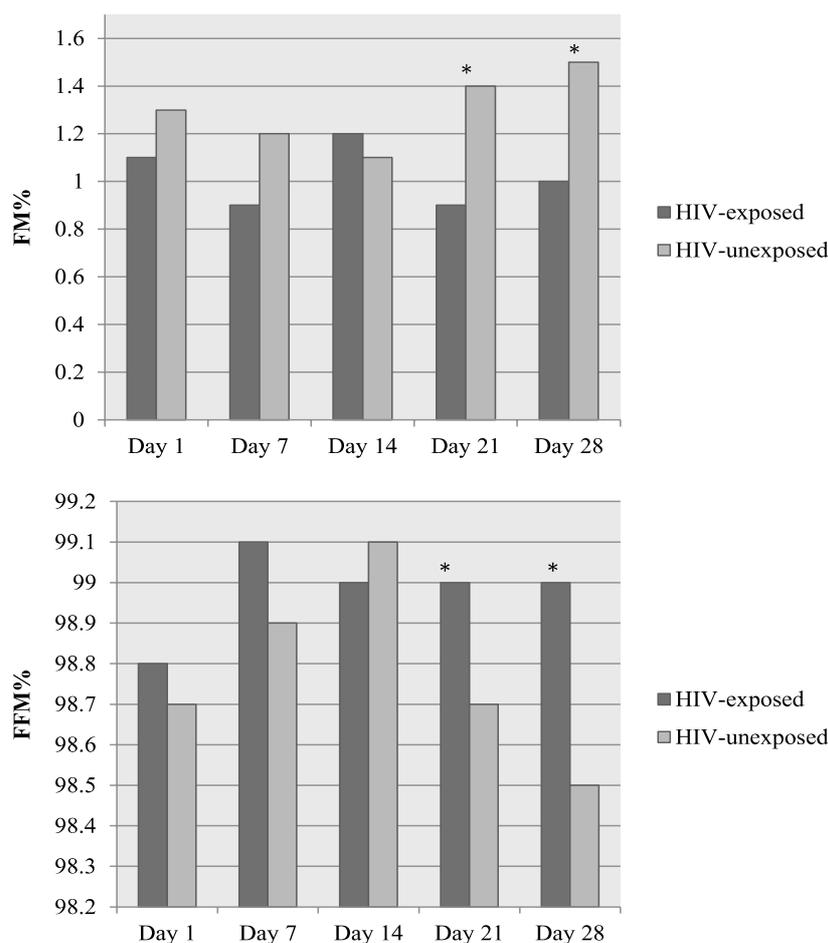
to HIV-uninfected mothers, and there is therefore a disparity in sample sizes between the infant and maternal groups. Supplementary Table 2 outlines the clinical characteristics and medical treatment of 103 who were included in this sub-study, divided into HIV-infected (27) and HIV-uninfected (76) mothers.  $\chi^2$  tests were used for nominal response variables, non-parametric Mann-Whitney tests for non-normal and ordinal response variables and one-way ANOVA tests for continuous response variables versus the nominal input variables. It was found that HIV-infected mothers had a significantly higher mean age than HIV-uninfected mothers ( $p \leq 0.01$ ).

One-way ANOVA versus HIV exposure of all study infants whose mothers used substances such as cigarettes, alcohol and narcotics during pregnancy showed no significant differences in body composition in terms of skinfold thickness, FM% and FFM% of infants at birth. Infants of mothers who smoked during pregnancy had a mean (SD) GA of 28.8 (2.32) weeks. However, these infants were heavier and had a higher FM% and lower FFM% at birth than infants whose mothers did not smoke (Supplementary Table 3). In addition, no statistically significant difference was detected in the body

composition of infants whose mothers received medication such as antibiotics and corticosteroids. Infants whose mothers were on antenatal corticosteroids were lighter in weight and displayed a lower FM% and higher FFM% than infants whose mothers did not take antenatal corticosteroids.

A high incidence of preterm birth was associated with mothers who suffered pre-eclampsia. By one-way ANOVA, these infants had a heavier birthweight with significantly lower FM% ( $p \leq 0.01$ ) and significantly higher FFM% ( $p \leq 0.01$ ). They also had significantly smaller TSF and SSSF (2.6 mm vs 2.9 mm,  $p = 0.03$ ) and (1.0 mm vs 1.7 mm,  $p = 0.02$ ), respectively, than infants of mothers without pre-eclampsia (Table 3).

All the HIV-infected mothers had received HAART at the time of delivery. Thirteen mothers (48%) received HAART for  $\geq 4$  weeks and 14 (52%) received HAART for  $\geq 20$  weeks. A one-way ANOVA test determined the relationship between duration of HAART and growth. Infants of mothers who received HAART for  $\geq 20$  weeks were generally heavier and had greater TSF and SSSF thickness measurements, indicating higher FM% and lower FFM% at birth than infants whose mothers received



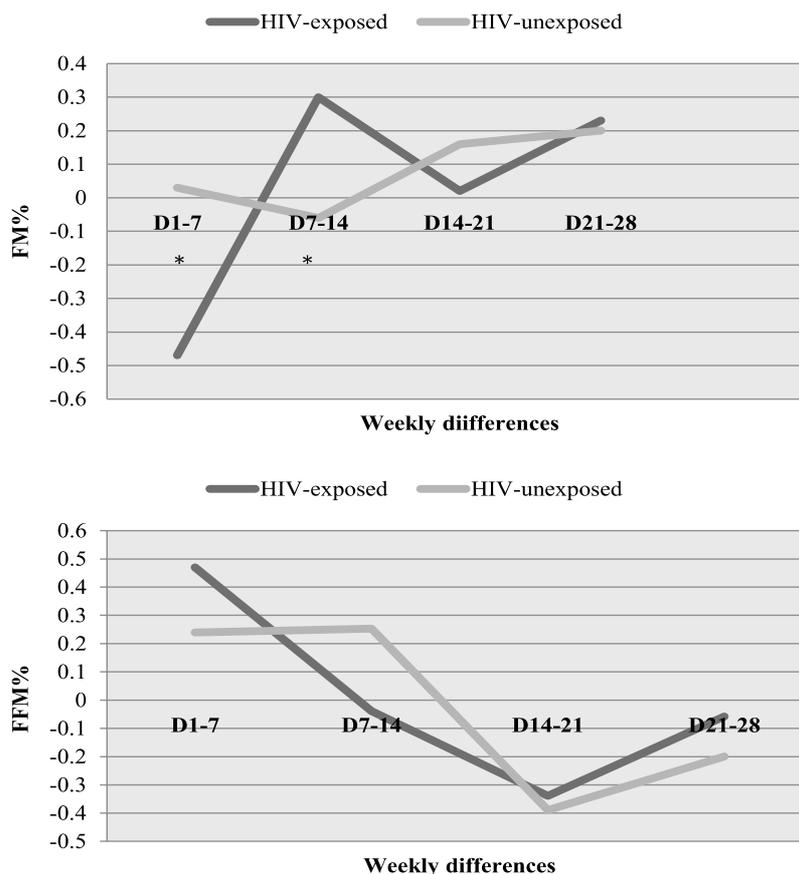
**Figure 2.** Comparison of body composition in terms of FM% and FFM% in HIV-exposed and -unexposed preterm infants. \*Statistically significant when  $p < 0.05$ . One-way ANOVA test was used.

HAART for  $\geq 4$ –20 weeks. However, no significant difference in body composition measurements in terms of skinfold thicknesses, FM% and FFM% were detected between the two groups at birth (Supplementary Table 4 and Figure 4).

A CD4 count was recorded in the medical files of 27 of the HIV-infected mothers (78%). Of these, two (10%) had a CD4 count  $< 200$ , eight (38%) had a CD4 count of  $\geq 200$ – $< 500$  and 11 (52%) had a CD4 count  $\geq 500$ . Using one-way ANOVA, infants whose mothers had a CD4  $< 200$  were the heaviest at birth [mean (SD) 1055 g (35.4)] and had the highest FM% [1.3% (0.97)], and consequently the greatest mean (SD) TSF and SSSF thickness measurements [2.7 mm (0.4) and 2.5 mm (0.7), respectively]. Infants whose mothers had a CD4 count of  $\geq 200$ – $< 500$  were the lightest at birth [763.8 g (94.1)] and the smallest TSF and SSSF measurements [2.4 mm (0.2) and 2.3 mm (0.4), respectively] and infants whose mothers had a CD4  $\geq 500$  had the least FM%, namely 1.2% (1.4) (Table 4).

## Discussion

HIV infection is increasingly prevalent in South Africa and HIV-infected mothers have a considerably higher risk of delivering a preterm infant [22,23]. The study was conducted to compare the body composition of HIV-exposed and HIV-unexposed preterm, VLBW and ELBW infants. Because all the HIV-infected mothers had received HAART, the effect of the duration of the treatment on the body composition of their infants was also assessed. There was a significant difference in the GA categories of the two groups: 23 of the HIV-exposed infants (77%) were extremely preterm (GA 25–28 weeks). Twenty-seven (26%) HIV-infected mothers and 30 (29%) HIV-exposed preterm infants were included in this study. This concurred with the current South African national consolidated guidelines for the prevention of mother-to-child transmission (PMTCT) released in April 2015 [31]: the HIV status of all mothers in the study was known at the time of delivery, and all the HIV-infected mothers



**Figure 3.** Comparison of weekly body composition increases/decreases in terms of FM% and FFM% in HIV-exposed and -unexposed preterm infants.

\*Statistically significant when  $p < 0.05$ . One-way ANOVA test was used.

**Table 3.** Anthropometric and body composition measurements of infants at birth whose mothers did or did not have pre-eclampsia.

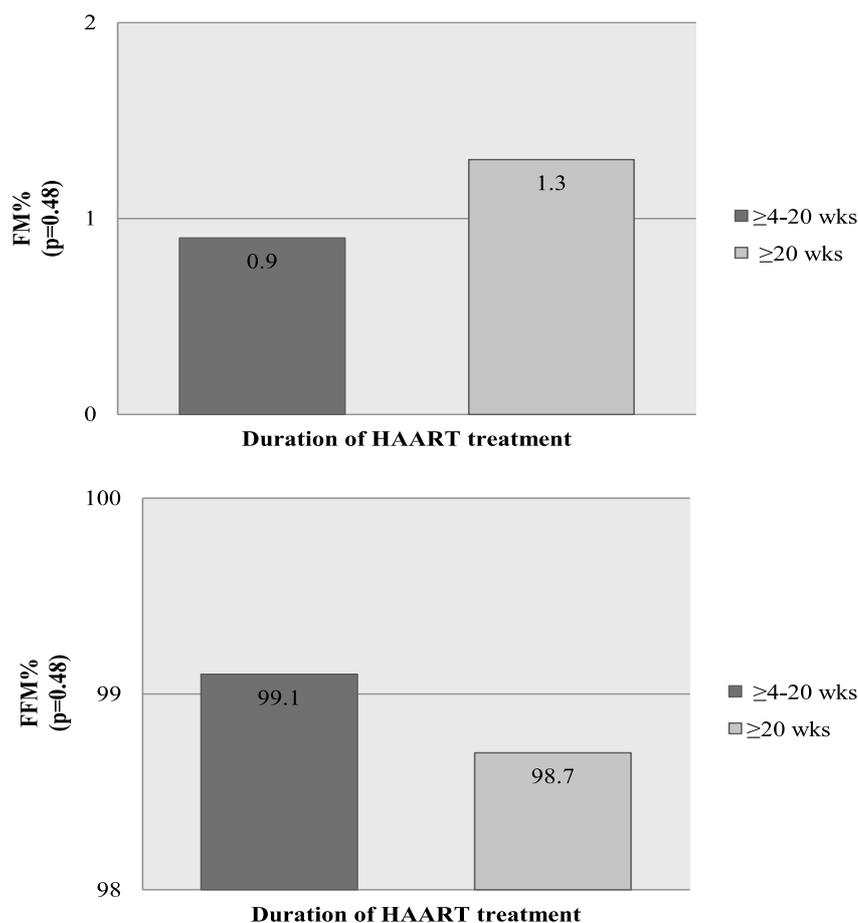
	No pre-eclampsia		p-value
	Pre-eclampsia n = 70 (68%)	n = 33 (32%)	
Day 1 of life, mean (SD)			
Weight, g	974.52 (170.37)	966.2 (156.3)	0.68
Length, cm	36.4 (2.99)	35.86 (2.6)	0.34
TSF, mm	2.6 (0.5)	2.9 (0.6)	<b>0.03</b>
SSSF, mm	2.4 (0.4)	2.6 (0.5)	<b>0.02</b>
FM, %	1.0 (0.1)	1.7 (0.15)	<b>&lt;0.01</b>
FFM, %	99 (0.1)	98.3 (0.15)	<b>&lt;0.01</b>

Notes: TSF, triceps skinfold thickness; SSSF, subscapular skinfold thickness; FM%, fat mass percentage; FFM%, fat-free mass percentage. Values are means and standard deviations (SD) unless otherwise indicated. Figures in bold are statistically significant. One-way ANOVA test was used.

were receiving HAART for  $\geq 4$  weeks at delivery. Only two mothers (7%) were on the second HAART regimen and four mothers (14%) were at high risk of transmission during pregnancy. According to the national consolidated guidelines and the South African prevention of mother-to-child transmission evaluation (SAPMTCTE) [31–43],

South Africa reduced early mother-to-child transmission (MTCT) to 3.5% by 8 weeks postpartum in 2008 and further reduced early MTCT to 2.7% and 2.6% in 2011–2012 and 2012–2013, respectively. In this study, none of the HIV-exposed preterm infants had a positive polymerase chain reaction (PCR) test at birth.

Recent studies in Africa provide evidence that HIV-exposed infants have lower birthweights and poorer growth rates than HIV-unexposed infants [28,44,45]. Studies in the U.S.A. [7] and the Democratic Republic of Congo (DRC) [46] concur that HIV-infected and HIV-exposed infants have a lower birthweight than HIV-unexposed infants. In this study, the HIV-exposed infants had a significantly lower birthweight and were significantly shorter in length than the HIV-unexposed preterm infants at birth. As expected, infants in both groups lost weight during the first week of life [10]. Throughout the study, HIV-exposed infants had a poorer growth rate than the HIV-unexposed infants. HIV-exposed infants generally had smaller weight gains throughout the 28-day follow-up period. There were statistically significant weight differences in HIV-exposed infants on day 28 of life and throughout days 1–28 of life.



**Figure 4.** Comparison of body composition in terms of FM% and FFM% at birth for the duration of HAART exposure. Note: One-way ANOVA test was used.

**Table 4.** Maternal CD4 count and body composition in 21 HIV-exposed infants at birth.

	CD4 <200 <i>n</i> = 2 (10%)	CD4 ≥200– <500 <i>n</i> = 8 (38%)	CD4 ≥500 <i>n</i> = 11 (52%)
<i>Day 1 of life, mean (SD)</i>			
Weight, g	1055.00 (35.36)	763.75 (94.10)	995.00 (130.84)
Length, cm	38.07 (1.98)	32.80 (0.89)	36.35 (2.98)
TSF, mm	2.65 (0.35)	2.40 (0.23)	2.64 (0.73)
SSSF, mm	2.52 (0.67)	2.28 (0.36)	2.48 (0.56)
FM, %	1.27 (0.97)	1.21 (1.11)	1.16 (1.37)
FFM, %	98.73 (0.97)	99.35 (0.34)	98.83 (1.37)

Notes: TSF, triceps skinfold thickness; SSSF, subscapular skinfold thickness; FM%, fat mass percentage; FFM%, fat-free mass percentage. Values are means and standard deviations (SD) unless otherwise indicated. One-way ANOVA test was used.

This study is one of the first in Africa to assess body composition in terms of FM and FFM in HIV-exposed and HIV-unexposed preterm infants. Existing literature on body composition and HIV focuses predominantly on older children and adults. A longitudinal observation study in the U.S.A. [7] compared the body composition of HIV-exposed children < 2 years by measuring their subscapular and triceps SFT. They found that, compared

with the standards contained in the National Health and Nutrition Examination Survey (NHANES), HIV-exposed children had significantly smaller skinfold thicknesses, which indicated significantly lower fat stores. In this study, HIV-exposed preterm infants generally had lower FM% for the duration of the study and had significantly lower TSF and SSSF thicknesses on days 21 and 28 of life, thus indicating significantly lower FM% and significantly higher FFM% than HIV-unexposed preterm infants on days 21 and 28 of life.

HIV-infected infants, children and adults benefit from HAART [47]. Although HAART is advantageous for maternal health and PMTCT, antenatal HAART has been associated with increased preterm delivery [18,22,23]. HIV as a disease and HIV treatment are associated with complicated side-effects such as altered fat distribution, especially an increase in central adiposity which can be a risk factor for morbidities such as cardiovascular diseases (CVD) and metabolic syndrome [4,47]. HIV-exposed preterm infants whose mothers were receiving HAART for a longer period (≥20 weeks) were generally heavier in weight with greater TSF and SSSF thicknesses, indicating higher FM% and lower FFM% at birth than in

HIV-exposed preterm infants whose mothers received HAART for  $\geq 4$ – $< 20$  weeks. Infants whose mothers had lower CD4 counts ( $< 200$ ) were heaviest at birth and had an altered body composition with higher FM% and lower FFM%. Seven HIV-infected mothers (58%) with CD4 counts of  $\geq 500$  who were on HAART for  $\geq 20$  weeks at the time of delivery gave birth to lighter and leaner infants, but the reason for this is not clear.

Pre-eclampsia is a pregnancy-specific disorder which often results in preterm labour and commonly affects around 10% of all pregnancies [48]. As far as we know, no study has examined the effect of pre-eclampsia and body composition in HIV-exposed and HIV-unexposed preterm infants. More than two-thirds of mothers (68%) in this study were diagnosed with pre-eclampsia. A Canadian study was one of the first to examine by GA the effect of pre-eclampsia on mean birthweight [49]. It found that infants of mothers with pre-eclampsia and a GA of  $< 37$  weeks had remarkably lower mean birthweights which is predominantly associated with preterm infants. [50]. In contrast, this study found that infants of mothers with pre-eclampsia were heavier at birth, but had significantly lower TSF and SSSF thickness, and therefore significantly lower FM% and significantly higher FFM% at birth than infants of mothers without pre-eclampsia.

Smoking in pregnancy results in a lower birthweight by a mean 150–250 g [51]. A study in the U.S.A. [52] found that newborns of mothers who smoked during pregnancy were considerably more likely to be small for gestational age (SGA) than large for gestational age (LGA) [52]. In this study, 31 mothers (30%) reported that they smoked during pregnancy. In contrast with previous reports [51,52], this study found that preterm infants (irrespective of HIV exposure) of mothers who smoked during pregnancy did not have significant differences in body composition. Infants of mothers who smoked were born very premature (28–32 weeks) and had a mean (SD) birthweight of 985.7 g (179.3), classified as ELBW, but were found to be heavier and had higher TSF and SSSF thickness measurements, thus indicating higher FM% and lower FFM% than infants of non-smokers during pregnancy. The explanation could be a range of pre-existing genetic or environmental factors which were not accounted for in this study.

This descriptive cross-sectional study demonstrated differences in the body composition of HIV-exposed and HIV-unexposed preterm infants. HIV-exposed infants were more likely to be extremely premature (25–28 weeks), were significantly smaller in weight and length at birth, and did not gain weight as well as the HIV-unexposed infants during the study. HIV-exposed infants presented with a significantly leaner body composition in terms of lower FM%, smaller skinfold thickness and higher FFM% than HIV-unexposed infants on days 21 and 28 of life. HIV-exposed infants whose mothers received

HAART for  $\geq 20$  weeks were heavier and had an altered body composition in terms of a higher FM%, greater skinfold thickness measurements and lower FFM% at birth than infants of mothers who received HAART for  $\geq 4$ – $< 20$  weeks. This study demonstrates that the distribution of body fat is altered in HIV-exposed preterm infants of mothers with a CD4 count  $< 500$  who receive HAART for  $\geq 20$  weeks. More research is needed to determine the effect of CD4 counts, ART and prophylactic medication on the body composition and fat distribution of HIV-exposed preterm infants which might predict possible health-related consequences for childhood and adulthood obesity.

A limitation of the study is the small number of preterm infants. Future studies with longer follow-up periods are required in order to identify the long-term effects of maternal HIV status and treatment duration on the body composition of HIV-exposed and -unexposed VLBW and ELBW preterm infants.

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No potential conflict of interest was reported by the authors.

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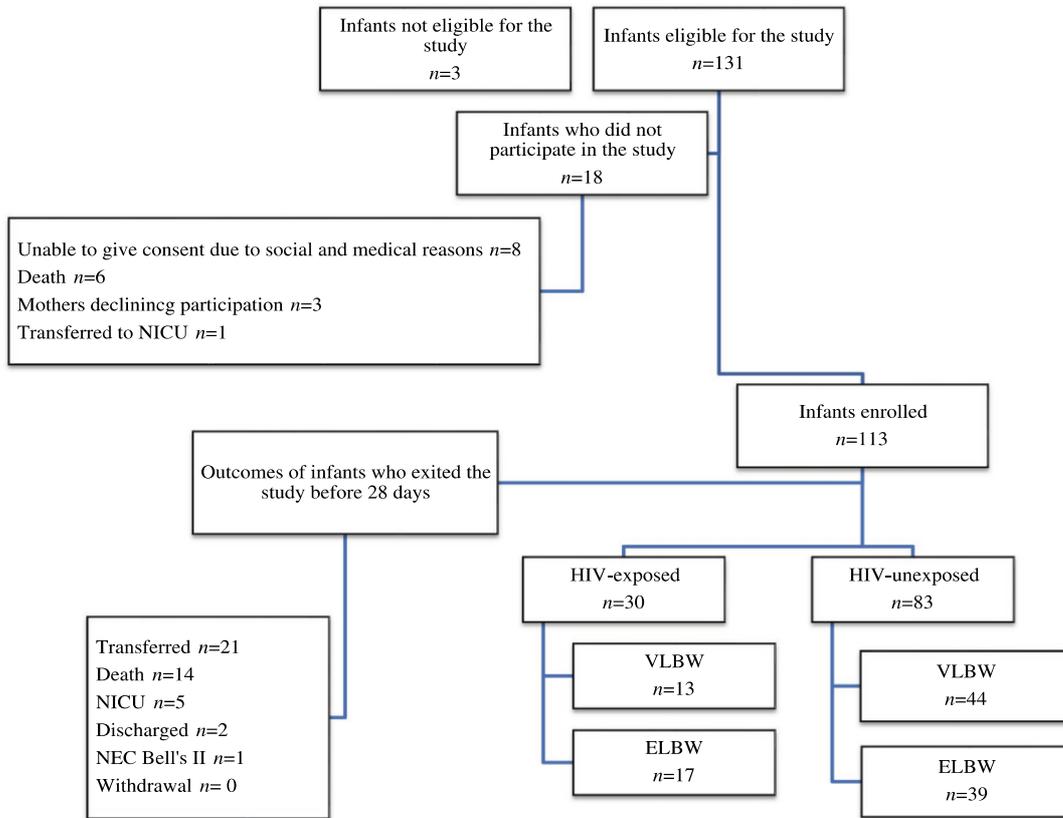
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**Appendix 1.**



**Figure A1.** Flow diagram of the study infants.

## **ORIGINAL ARTICLE 2**

# **How does the nutritional intake affect the body composition of HIV-exposed and HIV-unexposed preterm, very- and extremely-low birth weight infants?**

This article is under review by the journal of *Breastfeeding Medicine*.

## How does nutritional intake affect the body composition of HIV-exposed and HIV-unexposed preterm, very- and extremely-low birth weight infants?

Running title: Nutritional intakes on preterm body composition

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**Keywords:** body composition; breast milk fortifier; HIV-exposure; nutritional intakes; NPO; preterm infants.

## ABSTRACT

**Background:** Human breast milk (HBM) is considered inadequate in meeting protein requirements, especially for very low birth weight (VLBW) infants, which could affect body composition.

**Objectives:** To determine the effect of HBM on body composition of HIV-exposed and unexposed preterm VLBW and extremely-low birth weight (ELBW) infants. Furthermore, to ascertain the effect of breast milk fortification, and days nil per os (NPO) has on body composition.

**Methods:** A descriptive cross-sectional study was conducted. Preterm infants with a birth weight of  $\leq 1200$  g were included. Infant nutritional intakes and body composition measurements were recorded during the 28-day follow-up period.

**Results:** 110 of 113 preterm infants received HBM and 91 infants received fortified HBM. HIV-exposed and unexposed infants receiving fortified HBM displayed differences in fat mass percentage (FM%) (0.88% vs. 1.36%;  $p = 0.01$ ) and (0.97% vs. 1.49%;  $p = 0.03$ ) and fat-free mass percentage (FFM%) (98.98% vs. 98.68%  $p = 0.03$ ) and (99.02% vs. 98.49%;  $p = 0.02$ ), on day 21 and 28, respectively. Infants kept NPO displayed differences in FM% on day 7, 21 and 28 (0.9% vs 1.3%;  $p = 0.03$ ), (0.99% vs 1.4%;  $p = 0.02$ ) and (0.9% vs 1.6%  $p = 0.0004$ ) as well as differences in FFM% (99.1% vs 98.4%  $p = 0.0005$ ) on day 28 of life.

**Conclusion:** There were no significant differences in the body composition of infants who received HBM vs. fortified HBM. However, significant differences in body composition were reported between HIV exposure groups for infants who received fortified HBM. Infants who were kept NPO were generally smaller, shorter, and had lower FM% and more FFM%.

## INTRODUCTION

Over the past two decades, South Africa has experienced a serious HIV epidemic and is home to the world's largest HIV population.<sup>1</sup> HIV-infected pregnant mothers on highly active antiretroviral therapy (HAART) have a considerably higher risk (13–34%) of delivering an infant preterm.<sup>2 3</sup>

A recent study conducted in Africa by Adeteyo et al.<sup>4</sup> found that HIV-exposed uninfected (HIV-EU) infants had poorer growth than HIV-unexposed uninfected (HIV-UU) infants during infancy. Previous literature confirms that HIV-EU infants grew more poorly and had increased early mortality and morbidity rates compared with HIV-UU.<sup>4 5 6</sup> For the purposes of this article, the terms HIV-EU and HIV-UU infants will be referred to as HIV-exposed and HIV-unexposed infants, respectively.

The aggressive nutritional management recommended for preterm and small for gestational age (SGA) infants by the American Association of Pediatrics to achieve appropriate fetal growth rates and body composition, could result in an increased risk of cardiovascular diseases and metabolic syndrome later on in life. These risks are related to high early postnatal weight gain.<sup>7 8</sup> This was supported in a meta-analysis by Johnson et al.<sup>9</sup> detailing the relationship between nutritional intakes and body composition in preterm infants. Nutritional intakes include micronutrients and macronutrients (energy and protein) and their effect on the successful growth and body composition of preterm infants at term-equivalent age. Preterm infants, especially those born with a very low birth weight (<1.5 kg; VLBW), are a challenge to feed because of the high recommended daily allowance of energy, protein and nutrients required to achieve in-utero growth rates.<sup>8 10</sup>

Human breast milk (HBM) alone is considered inadequate to meet the nutritional requirements of preterm infants during early neonatal life, especially in infants born with very low birth weight (VLBW).<sup>11</sup> When fed in amounts that satisfy energy requirements, HBM provides only about 60% of the protein requirements.<sup>12 13</sup> Therefore, HBM from mothers with preterm infants is not nutritionally adequate and must be fortified to ensure adequate growth and body composition in terms of (fat mass) FM and (fat-free mass) FFM. There are three main fortification strategies namely: standard

fortification, super fortification and individualised (adjustable and targeted fortification). These strategies range from simple, quick, safe and inexpensive to complex, sophisticated and expensive.<sup>14 15 16 17</sup> Supplementation of HBM with a fortifier (energy, protein, vitamins and minerals) is common practice in many neonatal intensive care units (NICUs) to achieve nutritional requirements and successful intrauterine growth rates in preterm infants, while the many benefits of HBM such as better neurodevelopmental outcomes and a decrease in late-onset sepsis and necrotising enterocolitis (NEC) continue to be recorded.<sup>10 12 18</sup>

The Baby Friendly Hospital Initiative (BFHI) was launched by the World Health Organization (WHO) and United Nations Children's Fund as a global initiative to protect, promote and support breastfeeding practices by adhering to and implementing the minimum global criteria contained in "*The ten steps to successful breastfeeding*" guidelines.<sup>19</sup> These guidelines are critical to the prevention of mother-to-child transmission (PMTCT) programme.<sup>20 21 22</sup> The attending doctor should only prescribe a formula feed once all other options to administer HBM have been exhausted, such as when the mother is unable to produce or express breast milk, also known as mother's own milk (MOM), and donor breast milk (DBM) is unavailable.

There is a gap in published research regarding the relationship between HIV, breast milk intake, body composition and preterm infants and the effect thereof on the quality of growth and body composition, in terms of FM and FFM.<sup>9 23 24</sup> The purpose of the study was to evaluate the effect of HBM intake (and the fortification thereof) and the body composition of HIV-exposed and HIV-unexposed preterm VLBW and ELBW infants.

## **MATERIALS AND METHODS**

### **Study design and setting**

This descriptive cross-sectional study, which formed part of a larger cohort, was conducted from 16 May to 22 October 2016, in the Neonatal Unit of Tygerberg Children's Hospital (TBCH) situated in Tygerberg Academic Hospital (TAH), Cape Town, South Africa.

## **Sample size**

The sample sizes needed of the study populations were determined according to the live birth statistics for infants born with a birth weight of < 1 500 g (VLBW) at the institution. A power analysis for two-way analysis of variance (ANOVA) with a power of 90% to detect interaction effects of  $\delta = 0.49$  (regarded as a medium effect size) yielded a required sample size  $n = 23$  in each of the exposure groups. The total sample size of  $n = 113$  preterm infants consisted of  $n = 83$  HIV-unexposed preterm infants and  $n = 30$  HIV-exposed preterm infants.

## **Participants**

The study population, comprised of all HIV-exposed and HIV-unexposed preterm infants, who adhered to the inclusion criteria and whose mother gave written consent, were included in the study. The inclusion criteria stipulated the following: all male and female preterm infants born at TBCH with a gestational age (GA)  $\geq 25$  weeks and < 37 weeks and a birth weight  $\geq 500$  g  $\leq 1\ 200$  g born at TBCH.

## **Outcomes**

The primary outcome of the study was to determine the effect of HBM intakes on the body composition of HIV-exposed and HIV-unexposed preterm, VLBW and ELBW infants. This evaluation also included the effect of BM fortification, feeding intolerances and days on which infants were nil per os (NPO).

## **Methods**

Participants were included in this study for a 28-day follow-up period (day 1 was considered as the first 24 hours of life). The researchers collected the maternal medical background of each infant with inclusion. Infant clinical, medical and nutritional data were collected on a daily basis, and anthropometric and skinfold measurements were taken weekly.

The researchers documented all fluid input and output from the patients' fluid balance charts. The daily clinical assessments were done by the attending doctors and were recorded by the researchers or research assistants. For the duration of the study, milk feeds were advanced according to the standard ward protocol and feeds were

increased in daily increments of approximately 25-30 ml/kg/d. The time of initial feeding was at the discretion of the attending physician.

The primary nutritional intake prescribed for preterm infants (according to the national consolidation guidelines regarding the PMTCT of HIV)<sup>20 22</sup> for this research study was HBM, which included: MOM, DBM, pasteurised breast milk (PBM), and HBM fortified with a standard agent known as FM85.<sup>25</sup> Furthermore, in line with ward protocol, infants of HIV-infected and HIV-uninfected mothers received PBM or DBM or unpasteurised BM (expressed, donor or breastfed), respectively. The BM of HIV-infected mothers was pasteurised using the Pretoria pasteurisation method,<sup>26</sup> which was standard protocol in the ward.

The ward protocol prescribed the fortification of MOM in TBCH<sup>25</sup> for breastfed preterm infants who were born < 34 weeks gestational age and weighed < 1 800 g, and breastfed preterm infants who did not grow at a rate of 15 g/kg/day and had to receive full fluid requirements (150–180 ml/kg/day). For the duration of the study, the standard protocol applied, namely the BM fortifier was added at a ratio of 1 g to 20 ml MOM. The standard addition of 5 g of fortifier to 100 ml MOM increased the energy and protein concentration from  $\pm 67$  Kcal to 85 Kcal/100ml BM and 1,2 g to 2,2 g/100 ml, respectively.<sup>25</sup>

Additionally, the following data were assessed and recorded daily: feeding route, the volume of HBM, formula, total parenteral nutrition (TPN), Neonatalyte (glucose-electrolyte-containing solution) and saline provided to the infant, standard BM fortifier and medium-chain triglyceride oil, multivitamin supplementation, phototherapy and blood products received, medication administered, urinary output, stool frequency and colour/consistency, and feeding intolerance frequency.

The researcher assessed body composition by measuring skinfold thickness at two primary sites: triceps and subscapular. The skinfold thickness measurements of each infant were taken on day 1 of the study, and subsequently measured on a weekly basis. Upon exiting the study (day 28), the skinfold thickness was measured again. Five measurements were thus recorded: on days 1, 7, 14, 21 and 28. Skinfold thickness was determined by the use of Holtain skinfold callipers, with a calibration sensitivity of

0.1 mm and divisions of 0.2 mm. The same pair of callipers was used for all measurements and was calibrated before each measurement period. All skinfold measurements were taken on the right hand side of the body and only two measurements at each body site were assessed owing to the fragility of the study population and to ensure minimal invasion.<sup>27 28 29 30</sup> In cases where the results differed by more than  $> 0.5$  mm, a third measurement was taken. No skin fold measurements were done while infants weighed  $\leq 700$  g.

The Dauncey anthropometric method was used to determine total FM and FFM by assessing skinfold thickness at two sites (triceps and subscapular) in conjunction with nine other body dimensions. These body dimensions were circumferences (head, chest, mid-upper arm, mid-thigh and mid-calf) and lengths (upper arm, lower arm, supine and crown-rump length).<sup>31 32</sup>

### **Ethical approval**

Ethical approval was granted by the Health Research Ethics Committee of the Faculty of Medicine and Health Sciences, University of Stellenbosch and Tygerberg Academic Hospital, Ethics reference #N15/10/102.

### **Statistical analysis**

Data analyses were performed using Statistica version 13 (Dell Inc. (2016). Dell Statistica (data analysis software system.) in Stellenbosch, South Africa. When comparing continuous variables between two groups (anthropometric and body composition measurements for HIV-exposure), one-factor ANOVA was used; if residuals were not normally distributed, a nonparametric test, the Mann-Whitney test, was used to confirm conclusions for ANOVA. Nominal variables (e.g. GA and sex) were compared between groups with contingency tables using the maximum likelihood chi-square test. Descriptive statistics were reported in terms of the mean, standard deviation (SD) and maximum and maximum values. Confidence intervals of 95% were used to report the mean of each group and a  $p$ -value of  $< 0.05$  was used to determine statistical significance in this study.

## RESULTS

A total of 131 preterm infants conformed to the inclusion criteria and of these, 18 infants (14%) were excluded: eight (6%) of the exclusions were due to the mother being unable to give consent for medical or social reasons; six infants (5%) died before consent was obtained, three mothers (2%) declined participation and one infant (1%) was transferred to the NICU. The final study population consisted of 113 preterm infants; however, two of these infants died before enteral feeds were initiated and one infant exited the study due to being transferred to the NICU. Therefore, a total of 110 infants [HIV-exposed (n = 29) and HIV-unexposed infants (n = 81)] received MOM, DBM or PBM at a given point in time during the 28-day follow-up study (Supplementary Figure 1).

Table 1 below indicates the HBM composition<sup>33</sup> and nutritional intake of HIV-exposed and HIV-unexposed infants. No significant difference was noted in the HBM volume (ml/kg/day), energy density (kcal/kg/day) and macronutrient concentration, in terms of protein and fat (g/kg/day), in HIV-exposed and HIV-unexposed preterm infants.

**Table 1: Human breast milk composition and nutritional intakes of HIV-exposed and HIV-unexposed preterm infants**

	HIV-exposed Infants N= 29 (26%)	HIV-unexposed Infants N=81 (74%)	<i>p</i>
Volume of intake (ml/kg/day) $\bar{x}$ , $\pm$ SD			
Day 1	0.00 $\pm$ 0.00	0.28 $\pm$ 1.28	0.23
Day 7	69.76 $\pm$ 38.55	88.78 $\pm$ 46.56	0.09
Day 14	153.43 $\pm$ 49.3	148.88 $\pm$ 53.15	0.71
Day 21	150.60 $\pm$ 58.54	146.88 $\pm$ 61.48	0.79
Day 28	155.79 $\pm$ 58.78	143.245 $\pm$ 65.02	0.45
Day of life receiving full feed (days)	12.5 $\pm$ 3.71	11.375 $\pm$ 3.31	0.19
Human breast milk energy and macronutrient intake			
Energy (kcal/kg/day) $\bar{x}$ , $\pm$ SD			
Day 1	0.00 $\pm$ 0.00	0.19 $\pm$ 0.89	0.23
Day 7	46.74 $\pm$ 25.83	59.48 $\pm$ 31.19	0.09
Day 14	102.80 $\pm$ 33.03	99.75 $\pm$ 35.61	0.71
Day 21	100.90 $\pm$ 8.29	98.41 $\pm$ 5.16	0.79
Day 28	104.39 $\pm$ 39.39	96.97 $\pm$ 42.89	0.45
Protein (g/kg/day) $\bar{x}$ , $\pm$ SD			
Day 1	0.00 $\pm$ 0.00	0.01 $\pm$ 0.03	0.23
Day 7	1.53 $\pm$ 0.85	1.95 $\pm$ 1.02	0.09
Day 14	3.38 $\pm$ 1.09	3.75 $\pm$ 1.17	0.71
Day 21	3.31 $\pm$ 1.29	3.23 $\pm$ 1.35	0.79
Day 28	3.43 $\pm$ 1.29	3.15 $\pm$ 1.41	0.44
Fat (g/kg/day) $\bar{x}$ , $\pm$ SD			
Day 1	0.00 $\pm$ 0.00	0.013 $\pm$ 0.06	0.23
Day 7	3.21 $\pm$ 1.77	4.08 $\pm$ 2.14	0.09
Day 14	7.06 $\pm$ 2.27	6.85 $\pm$ 2.44	0.71
Day 21	6.93 $\pm$ 2.69	6.76 $\pm$ 2.83	0.79
Day 28	7.17 $\pm$ 2.70	6.59 $\pm$ 2.95	0.45

Values are means ( $\bar{x}$ ) and  $\pm$ Standard Deviations ( $\pm$ SD) unless otherwise indicated.

\* Statistically significant when ( $p = < 0.05$ )

Infants in both groups were generally initiated with minimal feeds on day 2–3 of life. Even though the HBM volume was similar between the two groups for the duration of the study period, it was noted that infants in the HIV-exposed group received slightly more HBM from day 14 of life onwards than the HIV-unexposed group. The macronutrient intake is directly proportional to the volume of HBM; therefore, HIV-unexposed infants generally had lower energy, protein and fat intakes when compared to HIV-exposed infants.

The HIV-exposed and HIV-unexposed preterm infants were compared per birth weight category (VLBW and ELBW) by a two-way ANOVA. According to this analysis, there was no statistically significant difference in the HBM composition and nutritional intakes throughout the 28-day follow-up period between the two groups.

### **Fortified breast milk**

For the duration of this study, the standard fortification formulation, which was in existence then, was used. From 2017, however, a reformulation has been implemented in South Africa.<sup>16 21</sup>

Of the 110 infants who received HBM, 91 (83%) received fortified HBM. Of these infants, 25 (27%) and 66 (73%) were HIV-exposed and HIV-unexposed preterm infants, respectively (Table 2). There was no significant difference ( $p = 0.48$ ) between the two groups in the mean number of days the infants received the BM fortifier. The HIV-exposed group did however receive the BM fortifier for fewer days 8.63 ( $\pm 7.14$ ) and for a maximum of 19 days compared with HIV-unexposed infants who received the fortifier for 9.64 ( $\pm 6.73$ ) days and for a maximum of 21 days.

**Table 2: Human breast milk fortification and nutrient intakes of HIV-exposed and HIV-unexposed preterm infants**

	HIV-exposed Infants N=25 (26%)	HIV-unexposed infants N=70 (74%)	<i>p</i>
<b>Fortifier (g/kg/day) <math>\bar{x}</math>, <math>\pm</math>SD</b>			
Day 1	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	–
Day 7	0.00 $\pm$ 0.00	0.08 $\pm$ 0.47	0.44
Day 14	4.33 $\pm$ 4.37	4.73 $\pm$ 4.32	0.69
Day 21	4.956 $\pm$ 4.73	5.16 $\pm$ 4.44	0.85
Day 28	6.76 $\pm$ 5.21	5.12 $\pm$ 4.68	0.20
<b>NOD receiving a fortifier <math>\bar{x}</math>, <math>\pm</math>SD</b>	8.64 $\pm$ 7.14	9.64 $\pm$ 6.73	0.48
<b>Fortifiers energy and macronutrient intake</b>			
<b>Energy (kcal/kg/day) <math>\bar{x}</math>, <math>\pm</math>SD</b>			
Day 1	0.0 $\pm$ 0.00	0.00 $\pm$ 0.00	–
Day 7	0.0 $\pm$ 0.00	0.00 $\pm$ 0.00	–
Day 14	15.07 $\pm$ 15.20	16.45 $\pm$ 15.02	0.69
Day 21	17.76 $\pm$ 16.47	17.95 $\pm$ 15.45	0.85
Day 28	23.51 $\pm$ 18.12	17.25 $\pm$ 15.93	0.16
<b>Protein (g/kg/day) <math>\bar{x}</math>, <math>\pm</math>SD</b>			
Day 1	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	–
Day 7	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	–
Day 14	0.87 $\pm$ 0.87	0.95 $\pm$ 0.86	0.69
Day 21	0.99 $\pm$ 0.19	1.03 $\pm$ 0.11	0.85
Day 28	1.35 $\pm$ 1.04	1.02 $\pm$ 0.94	0.20
<b>Fat (g/kg/day) <math>\bar{x}</math>, <math>\pm</math>SD</b>			
Day 1	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	–
Day 7	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	–0.69
Day 14	0.02 $\pm$ 0.02	0.02 $\pm$ 0.02	0.85
Day 21	0.02 $\pm$ 0.02	0.02 $\pm$ 0.02	0.13
Day 28	0.03 $\pm$ 0.02	0.02 $\pm$ 0.02	
<b>Fortified breast milk energy and macronutrient intake</b>			
<b>Energy (kcal/kg/day) <math>\bar{x}</math>, <math>\pm</math>SD</b>			
Day 1	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	–
Day 7	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	–
Day 14	145.94 $\pm$ 19.06	141.54 $\pm$ 2.68	0.41
Day 21	153.26 $\pm$ 19.20	147.015 $\pm$ 16.21	0.25
Day 28	151.47 $\pm$ 26.28	149.95 $\pm$ 14.02	0.80
<b>Protein (g/kg/day) <math>\bar{x}</math>, <math>\pm</math>SD</b>			
Day 1	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	–
Day 7	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	–
Day 14	5.41 $\pm$ 0.87	5.29 $\pm$ 0.77	0.61
Day 21	5.76 $\pm$ 0.84	5.53 $\pm$ 0.66	0.31
Day 28	5.75 $\pm$ 1.16	5.69 $\pm$ 0.53	0.99
<b>Fat (g/kg/day) <math>\bar{x}</math>, <math>\pm</math>SD</b>			
Day 1	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	–
Day 7	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	–
Day 14	8.32 $\pm$ 0.87	7.96 $\pm$ 0.88	0.17
Day 21	8.53 $\pm$ 0.83	8.17 $\pm$ 0.89	0.20
Day 28	8.28 $\pm$ 1.18	8.21 $\pm$ 0.85	0.81

Values are means ( $\bar{x}$ ) and  $\pm$ Standard Deviations ( $\pm$ SD) unless otherwise indicated.

\*Statistically significant when ( $p = < 0.05$ )

NOD; Number of days

For the duration of the study, there were no statistically significant differences in body composition, in terms of skinfold thickness measurements, fat mass percentage (FM%) and fat-free mass percentage (FFM%), between infants who received fortified HBM and those who did not. However, it was noted that, for the duration of the study, infants who received the fortifier were generally smaller and did not grow at a rate of 15 g/kg/day (Supplementary Table 1).

Comparisons for a two-way ANOVA for infants receiving the fortified HBM and HIV-exposure could not be determined from day 7 onwards as there were no HIV-exposed infants in the group “infants receiving HBM alone.” Therefore the anthropometric and body composition measurements were determined for infants receiving fortified BM only and HIV-exposure (**Table 3**).

**Table 3: Weekly anthropometric and body composition measurements of infants receiving fortified breast milk**

	Infants receiving fortified breast milk		<i>p</i>
	N = 91 (83%)		
	HIV-exposed N = 25 (28%)	HIV-unexposed N = 66 (72%)	
<b>^ Day 1 of life <math>\bar{x}</math>, <math>\pm</math>SD</b>			
Weight (g)	934.40 $\pm$ 155.46	996.37 $\pm$ 146.72	0.07
Length (cm)	35.58 $\pm$ 2.76	36.84 $\pm$ 2.79	0.06
TSF (mm)	2.62 $\pm$ 0.59	2.75 $\pm$ 0.49	0.12
SSSF (mm)	2.5 $\pm$ 0.47	2.48 $\pm$ 0.38	0.95
FM (%)	1.21 $\pm$ 1.09	1.29 $\pm$ 0.87	0.49
FFM (%)	98.79 $\pm$ 1.09	98.71 $\pm$ 0.85	0.49
<b>^ Day 7 of life <math>\bar{x}</math>, <math>\pm</math>SD</b>			
Weight (g)	904.20 $\pm$ 139.03	942.41 $\pm$ 152.99	0.27
Length (cm)	35.53 $\pm$ 1.74	36.69 $\pm$ 1.68	0.05*
TSF (mm)	2.42 $\pm$ 0.37	2.62 $\pm$ 0.38	0.03*
SSSF (mm)	2.26 $\pm$ 0.40	2.35 $\pm$ 0.34	0.19
FM (%)	0.89 $\pm$ 0.65	1.24 $\pm$ 0.69	0.13
FFM (%)	99.11 $\pm$ 0.65	98.90 $\pm$ 1.17	0.21
<b>Day 14 of life <math>\bar{x}</math>, <math>\pm</math>SD</b>			
Weight (g)	919.16 $\pm$ 179.60	999.29 $\pm$ 159.59	0.04*
Length (cm)	35.83 $\pm$ 1.78	36.66 $\pm$ 1.89	0.07
TSF (mm)	2.52 $\pm$ 0.33	2.63 $\pm$ 0.39	0.30
SSSF (mm)	2.34 $\pm$ 0.26	2.39 $\pm$ 0.37	0.55
FM (%)	1.10 $\pm$ 0.60	1.13 $\pm$ 0.69	0.86
FFM (%)	99.04 $\pm$ 1.36	99.14 $\pm$ 1.60	0.84
<b>Day 21 of life <math>\bar{x}</math>, <math>\pm</math>SD</b>			
Weight (g)	1012.96 $\pm$ 196.43	1112.45 $\pm$ 190.36	0.02*
Length (cm)	36.66 $\pm$ 1.96	37.55 $\pm$ 1.63	0.09
TSF (mm)	2.53 $\pm$ 0.38	2.75 $\pm$ 0.40	0.02*
SSSF (mm)	2.31 $\pm$ 0.45	2.58 $\pm$ 0.43	<0.01*
FM (%)	0.88 $\pm$ 0.62	1.36 $\pm$ 0.73	0.01*
FFM (%)	98.98 $\pm$ 0.97	98.68 $\pm$ 0.798	0.03*
<b>Day 28 of life <math>\bar{x}</math>, <math>\pm</math>SD</b>			
Weight (g)	1026.20 $\pm$ 144.17	1203.92 $\pm$ 215.74	<0.01*
Length (cm)	36.59 $\pm$ 1.64	37.99 $\pm$ 1.79	<0.01*
TSF (mm)	2.55 $\pm$ 0.41	2.91 $\pm$ 0.59	<0.01*
SSSF (mm)	2.39 $\pm$ 0.40	2.71 $\pm$ 0.52	0.01*
FM (%)	0.97 $\pm$ 0.74	1.49 $\pm$ 0.85	0.03*
FFM (%)	99.02 $\pm$ 0.74	98.49 $\pm$ 0.85	0.02*

Values are means ( $\bar{x}$ ) and  $\pm$ Standard Deviations ( $\pm$ SD) unless otherwise indicated.

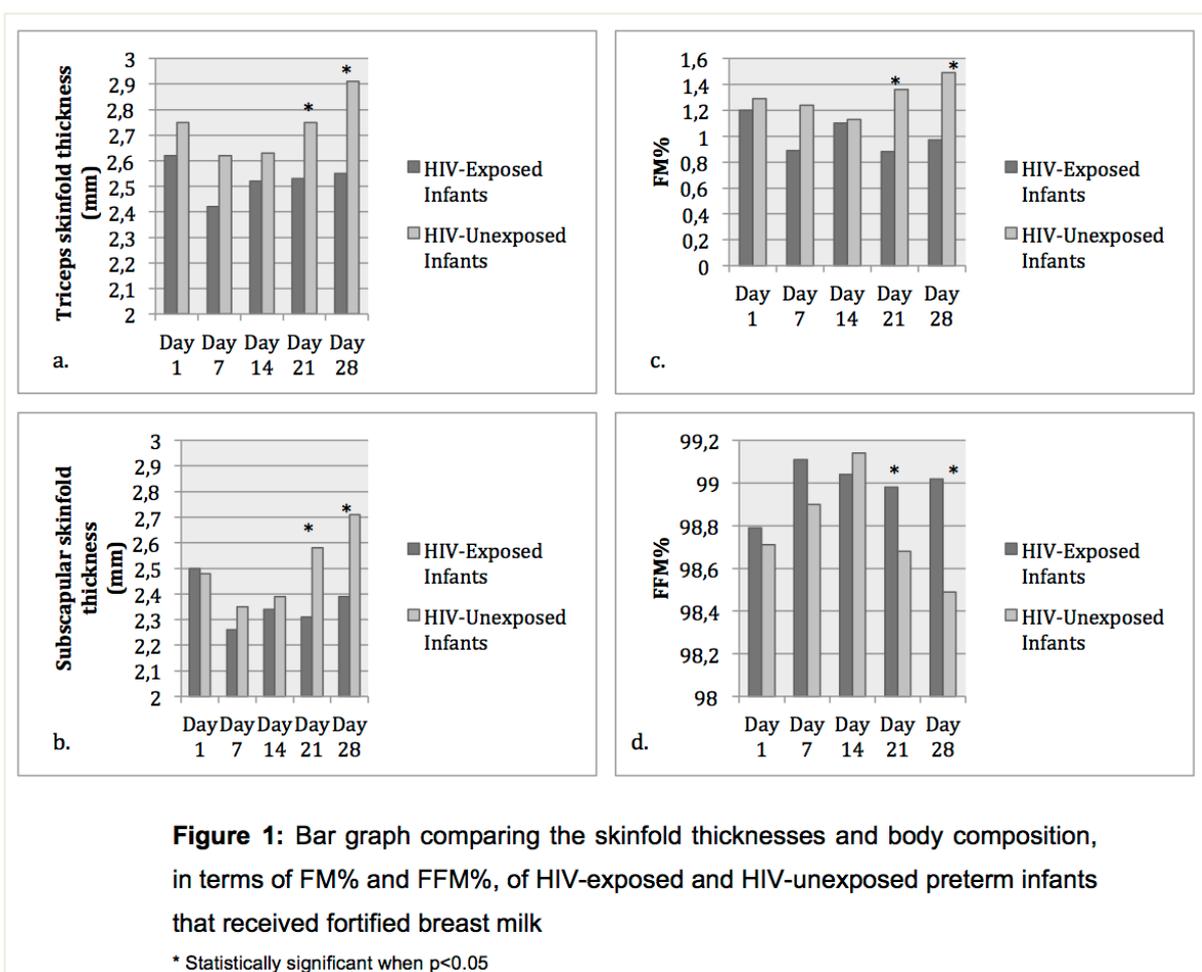
\*Statistically significant when ( $p = < 0.05$ )

\*\*Extremely statistically significant when ( $p = < 0.001$ )

^ These values are based on unfortified breast milk, refer to Table 2.

Fat-free mass percentage; FM%, Fat mass percentage; Subscapular skinfold thickness; TSF, Triceps skinfold thickness

Significant differences were found for infants who received fortified HBM and HIV exposure for weight and length [on days 14, 21 and 28 ( $p = 0.04$ ,  $p = 0.02$  and  $< p = 0.01$  and day 28  $< p = 0.01$ ) of life, respectively]. Significant differences were also found between exposures for triceps skinfolds (TSF), subscapular skinfolds (SSSF), fat mass% (FM%) and fat-free mass% (FFM%) [on day 21 and 28 ( $p = 0.02$  and  $< p = 0.04$ ), ( $< p = 0.01$  and  $p = 0.01$ ), ( $p = 0.01$  and  $p = 0.03$ ) and ( $p = 0.03$  and  $p = 0.02$ ) of life, respectively] (Figure 1).



### Formula and total parenteral nutrition (TPN)

During the 28-day follow-up study period, five infants (4%) received infant formula for an average of 7.6 days and 11 infants (10%) received TPN for an average of five days. Owing to the small number of infants who received these nutritional intakes for a short period of time, no conclusive evidence regarding body composition could be determined. However, as expected, the 10 infants (91%) who received TPN were

classified as ELBW and had a significantly lower ( $p = 0.0012$ ) mean birth weight 822.73 g ( $\pm 132$  g) when compared with infants who did not receive TPN during the 28-day follow-up period. Infants who received TPN displayed a consistent trend of being leaner with smaller TSF and SSSF thicknesses, lower FM% and higher FFM% for the duration of the study.

All five infants who received formula were HIV-unexposed. Only one HIV-exposed infant (9%) received TPN and the remainder, i.e. 10 (91%) were HIV-unexposed infants, therefore adhering to BFHI and PMTCT guidelines. Of the HIV-unexposed infants who received TPN, seven (70%) completed the 28-day follow-up study, one infant was diagnosed with NEC Bells stage II and two infants passed away.

### **The effect of Nil per os (NPO)**

Table 4 depicts the anthropometric and body composition measurements of all 113 preterm infants included in this sub-study, divided into the total number of days infants were kept NPO at any given time during the 28-day follow-up period. For the purposes of this study, feeding intolerance was recorded with the occurrence of a vomit or aspirate. A total of 41 infants (36%) were NPO due to suspected intolerance during the study period. Of these infants, more than three quarters (76%) were kept NPO for  $\leq 3$  days and the remainder for  $> 4 - \leq 8$  days. No infants were kept NPO for 6 days.

Infants who were NPO at any given point during the study revealed that, generally, the longer total number of days they were kept NPO, the smaller, shorter and leaner they were and the higher their FFM% was. These infants displayed significant differences in FM% on day 7 ( $p = 0.04$ ), day 21 ( $p = 0.04$ ) and day 28 ( $p = 0.02$ ) of life as well as significant differences in FFM% on day 21 ( $p = 0.02$ ) and day 28 ( $p = 0.03$ ) of life. Skinfold thickness was measured concurrently with FM%, and infants who were kept NPO had significant differences in TSF thicknesses on day 21 ( $p = 0.02$ ) and day 28 ( $p = 0.04$ ) of life, as well as significantly different SSSF thicknesses on day 28 ( $p = 0.02$ ) of life (Table 4).

**Table 4: The effect of nil per os (NPO) on the anthropometry and body composition of preterm infants**

	Infants who were not NPO N = 72 (64%)	Infants who were NPO N = 41 (36%)							
	<b>NPO for 0 days</b> N = 72 (64%)	<b>NPO for 1 day</b> N = 14 (34%)	<b>NPO for 2 days</b> N = 10 (24%)	<b>NPO for 3 days</b> N = 7 (17%)	<b>NPO for 4 days</b> N = 3 (7%)	<b>NPO for 5 days</b> N = 3 (7%)	<b>NPO for 7 days</b> N = 2 (5%)	<b>NPO for 8 days</b> N = 2 (5%)	<i>p</i>
<b>Day 1 of life <math>\bar{x}</math>, <math>\pm</math>SD</b>									
Weight (g)	985.14 $\pm$ 162.23	962.86 $\pm$ 178.56	963.00 $\pm$ 173.21	967.14 $\pm$ 62.11	920.00 $\pm$ 135.28	890.00 $\pm$ 251.198	1065.00 $\pm$ 49.5	680.00 $\pm$ 28.29	0.41
Length (cm)	36.55 $\pm$ 3.12	35.79 $\pm$ 3.19	35.60 $\pm$ 1.78	35.46 $\pm$ 1.05	34.74 $\pm$ 0.64	34.72 $\pm$ 2.59	37.28 $\pm$ 1.03	34.5 $\pm$ 0.71	0.41
TSF (mm)	2.74 $\pm$ 0.49	2.81 $\pm$ 0.55	2.57 $\pm$ 0.35	2.66 $\pm$ 0.97	2.63 $\pm$ 0.70	---	2.75 $\pm$ 0.212	---	0.57
SSSF (mm)	2.46 $\pm$ 0.40	2.32 $\pm$ 0.26	2.29 $\pm$ 0.44	2.56 $\pm$ 0.56	2.38 $\pm$ 0.43	2.60 $\pm$ 0.92	2.95 $\pm$ 0.07	---	0.46
FM (%)	1.26 $\pm$ 0.81	1.27 $\pm$ 0.89	0.96 $\pm$ 0.83	1.31 $\pm$ 1.66	1.15 $\pm$ 1.3	---	1.49 $\pm$ 0.66	---	0.69
FFM (%)	98.75 $\pm$ 0.81	98.73 $\pm$ 0.89	99.04 $\pm$ 0.83	98.7 $\pm$ 1.66	98.85 $\pm$ 1.29	---	98.51 $\pm$ 0.66	---	0.69
<b>Day 7 of life <math>\bar{x}</math>, <math>\pm</math>SD</b>									
Weight (g)	952.18 $\pm$ 154.1	948.64 $\pm$ 183.5	845.71 $\pm$ 175.68	897.143 $\pm$ 81.79	830.00 $\pm$ 105.83	988.00 $\pm$ 110.31	1065.00 $\pm$ 49.49	640.00 $\pm$ 14.14	0.08
Length (cm)	36.68 $\pm$ 1.63	36.61 $\pm$ 1.96	34.58 $\pm$ 3.74	35.73 $\pm$ 1.03	---	34.98 $\pm$ 2.18	35.70 $\pm$ 0.21	31.63 $\pm$ 0.32	0.09
TSF (mm)	2.64 $\pm$ 0.42	2.57 $\pm$ 0.49	2.29 $\pm$ 0.28	2.36 $\pm$ 0.31	2.22 $\pm$ 0.33	2.63 $\pm$ 0.35	2.85 $\pm$ 0.64	---	0.10
SSSF (mm)	2.36 $\pm$ 0.36	2.30 $\pm$ 0.45	2.17 $\pm$ 0.40	2.32 $\pm$ 0.58	2.07 $\pm$ 0.23	2.33 $\pm$ 0.59	2.90 $\pm$ 0.14	---	0.12
FM (%)	1.29 $\pm$ 0.66	0.92 $\pm$ 0.70	0.49 $\pm$ 0.52	0.53 $\pm$ 0.29	---	1.57 $\pm$ 1.24	1.43 $\pm$ 1.00	---	0.04 *
FFM (%)	98.86 $\pm$ 1.17	99.07 $\pm$ 0.70	99.51 $\pm$ 0.52	99.47 $\pm$ 0.29	---	98.43 $\pm$ 1.24	98.57 $\pm$ 1.00	---	0.06
<b>Day 14 of life <math>\bar{x}</math>, <math>\pm</math>SD</b>									
Weight (g)	1009.56 $\pm$ 116.68	952.08 $\pm$ 200.44	983.17 $\pm$ 111.33	930.00 $\pm$ 35.59	856.67 $\pm$ 113.72	920.00 $\pm$ 236.43	1080.00 $\pm$ 77.78	675.00 $\pm$ 91.92	0.16
Length (cm)	36.65 $\pm$ 1.96	36.66 $\pm$ 2.11	36.84 $\pm$ 0.87	36.49 $\pm$ 0.58	35.20 $\pm$ 1.06	34.93 $\pm$ 3.38	36.65 $\pm$ 0.07	32.23 $\pm$ 0.88	0.30
TSF (mm)	2.63 $\pm$ 0.35	2.69 $\pm$ 0.50	2.60 $\pm$ 0.33	2.45 $\pm$ 0.33	2.67 $\pm$ 0.55	2.45 $\pm$ 0.35	3.05 $\pm$ 0.07	---	0.32
SSSF (mm)	2.41 $\pm$ 0.34	2.31 $\pm$ 0.36	2.38 $\pm$ 0.45	2.26 $\pm$ 0.39	2.13 $\pm$ 0.23	2.43 $\pm$ 0.04	2.73 $\pm$ 0.25	---	0.46
FM (%)	1.21 $\pm$ 0.60	0.87 $\pm$ 0.66	0.84 $\pm$ 0.74	0.98 $\pm$ 0.95	0.83 $\pm$ 1.17	0.89 $\pm$ 0.74	1.77 $\pm$ 0.16	---	0.42
FFM (%)	99.04 $\pm$ 1.51	99.14 $\pm$ 0.66	100.37 $\pm$ 2.95	99.02 $\pm$ 0.95	99.17 $\pm$ 1.17	99.11 $\pm$ 0.74	97.52 $\pm$ 0.85	---	0.34
<b>Day 21 of life <math>\bar{x}</math>, <math>\pm</math>SD</b>									
Weight (g)	1131.98 $\pm$ 192.70	1029.18 $\pm$ 215.99	1108.17 $\pm$ 153.38	1022.33 $\pm$ 103.13	905.00 $\pm$ 117.58	1026.67 $\pm$ 272.27	1130.00 $\pm$ 56.59	720.00 $\pm$ 155.56	0.09
Length (cm)	37.58 $\pm$ 1.74	37.47 $\pm$ 1.78	37.74 $\pm$ 1.07	37.29 $\pm$ 0.831	35.78 $\pm$ 1.59	36.12 $\pm$ 3.14	37.33 $\pm$ 0.18	32.83 $\pm$ 0.32	0.29
TSF (mm)	2.76 $\pm$ 0.36	2.48 $\pm$ 0.53	2.90 $\pm$ 0.30	2.41 $\pm$ 0.42	2.37 $\pm$ 0.15	2.43 $\pm$ 0.45	3.00 $\pm$ 0.00	---	0.02 *
SSSF (mm)	2.60 $\pm$ 0.41	2.25 $\pm$ 0.49	2.63 $\pm$ 0.42	2.33 $\pm$ 0.49	2.07 $\pm$ 0.13	2.45 $\pm$ 0.56	2.70 $\pm$ 0.71	---	0.12
FM (%)	1.38 $\pm$ 0.61	0.922 $\pm$ 0.94	1.55 $\pm$ 0.61	0.52 $\pm$ 0.79	0.53 $\pm$ 0.06	0.77 $\pm$ 1.05	1.71 $\pm$ 0.29	---	0.04 *
FFM (%)	98.62 $\pm$ 0.61	99.08 $\pm$ 0.94	98.45 $\pm$ 0.61	99.48 $\pm$ 0.79	99.47 $\pm$ 0.06	99.77 $\pm$ 1.53	96.93 $\pm$ 1.63	---	0.02 *

	Infants who were not NPO N = 72 (64%)	Infants who were NPO N = 41 (36%)							
	<b>NPO for 0 days</b> N = 72 (64%)	<b>NPO for 1 day</b> N = 14 (34%)	<b>NPO for 2 days</b> N = 10 (24%)	<b>NPO for 3 days</b> N = 7 (17%)	<b>NPO for 4 days</b> N = 3 (7%)	<b>NPO for 5 days</b> N = 3 (7%)	<b>NPO for 7 days</b> N = 2 (5%)	<b>NPO for 8 days</b> N = 2 (5%)	<i>p</i>
<b>Day 28 of life <math>\bar{x}</math>, <math>\pm</math>SD</b>									
Weight (g)	1205.05 $\pm$ 218.61	1127.73 $\pm$ 244.75	1108.25 $\pm$ 142.68	1141.50 $\pm$ 120.29	989.00 $\pm$ 74.72	1178.33 $\pm$ 317.19	1177.50 $\pm$ 102.53	735.00 $\pm$ 134.4	0.18
Length (cm)	37.91 $\pm$ 1.77	37.33 $\pm$ 2.37	37.78 $\pm$ 1.45	37.82 $\pm$ 0.98	36.23 $\pm$ 1.24	36.63 $\pm$ 4.49	37.85 $\pm$ 0.92	34.08 $\pm$ 0.32	0.40
TSF (mm)	2.93 $\pm$ 0.57	2.73 $\pm$ 0.52	2.80 $\pm$ 0.29	2.33 $\pm$ 0.45	2.35 $\pm$ 0.33	2.28 $\pm$ 0.53	2.65 $\pm$ 0.49	---	0.04 *
SSSF (mm)	2.79 $\pm$ 0.49	2.47 $\pm$ 0.41	2.35 $\pm$ 0.06	2.24 $\pm$ 0.45	2.12 $\pm$ 0.14	2.40 $\pm$ 0.71	2.55 $\pm$ 0.49	---	0.02 *
FM (%)	1.622 $\pm$ 0.79	1.13 $\pm$ 0.75	1.31 $\pm$ 0.49	0.56 $\pm$ 0.79	0.67 $\pm$ 0.52	0.30 $\pm$ 0.68	1.08 $\pm$ 0.37	---	0.02 *
FFM (%)	98.38 $\pm$ 0.79	98.87 $\pm$ 0.75	98.69 $\pm$ 0.46	99.45 $\pm$ 0.79	99.34 $\pm$ 0.52	---	98.91 $\pm$ 0.37	---	0.03 *

Values are means ( $\bar{x}$ ) and  $\pm$ Standard Deviations ( $\pm$ SD) unless otherwise indicated.

\* Statistically significant when ( $p = < 0.05$ )

--- Indicates that no measurements were obtained for the following reasons: Length, infants received NCPAP therefore length measurements were unable to be conducted; TSF and SSSF, the researchers did not conduct skinfolds on infants if they appeared critically ill or had a weight  $\leq 700$  g; FM% and FFM%, unable to calculate values according to the Dauncey anthropometric method if all anthropometric and body composition measurements were not conducted. Fat-free mass percentage; FM%, fat mass percentage; NPO, nil per os; Subscapular skinfold thickness; TSF, Triceps skinfold thickness

## DISCUSSION

This study represents one of the first to assess the nutritional intakes and the its effects on the quality of growth and body composition of HIV-exposed and HIV-unexposed preterm infants in a tertiary academic hospital in a resource-poor setting of South Africa.

Very few study infants received nutritional intakes other than HBM or fortified HBM and for a very short period of time, thereby adhering to the BFHI and PMTCT guidelines.<sup>21</sup> Conclusive evidence could not be determined for the effect of nutritional intakes, such as formula milk or TPN, and the possible effect of these, on the body composition of HIV-exposed and HIV-unexposed preterm infants.

The European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) recommends enteral and parenteral energy intakes of 110–135 kcal/kg/day and 110–120 kcal/kg/day, respectively and a protein intake of 3.5–4 g/kg/day for VLBW preterm infants.<sup>34</sup> According to clinical guidelines,<sup>25</sup> VLBW and ELBW preterm infants are expected to reach full fluid requirements (150–180 ml/kg/day) between day  $\pm$ 10–14 of life. HIV-exposed preterm infants achieved full fluid requirements by day 12.5 ( $\pm$ 3.71) of life, whereas the HIV-unexposed preterm infants achieved full fluid requirements on day 11.375 ( $\pm$ 3.31) of life. The majority of the study infants ( $n = 19$  and  $n = 91$ ) received nutritional intakes from HBM only and fortified HBM respectively, during the 28-day follow-up period. Therefore, the effect of nutritional intakes on body composition was primarily focused on HIV-exposed and HIV-unexposed preterm infants who received only HBM and fortified HBM.

K Strydom, E van Niekerk and M.A Dhansay<sup>35</sup> conducted one of the first studies in Africa to assess the body composition of HIV-exposed and HIV-unexposed preterm infants. This study found that HIV-exposed preterm infants had significantly lower birth weights and poorer growth rates, and presented with a significantly leaner body composition in terms of FM%, lower skinfold thickness measurements and FFM% when compared with their HIV-unexposed counterparts.

A Cochrane review by Brown<sup>36</sup> comprising fourteen randomised controlled trials and 1 071 preterm infants addressed multi-nutrient fortification of HBM for preterm infants.

This review found that multi-nutrient fortification of HBM had small but statistically significant increases in growth rates (weight, length and head circumference) for preterm infants during hospital admission, but there was a lack of data on the long-term effects for growth and development. A recent review on fortification strategies of HBM for improved in-hospital growth of preterm infants conducted in a resource-poor setting such as South Africa, concluded that standard fortification did not lead to suitable in-hospital growth for preterm infants, and alternative fortification strategies should be considered.<sup>12</sup> In our study, the inclusion criteria comprised the smallest and most preterm infants (GA  $\leq$  37 weeks and a birth weight  $\leq$  1 200 g) born in the institution. It was anticipated that these infants would generally have been smaller and not grow at the required rate of 15 g/kg/day and therefore would require supplementation of fortified HBM according to the fortifying HBM indications. According to the simplified energy and protein requirement guidelines commonly used in TBCH, it is recommended that preterm infants receive 110–150 Kcal/kg/day energy and a minimum of 9% protein of total energy.<sup>25</sup> When comparing the composition and nutritional intakes, these requirements are met when HBM is fortified with a standard agent (Table 2). It is however noted that the HBM composition used in our study was based on the average composition of preterm MOM.<sup>33</sup>

There were no significant differences in body composition in terms of FM% and FFM% of infants who received fortified HBM and infants who did not. However, it was found that infants who were receiving fortifier generally had smaller skin fold measurements, indicating less FM% and higher FFM% compared with infants who did not receive fortifier (Supplementary Table 1). There were no HIV-exposed infants who received BM alone from day 7 onwards. This could be because HIV-exposed preterm infants had poorer growth rates when compared with their HIV-unexposed counterparts. When comparing HIV-exposure and infants who receive fortified HBM, it was noted that the HIV-exposed infants were generally smaller, shorter and leaner for the duration of the study when compared with the HIV-unexposed infants. Significant differences were found in terms of TSF, SSSF, FM% and FFM%, especially nearer to the end of the study, on days 21 and 28 of life.

A review by Hay and Thureen<sup>37</sup> stated that preterm FFM growth was dependent on

protein intake and insufficient nutrition and that decreased protein intakes could lead to muscle mass breakdown. Therefore, while growth alone is an important factor and requires increased amounts of amino acids and protein included in the nutritional intakes of preterm infants it is also essential for cell and tissue replication and growth.<sup>38</sup>

37 39

This study noted that the weight and body composition of preterm infants who were kept NPO at any given point in time during the 28-day study period generally had a negative association and displayed significant differences in FM% on day 7 ( $p = 0.04$ ), day 21 ( $p = 0.04$ ) and day 28 ( $p = 0.02$ ) of life, and significant differences in FFM% on day 21 ( $p = 0.02$ ) and day 28 ( $p = 0.03$ ) of life. This study also found that infants who were NPO for the longest duration, a total number of 8 days during the 28-day study period, were the smallest; weighing generally  $<700$  g for the duration of the study. Therefore the researchers were unable to conduct skinfold thickness measurements using the Dauncey anthropometric method and consequently body composition measures in terms of FM and FFM were inconclusive. Limited literature exists elucidating the relationship between preterm infants being kept NPO and the effect it could have on weight, growth and body composition.<sup>40</sup> For this reason, a more comprehensive study should be conducted in this area.

Recommendations for future studies include a longer follow up period to identify the effects nutritional intake has on the long-term growth and body composition of HIV-exposed and HIV-unexposed preterm infants. This study made use of reference values for the calculation of nutrient content for the feeds that were prescribed to the infants. None of these feeds (BM, DMB, PBM or fortified HBM) were analysed for nutrient content. Future studies should consider milk analyses to determine the exact quantity of micronutrients, energy and macronutrients in terms of protein and fat. The strengths of this study are that it was one of the first studies in Africa to assess the effects of nutritional intakes on the growth and body composition of HIV-exposed and HIV-unexposed preterm infants.

## **CONCLUSION**

This descriptive cross-sectional study of HIV-exposed and HIV-unexposed preterm VLBW and ELBW infants was conducted at an institution that has been BFHI

accredited since 2005. For this reason, the majority of the study infants received nutritional intakes from HBM and fortified HBM. HIV-unexposed preterm infants achieved full fluid requirements earlier than HIV-exposed preterm infants. Preterm infants receiving fortified HBM were smaller and presented with a poorer growth rate of <15 g/kg/day. There were no significant differences in body composition of infants receiving HBM or fortified HBM. Significant differences in weight, skinfold thickness and body composition measurements (in terms of FM% and FFM%) were found for HIV-exposed and HIV-unexposed preterm infants who received fortified HBM. This study found that infants who were kept NPO for any duration during the 28-day follow-up period were generally smaller, shorter, had lower FM% and higher FFM%.

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## **CONFLICTS OF INTERES**

The authors declare no conflict of interest with respect to the research, authorship, and/or publication of this article.

## **AVAILABILITY OF DATA AND MATERIALS**

Please contact author for data requests.

## AUTHORS' CONTRIBUTIONS

K. Strydom, E. van Niekerk and M.A Dhansay equally contributed to the design and conceptualisation of the research study; D. G. Nel did the statistical analyses; K. Strydom and E. van Niekerk reviewed the data. K Strydom performed data collection, contributed to the acquisition and analysis of the data, interpreted the data and drafted this manuscript. E. van Niekerk and M. A. Dhansay helped to draft and critically reviewed the manuscript. All authors critically revised and approved the final version of the manuscript and agreed to be fully accountable for ensuring the integrity and accuracy of the work.

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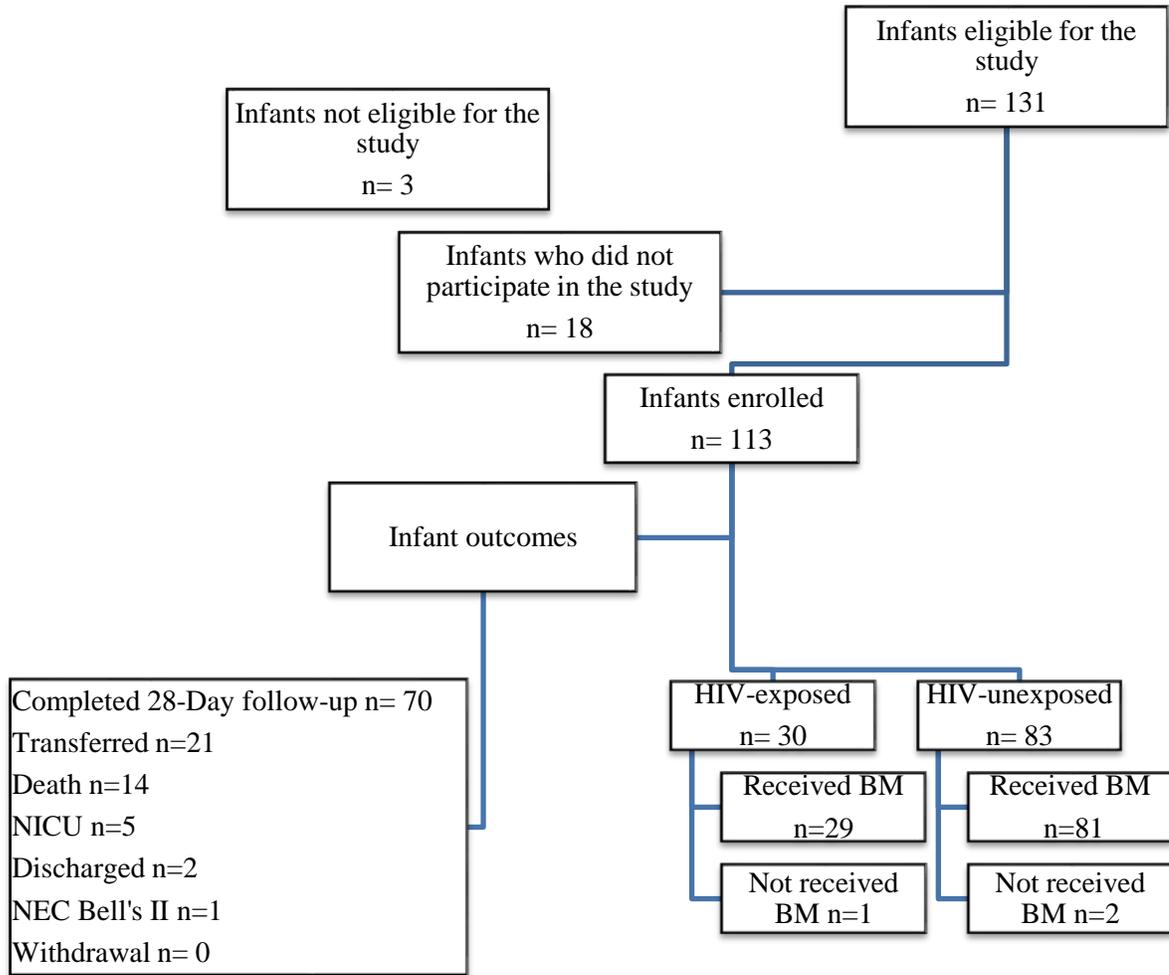
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**SUPPLEMENTARY DATA**

**Supplementary Figure 1.** Flow diagram of infants included in the study



**Supplementary Table 1: Weekly anthropometric and body composition measurements of infants receiving only human breast milk or fortified human breast milk**

	Infants receiving fortified breast milk N = 91 (83%)	Infants receiving breast milk alone N = 19 (17%)	<i>p</i>
<b>Day 1 of life <math>\bar{x}</math>, <math>\pm</math>SD</b>			
Weight (g)	979.34 $\pm$ 17.06	936.83 $\pm$ 150.89	0.27
Length (cm)	36.50 $\pm$ 2.83	34.89 $\pm$ 2.67	0.017 *
TSF (mm)	2.72 $\pm$ 0.51	2.72 $\pm$ 0.60	0.99
SSSF (mm)	2.49 $\pm$ 0.40	2.22 $\pm$ 0.43	0.009*
FM (%)	1.27 $\pm$ 0.91	1.03 $\pm$ 0.79	0.30
FFM (%)	98.73 $\pm$ 0.91	98.97 $\pm$ 0.79	0.30
<b>Day 7 of life <math>\bar{x}</math>, <math>\pm</math>SD</b>			
Weight (g)	932.62 $\pm$ 149.59	926.23 $\pm$ 211.25	0.89
Length (cm)	36.41 $\pm$ 1.75	34.66 $\pm$ 2.91	0.014 *
TSF (mm)	2.57 $\pm$ 0.38	2.66 $\pm$ 0.72	0.54
SSSF (mm)	2.32 $\pm$ 0.36	2.44 $\pm$ 0.71	0.39
FM (%)	1.16 $\pm$ 0.69	1.08 $\pm$ 0.76	0.79
FFM (%)	98.96 $\pm$ 1.07	98.92 $\pm$ 0.76	0.93
<b>Day 14 of life <math>\bar{x}</math>, <math>\pm</math>SD</b>			
Weight (g)	979.13 $\pm$ 165.65	1065.0 $\pm$ 203.72	0.27
Length (cm)	36.44 $\pm$ 1.89	36.53 $\pm$ 3.49	0.93
TSF (mm)	2.60 $\pm$ 0.38	2.69 $\pm$ 0.35	0.62
SSSF (mm)	2.37 $\pm$ 0.34	2.48 $\pm$ 0.39	0.51
FM (%)	1.13 $\pm$ 0.66	1.04 $\pm$ 0.67	0.80
FFM (%)	99.11 $\pm$ 1.54	98.96 $\pm$ 0.67	0.85
<b>Day 21 of life <math>\bar{x}</math>, <math>\pm</math>SD</b>			
Weight (g)	1085.72 $\pm$ 196.32	1178.60 $\pm$ 224.64	0.31
Length (cm)	37.30 $\pm$ 1.76	37.36 $\pm$ 2.82	0.95
TSF (mm)	2.69 $\pm$ 0.41	2.74 $\pm$ 0.31	0.77
SSSF (mm)	2.50 $\pm$ 0.45	2.70 $\pm$ 0.52	0.34
FM (%)	1.22 $\pm$ 0.73	1.36 $\pm$ 0.65	0.68
FFM (%)	98.76 $\pm$ 0.85	98.64 $\pm$ 0.65	0.76
<b>Day 28 of life <math>\bar{x}</math>, <math>\pm</math>SD</b>			
Weight (g)	1156.27 $\pm$ 211.88	1277.50 $\pm$ 344.419	0.27
Length (cm)	37.56 $\pm$ 1.86	38.35 $\pm$ 2.90	0.42
TSF (mm)	2.80 $\pm$ 0.57	2.58 $\pm$ 0.32	0.43
SSSF (mm)	2.17 $\pm$ 0.51	2.78 $\pm$ 0.34	0.54
FM (%)	1.33 $\pm$ 0.84	1.09 $\pm$ 0.49	0.57
FFM (%)	98.66 $\pm$ 0.85	98.91 $\pm$ 0.49	0.56

Values are means ( $\bar{x}$ ) and  $\pm$ Standard Deviations ( $\pm$ SD) unless otherwise indicated.

\* Statistically significant when ( $p = < 0.05$ )

\*\* Extremely statistically significant when ( $p = < 0.001$ )

^ These values are based on unfortified breast milk, refer to Table 2.

Fat free mass percentage; FM%, fat mass percentage; Subscapular skinfold thickness; TSF, Triceps skinfold thickness

# CHAPTER 4

## **SUMMARY, CONCLUSION AND RECOMMENDATIONS**

## 4.1 SUMMARY OF STUDY OBJECTIVES AND DESIGN

### *“FROM LITTLE ACORNS MIGHTY OAKS GROW”*

*- ANONYMOUS-*

In 2010, “an estimated 14.9 million babies worldwide were born preterm, and this figure rises every year”. Fifty-two percent of global live births occur in sub-Saharan Africa and South Asia, and of these 60% are preterm deliveries (Lancet 2012<sup>1</sup>). HIV is highly prevalent in South Africa and HIV-infected pregnant mothers have a considerably higher risk (13–34%) of delivering an infant preterm.<sup>1 2</sup> HIV-exposed infants seem to have poorer growth and greater early mortality and morbidity rates than their HIV-unexposed counterparts.<sup>3 4</sup> Changes in body fat distribution, as well as glucose and bone metabolism in HIV-positive children can increase their risk of future morbidities.<sup>5</sup>

There is an evident gap in documented research regarding nutritional interventions and the effect thereof on the quality of growth and body composition of the preterm infant,<sup>6 7 8 9</sup> especially in the context of HIV infection.<sup>9 10</sup> According to published research, the best method for assessing preterm body composition, in terms of fat mass (FM) and fat-free mass (FFM), is Dauncey’s anthropometric model.<sup>11 12 13 14</sup> The use of this method to determine body composition is accurate, safe, inexpensive, non-invasive and can be performed at the patient’s bedside<sup>11 12 13 14</sup>, thereby avoiding excessive disturbance to these fragile infants.<sup>12</sup>

Human breast milk (HBM) is globally accepted as the optimal and ideal nutritional intervention for full-term and preterm infants. The benefits of HBM include better neurodevelopmental outcomes, improved gastrointestinal function, improvements in host defence and a decrease in late-onset sepsis and necrotising enterocolitis (NEC).<sup>15 16 17 18</sup>

The composition of preterm and term milk changes over time: HBM from mothers with preterm infants is initially higher in protein and fat, when compared with term milk.<sup>19 20 21</sup> A study by Hsu et al.<sup>18</sup> examined the change in macronutrient and micronutrient composition of preterm milk over a four-week post-partum period. The results showed that the composition of fat increased gradually but plateaued after two-weeks post-partum. Research has shown that the composition of protein decreases slowly over the following weeks of lactation.<sup>22</sup> As preterm milk is low in calcium, the bone mineral density and bone mass of preterm infants may be of concern in the long term.

Olhager and Forsum<sup>23</sup> stated that despite its benefits, preterm milk alone does not meet the increased nutritional requirements of infants born with a very-low birth weight (VLBW), as preterm milk provides only approximately 60% of their protein requirements.<sup>24 25</sup> Therefore, HBM from mothers with preterm infants is not nutritionally adequate and must be fortified to ensure adequate growth. It is particularly challenging to feed and meet the nutritional requirements of these infants owing to the high recommended daily allowance of energy, protein and other nutrients to achieve in-utero growth rates, in conjunction with the slow feeding progressions, small feeding volumes, feeding intolerance and gastrointestinal immaturity.<sup>15 16 18 26 27 28</sup>

When there is an insufficiency in mother's own milk (MOM) or if MOM is unavailable, pasteurised donor breast milk (DBM) is the first line of nutritional intervention for preterm infants.<sup>29</sup> Donor breast milk usually comes from a mother who has an abundance of milk, more than her own infant can consume, and is from mothers with more mature milk (later on in lactation). The composition of DBM is lower in fat and protein and requires fortification to achieve the nutritional requirements of VLBW infants to ensure adequate growth. Supplementation of HBM with a fortifier (protein, nutrients, vitamins and minerals) is common practice in many neonatal intensive care unit (NICUs) to achieve the nutritional requirements and successful intrauterine growth rates in preterm infants, while the benefits of HBM are continually derived.<sup>24 25 30</sup>

The primary objective of the study was to evaluate and compare body composition in terms of the FM and FFM of HIV-exposed and HIV-unexposed, preterm, VLBW and extremely-low birth weight (ELBW infants).

The secondary research objectives were as follows:

- i. To evaluate the effect of HIV-infected mothers' treatment duration of highly active antiretroviral therapy (HAART) on the body composition of HIV-exposed, preterm, VLBW and ELBW infants.
- ii. To evaluate the effect of nutritional intake according to the ward protocol on the body composition of HIV-exposed and HIV-unexposed, preterm, VLBW and ELBW infants.
- iii. To evaluate the effect of breast milk fortification on the body composition of HIV-exposed and HIV-unexposed, preterm, VLBW and ELBW infants.
- iv. To evaluate the effect of feeding intolerances and days that infants are nil per os (NPO) on the body composition of HIV-exposed and HIV-unexposed, preterm, VLBW and ELBW infants.

A cross-sectional descriptive study with an analytical component was conducted in the neonatal units of Tygerberg Children's Hospital (TBCH), Cape Town, South Africa between May 2016 and October 2016. Participants were included if the following inclusion criteria were met: (i) all male and female HIV-exposed and HIV-unexposed preterm infants with a gestational age of 25–37 weeks; (ii) birth weight ( $\geq 500$  g– $\leq 1\ 200$  g); (iii) receiving nutritional support in the form of enteral nutrition and/or parenteral nutrition [EN: breast milk and supplementation thereof (FM85), formula feed) and parenteral nutrition (TPN)]; and (iv) written parenteral consent. Infants from multiple gestations meeting these criteria were included in the study. Infants were exited from the study if the following occurred: (i) day 28 of the study completed; (ii) transferred to neonatal or paediatric intensive care unit (NICU/PICU); (iii) discharged within the 28 days of recruitment; (iv) confirmed NEC: Bells stage II or III; (v) mothers admitted to intensive care unit (ICU) or those who gave their

infant/s up for adoption; (vi) if the mother of an eligible child could not be found after 48 hours of recruitment; and (vii) death.

Participants were included in this study for a 28-day follow-up period. Maternal medical background, infant clinical, medical and nutritional data were collected on a daily basis by the researcher. These included: medical information, medications, supplementation and all fluid input and output. Maternal antiretroviral (ARV) treatment was documented for the HIV-exposed preterm infants. The daily clinical assessments were done by the attending doctors and were documented. Furthermore, the following data was assessed and recorded daily: supplementation with medium-chain triglyceride oil, multivitamin and iron supplementation, phototherapy and blood products received, medication administered, urinary output, stool frequency, colour and consistency and the occurrence, frequency, colour and consistency of feeding intolerances.

The researcher assessed the daily infant nutritional intakes, which included feeding route and volume of breast milk, infant formula, TPN, Neonatolyte (glucose-electrolyte-containing-solution) and saline. The time of initial feeding was at the discretion of the attending physician. Infants of HIV-infected mothers received pasteurised expressed breast milk (PBM), which was pasteurised using the Pretoria flash method. Infants of HIV-uninfected mothers received unpasteurised breast milk. The protocol for the fortification and supplementation with FM85 (breast milk fortifying agent available in South Africa) was applied for the duration of the study.

All anthropometric and body composition measurements were conducted on day 1 of the preterm infants' life and subsequently on a weekly basis: days 7, 14, 21 and 28 of life. Anthropometric measurements included: daily weights (g) and weekly lengths (cm). Body composition was determined using the Dauncey anthropometric method. Fat mass (FM) and FFM were determined by assessing skinfold thickness at two primary sites (triceps and subscapular) in conjunction with nine body dimensions: circumferences (head, chest, mid-upper arm, mid-thigh and mid-calf) and lengths (upper arm, lower arm, supine and crown-rump). No anthropometric measurements were conducted if the infant was

receiving nasal continuous positive airway pressure (NCPAP) and no skinfold measurements were done for the period that infants had a weight  $\leq 700$  g.

Of the 131 preterm infants initially meeting the inclusion criteria, eight mothers were unable to give consent for medical or social reasons, three mothers declined consent, one infant was transferred to the NICU before inclusion and six infants died before consent was obtained. This left a sample size of 113 preterm infants in this descriptive cross-sectional study. Of these, 30 infants (27%) were HIV-exposed and 83 (73%) were HIV-unexposed.

Of the 113 infants enrolled in the study, 70 (62%) completed the 28-day follow-up study period. Reasons for not completing the 28-day follow-up were: 21 were transferred to other hospitals, five were sent to the NICU, two were discharged, one was diagnosed as NEC Bells II and 14 deaths occurred. There were no withdrawals from the study.

There were ten pairs of twins in the study; therefore the maternal study population comprised a total of 103 mothers. Of these, 27 (26%) were HIV-infected and 76 (74%) were HIV-uninfected.

## **4.2 ADDRESSING STUDY OBJECTIVES**

In this section, each objective will be described and interpreted within the context of the results of the research.

### **4.2.1 Body composition and HIV-exposure**

To our knowledge, this is one of the first studies to assess the body composition, in terms of FM and FFM, of HIV-exposed and HIV-unexposed preterm infants (refer to glossary of terms for definitions). Existing literature on body composition and HIV focuses predominantly on: HIV-exposed uninfected (HIV-EU) infants, HIV-infected and HIV-unexposed infants, children and adults. In this study, HIV-exposed infants were born significantly more preterm ( $p = 0.01$ ; GA 25–28 weeks), and were generally smaller, shorter and leaner at birth when compared with the HIV-unexposed infants. HIV-exposed

preterm infants presented with significantly lower birth weights and lengths [929 g ( $\pm 155$ g); ( $p = 0.05$ )] and [35.1 cm ( $\pm 2.8$  cm); ( $p = 0.01$ )], respectively.

As expected<sup>31</sup> an almost equal weight loss was seen in the first week of life for both the HIV-exposed and HIV-unexposed groups [ $-58.5$ g ( $\pm 60.6$ g) and  $-58.2$ g ( $\pm 60.15$ g); ( $p = 0.32$ )]. This study found that HIV-exposed preterm infants generally had a lower FM% for the duration of the study and were found to have significantly smaller triceps skinfold (TSF) and sub-scapular skinfold (SSSF) thicknesses [on day 7, day 21, day 28 ( $p = 0.03$ ,  $p = 0.02$  and  $p = <0.01$ ) and day 21 and day 28 ( $p = <0.01$  and  $p = <0.01$ ) of life, respectively]. This indicates a significantly lower FM% and a significantly higher FFM% when compared with HIV-unexposed preterm infants on day 21 ( $p = 0.01$  and  $p = 0.03$ ) and day 28 ( $p = 0.03$  and  $p = 0.03$ ) of life. This study failed to show significant differences in FM%, FFM% and TSF thickness measurements for HIV-exposures for the duration of the study (day 1–28 of life). However, significant differences ( $p = 0.01$ ) in SSSF thickness were found for HIV-exposures for the duration of the study (day 1–28 of life).

Environmental factors (maternal socio-economic environment and nutritional status) could have an effect on early life programming and determine or influence an infant's risk of developing diseases later on in life.<sup>32</sup> Barker et al.<sup>33</sup> and Hales et al.<sup>34</sup> conducted studies which reported that infants born with lower birth weights died of ischemic heart disease later on in life and had a higher prevalence of type 2 diabetes when compared with adults who were born with a normal weight at birth.<sup>35</sup> Barker et al.<sup>36 37</sup> further went on to study small for gestational age (SGA) and preterm birth and the effects these had on the risk of diseases later on in life. They reported that the low birth weight of infants born prematurely could be an indicator for developing lifestyle diseases later on, such as obesity, type 2 diabetes, hypertension and cardiovascular disease (CVD). Existing literature on body composition and HIV predominantly focuses on older children and adults and there is limited literature regarding early life programming and HIV.

A longitudinal observation study found that when compared with the National Health and Nutrition Examination Survey (NHANES) standards, HIV-EU children  $< 2$  years had

significantly smaller TSF and SSSF thicknesses, indicating significantly lower fat stores.<sup>38</sup> Even though the researchers were unable to compare the results of this study to available literature it was found that our results were supportive of this trend in body composition. The current study indicated that HIV-exposed infants generally had smaller body composition measurements (TSF, SSSF and FM%) at weekly measurements on day 1, 7, 14, 21 and 28 of life, indicating that they were leaner for the duration of the study. (Refer to article 1: *The effect of maternal HIV status and treatment duration on body composition of HIV-exposed and HIV-unexposed preterm, very- and extremely-low birth weight infants*, and Table 2: *Weekly anthropometric and body composition measurements*.) The researcher therefore rejects the null hypothesis (see 2.3.3) as clear differences existed in the body composition of HIV-exposed and HIV-unexposed preterm, VLBW and ELBW infants. HIV-exposed infants were smaller and leaner than HIV-unexposed infants. The researchers further hypothesise that early life programming affects the long-term outcomes of HIV-exposed preterm infants.

#### **4.2.2 Effect of HIV-infected mothers' treatment duration on body composition**

Research reveals that HIV-infected infants, children and adults benefit from HAART.<sup>39</sup> Even though HAART is advantageous for maternal health and for the prevention of mother-to-child transmission (PMTCT), antenatal HAART has been associated with increased preterm delivery.<sup>1 2 40</sup> HIV as a disease and HIV treatment are associated side effects such as altered fat distribution, especially an increase in central adiposity, which can be a risk factor for morbidities such as CVD and metabolic syndrome.<sup>10 39</sup> To our knowledge, the relationship between body fat distribution and the body composition of HIV-infected or HIV-exposed preterm infants receiving antiretroviral therapy (ART) as prophylaxis has not yet been determined.

This study included 27 HIV-infected mothers who were all on HAART at the time of delivery. This concurs to the most recent South African national consolidated guidelines for PMTCT released in April 2015.<sup>41 42</sup> The HIV-status of all mothers included in this study was known at the time of delivery and all HIV-infected study mothers had received HAART for  $\geq 4$  weeks at delivery. According to the guidelines, in 2011, mother-to-child

transmission (MTCT) decreased to 2.7%.<sup>41</sup> In this study none of the HIV-exposed preterm infants had a positive polymerase chain reaction (PCR) test for the duration of the study. Even though the researchers only recorded the PCR at birth and did not conduct any follow-up PCR tests, the low transmission rates and positive statistics are very encouraging, indicating that Tygerberg academic hospital (TAH) is implementing and adhering to PMTCT governmental guidelines and policies.

All HIV-infected mothers had received HAART at the time of delivery. Thirteen mothers (48%) received HAART for  $\geq 4$ – $< 20$  weeks and 14 (52%) received HAART for  $\geq 20$  weeks. This study failed to show significant differences between the duration mothers received HAART and TSF, SSSF, FM% and FFM% measurements at birth among HIV-exposed infants. However, this study found that HIV-exposed preterm infants whose mothers received HAART  $\geq 20$  weeks were generally heavier and had larger TSF and SSSF thickness measurements, indicating increased FM% and lower FFM% at birth when compared with infants whose mothers were on HAART for  $\geq 4$ – $< 20$  weeks. Increased skinfold measurements and FM% indicates an altered body fat distribution which could increase the risk factors for future morbidities of these infants.<sup>5 10</sup> Low maternal CD4 count ( $< 200$  cells/ $\mu$ L) was associated with heavy birth weights, higher FM% and lower FFM%. The researcher therefore accepts the null hypothesis (see 2.3.3) that HIV-infected mothers' treatment duration of HAART will have no effect on the growth and body composition of HIV-exposed, preterm, VLBW and ELBW infants. However, this could be due to small samples and detection as it is clear that, clinically, there was a significant difference in weight as infants born to mothers on HAART  $\geq 20$  weeks were almost 100g heavier at birth when compared with infants born to mothers on HAART  $\geq 4$ – $< 20$  weeks. For the scope of this study and for the purposes of addressing the hypothesis, the researchers only compared the weight and body composition of treatment durations at birth.

### 4.2.3 Nutritional intakes and body composition

This study represents one of the first to assess the nutritional intakes and the effect thereof on the quality of growth and body composition of HIV-exposed and HIV-unexposed preterm infants. There is increasing interest in the relationship between nutritional status during early infancy and childhood and the increased risk of adverse health effects as adults.<sup>23 28 31 43 44</sup>

Tygerberg Children's Hospital has been an accredited Baby Friendly Hospital Initiative since 2005 and implements the PMTCT national consolidated guidelines.<sup>41 42</sup> Only five (4%) of the included infants received infant formula for an average of 7.6 days and 11 infants (10%) received TPN for an average of five days. This study failed to provide conclusive evidence on the effects of nutritional intakes, such as infant formula and TPN, and their possible effect on the body composition of HIV-exposed and HIV-unexposed preterm infants.

Human breast milk is globally known to provide the optimal nutritional intake for infants. However, breast milk from mothers with preterm infants is considered nutritionally inadequate and must be supplemented with a fortifying agent to ensure adequate growth of these infants of  $> 15$  g/kg/day.<sup>23 24 25 30 45</sup> One hundred and ten infants [HIV-exposed ( $n = 29$ ) and HIV-unexposed ( $n = 81$ )] received expressed breast milk (EBM), DBM or PBM at a given point in time during the 28-day follow-up study. In this current study, no significant differences in body composition in terms of FM% and FFM% were noted for HIV exposure. The majority of study infants ( $n=110$ ) received nutritional intakes from breast milk only ( $n = 19$ ) and fortified breast milk ( $n = 91$ ), during the 28-day follow-up period. The indication for the use of a fortifier was the infants were generally smaller, and did not grow at the required rate of 15 g/kg/day. There were no significant differences in body composition in terms of FM% and FFM% of infants who received fortified breast milk and infants who did not. These findings are in agreement with a recent review conducted in South Africa, which concluded that standard fortification strategies of human breast milk did not lead to sufficient in-hospital growth of preterm infants. The authors recommended that the super fortification strategy or patient-specific requirements should be

implemented, as it would lead to higher protein intakes and adequate growth of the preterm infant.<sup>24 46</sup> The researcher, therefore, fails to reject the null hypotheses (see 2.3.3) that there is no difference in body composition between VLBW and ELBW preterm infants who receive breast milk only and infants who receive fortified breast milk.

*For the purposes of this study, the researcher followed ward protocol for the fortification of HBM in TBCH. The fortifier was FM85 and the standard fortification strategy was used.<sup>45</sup>*

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The term ‘*feeding intolerance*’ is commonly used in the literature, but there is little consensus regarding its definition. Fanaro<sup>47</sup> defined a feeding intolerance as a “combination of clinical signs suggesting an inability by the subject to tolerate enteral nutrition”. Moore and Wilson<sup>48</sup> conducted a concept analysis in an attempt to find a universally accepted definition for the term. All known definitions from dictionaries, medical textbooks, literature and biomedical databases were used to compose the most comprehensive definition: “a feeding intolerance in the premature infant is the inability to digest enteral feedings presented as GRV (gastric residual volume) more than 50%, abdominal distension or emesis or both, and the disruption of the patient’s feeding plan.” For the purposes of this study, feeding intolerance was recorded with the occurrence of vomiting or aspiration, at which stage infants were NPO.

A total of 41 infants (36%) were NPO because of a suspected intolerance during the 28-day study period. Infants who were NPO at any given point during the study, but especially for longer durations, were found to be generally lighter, shorter, leaner and had a higher FFM%. Significant differences in body composition were found for infants who were kept NPO during the 28-day follow-up period. These infants displayed significant differences in FM% on day 7 ( $p = 0.04$ ), day 21 ( $p = 0.04$ ) and day 28 ( $p = 0.02$ ) of life as well as significant differences in FFM% on day 21 ( $p = 0.02$ ) and day 28 ( $p = 0.03$ ) of life. Skinfold thickness was congruent with FM% and infants that were NPO had significant differences in TSF thickness on day 21 ( $p = 0.02$ ) and day 28 ( $p = 0.04$ ) of life, as well as significantly different SSSF thicknesses on day 28 ( $p = 0.02$ ) of life. (Refer to article 2:

*How does nutritional intake affect the body composition of HIV-exposed and HIV-unexposed preterm, very- and extremely-low birth weight infants?* and Table 4: *The effect of nil per os (NPO) on the anthropometry and body composition of preterm infants.*) This study concludes that keeping an infant NPO and providing inadequate nutrition during early life may be associated with an increase in morbidity, poor development (neurologically and physiologically) and impaired cognitive performance later on in life.<sup>23</sup>  
<sup>26 49</sup> The researcher therefore rejects the null hypothesis (see 2.3.3) as preterm infants who were NPO were smaller, had lower FM% and higher FFM% than infants who were not NPO at any point during the study. Limited literature exists elucidating the relationship between preterm infants being kept NPO and the effect it could have on weight changes.

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It is increasingly evident that preterm infants who are NPO or not meeting their nutritional requirements do not achieve growth rates in relation to size, anthropometry and body composition when compared with their term counterparts at term-equivalent age. Therefore, these infants are at risk of developing adverse outcomes later on in life such as neurodevelopmental delays, decreased immunity and stunting.<sup>43 51 52 53</sup> On the other hand, infants who experience rapid catch-up growth and intra-abdominal adiposity could be at risk of developing metabolic syndrome and CVD later on in life.<sup>6 7 8 26 54 55</sup>

Recent literature has reported that the risk of diseases such as obesity, CVD and type 2 diabetes is influenced by more than just an excess energy intake and sedentary lifestyle. The very early life of an infant in-utero and the environment that he or she is exposed to could influence the infant's risk of disease later on in life.<sup>56 57</sup> Maternal risk factors during pregnancy could also have an effect on an infant's early life programming in the prenatal period. Environmental factors such as maternal obesity, gestational diabetes and smoking have been found to be precursors to obesity later on in life. This study found that infants whose mothers smoked cigarettes during pregnancy were heavier and had a higher FM% and lower FFM% at birth than infants whose mothers did not smoke. (Refer to article 1: *The effect of maternal HIV status and treatment duration on body composition of HIV-exposed and HIV-unexposed preterm, very- and extremely-low birth weight infants.*)

Previous literature has reported that infants of mothers who smoked during pregnancy were considerably more likely to be SGA.<sup>58 59</sup> However, more recently studies have linked smoking during pregnancy with childhood and adult obesity.<sup>60 61 62 63</sup> The researchers hypothesise that prenatal smoking is a risk factor for fetal growth restriction and lower birth weights and subsequently can be associated with the risk of developing obesity later on in life.

Infants of mothers who had received HAART for 20 weeks or longer were generally heavier and had higher FM% and lower FFM% at birth when compared with infants whose mothers were receiving HAART 4–20 weeks. This study also found that infants whose mothers had CD4 counts < 200 cells/ $\mu$ L were the heaviest at birth and had the highest FM% when compared with infants whose mothers had higher CD4 counts (CD4  $\geq$  200–< 500 cells/ $\mu$ L and CD4  $\geq$  500 cells/ $\mu$ L). (Refer to article 1: *The effect of maternal HIV status and treatment duration on body composition of HIV-exposed and HIV-unexposed preterm, very- and extremely-low birth weight infants.*) Infants of mothers who are HIV-positive could be exposed to a maternal immunosuppressed environment in-utero which could cause epigenetic changes and oxidative stress, thereby increasing the risk of metabolic and cardiovascular diseases. (Refer to article 1: *The effect of maternal HIV status and treatment duration on body composition of HIV-exposed and HIV-unexposed preterm, very- and extremely-low birth weight infants.*)

The thrifty phenotype hypothesis states that maternal undernutrition results in endocrine changes (insulin resistance) that decrease nutrient supply to the somatic cells of the fetus and rather nourish the heart and brain. The fetus adapts to this environment in-utero and when born into a world of abundance, the increased insulin resistance could result in the infant developing obesity and type 2 diabetes later on in life.<sup>64 65</sup> Body composition studies indicate that babies with higher abdominal FM% and lower overall FFM%, known as *'thin-fat babies'*, are at a higher risk of developing type 2 diabetes later on in life. Therefore, the thrifty phenotype hypothesis extends further than only maternal undernutrition and could include environmental factors, which result in altered body composition of preterm infants.<sup>66</sup>

This study found that infants who were exposed to environmental factors such as smoking, low CD4 counts and were exposed to chronic medication (HAART  $\geq$  20 weeks) had increased birth weights and higher FM%. These infants are already pre-disposed due to their low birth weights to risk factors later on in life. HIV-exposed preterm infants were found to be the smallest and leanest, and therefore have an even higher increased risk of developing diseases such as obesity, type 2 diabetes and CVD later on in life.

### 4.3 LIMITATIONS OF THE STUDY

The following limitations could have impacted the quality of results of this descriptive cross-sectional study:

- i. Owing to the slow enrolment of HIV-exposed preterm infants and a limited time frame for data collection, the statistician recalculated the sample size for the HIV-exposed group. Refer to Chapter 2: *Methodology* section 2.6.1 *Sample size*
- ii. When infants were receiving NCPAP no anthropometric measurements (weight, length, head circumferences) were conducted for this time period. This was a limitation to the current study, as all anthropometric and body composition measurements were needed to determine FM% and FFM% using the Dauncey anthropometric method on the five occasions during the study period (Day 1, 7, 14, 21 and 28).
- iii. Owing to the fragility and friability of the preterm infants' skin, no skinfold measurements were conducted for the time period that infants had a weight of  $\leq$  700 g. This was a limitation to the current study, as all anthropometric and body composition measurements were needed to determine FM% and FFM% using the Dauncey anthropometric method on the five occasions during the study period (Day 1, 7, 14, 21 and 28).
- iv. During the 28-day recruitment period 21 infants were transferred to other hospitals before the end of the 28-day follow-up period.

**The following data and results could have been collected to further strengthen the**

**results of the study:**

- i. Pre-existing maternal body composition and environmental factors could have been accounted for and would have presented interesting body composition results.
- ii. Body composition measurements, in terms of weight, TSF, SSSF, FM%, FFM%, of HIV-exposed and HIV-unexposed infants at 6 months would have helped to determine the quality of catch-up growth and body composition and to draw associations with HIV-exposure.
- iii. A secondary PCR at 10 weeks could have been used to determine HIV MTCT risk and assess the effects on body composition.
- iv. Recording maternal viral loads at birth could have further indicated maternal HIV stage. This information may have been interesting for identifying the effect of maternal HIV viral loads on the body composition of HIV-exposed infants.
- v. Reference values were used for the calculation of nutrient content for the feeds (HBM, DBM, PBM or fortified breast milk) that were prescribed to the infants. Analysing the micronutrient, energy and macronutrients content (in terms of protein and fat) could have led to a better understanding of the quality of growth and body composition distribution in terms of FM% and FFM%.

**4.4 RECOMMENDATIONS****4.4.1 Practical recommendations to address the research question**

- i. To the researchers' best knowledge, this was the first study to assess the body composition of HIV-exposed and HIV-unexposed, VLBW and ELBW preterm infants. More studies should be conducted using different body composition assessment methods such as the gold standard; air displacement plethysmography (ADP), using the PEA POD, and dual-energy X-ray absorptiometry to confirm the differences in body composition of these infants.

- ii. The effect of HIV, the immunosuppressed state of an HIV-positive mother and the treatment duration and exposure on the growth and body composition of HIV-exposed preterm VLBW and ELBW infants should be explored.

#### **4.4.2 Recommendations for future research**

- i. A prospective study should investigate long-term outcomes of body composition in HIV-exposed and HIV-unexposed preterm infants. An important research question that remains is: *What are the long term outcomes of body composition and the effect of the quality of catch-up growth in HIV-exposed and HIV-unexposed preterm infants and the incidence of morbidities associated with central obesity such as CVD and metabolic syndrome?*
- ii. A longer follow up study period may have contributed to the identification of the long-term effects such as maternal HIV status and treatment duration on the body composition of HIV-exposed and unexposed VLBW and ELBW preterm infants, as well as the effects nutritional intake has on the long-term growth and body composition of HIV-exposed and HIV-unexposed preterm infants.
- iii. Future studies should conduct milk analyses on the nutritional intake samples (breast milk, donor breast milk, pasteurised breast milk and fortified breast milk) infants received to determine the micronutrient, energy and macronutrient composition and the effect these could have on the quality of growth and body composition of preterm infants.
- iv. Analysing the breast milk composition of HIV-positive and HIV-negative mothers should be examined and their possible effect on the growth and body composition of HIV-exposed and HIV-unexposed preterm infants.

## 4.5 KEY POINTS

<b>Key points to take home</b>	
<b>1.</b>	Mothers receiving HAART could have an increased risk of preterm delivery
<b>2.</b>	At birth, HIV-exposed uninfected preterm infants are significantly smaller (in weight and length), generally leaner (in terms of FM%) and do not gain weight as well in the first 28 days of life, when compared with HIV-unexposed preterm infants.
<b>3.</b>	HIV-exposed preterm infants have significantly leaner body composition in terms of FM% and skinfold thickness (triceps and biceps) on day 21 and day 28 of life.
<b>4.</b>	The treatment duration of maternal HAART affects the postnatal body composition of preterm infants.
<b>5.</b>	HIV-unexposed preterm infants reach full fluid requirements earlier [day 11.375 ( $\pm 3.31$ ) of life] than HIV-exposed preterm infants [day 12.5 ( $\pm 3.71$ ) of life].
<b>6.</b>	Preterm infants receiving fortified breast milk were smaller and presented with a poorer growth rate $< 15$ g/kg/day.
<b>7.</b>	No significant differences in body composition were found in infants receiving HBM vs. fortified HBM.
<b>8.</b>	The duration infants are kept NPO has an effect on their growth and body composition as these infants were generally smaller, shorter, had lower FM% and higher FFM%.

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# ADDENDA

## ADDENDUM A: CONSENT FORMS (ENGLISH, AFRIKAANS, ISIXHOSA)

### PARTICIPANT INFORMATION LEAFLET AND CONSENT FORM

#### TITLE OF THE RESEARCH PROJECT:

**Body composition, Growth** and nutritional intake of **HIV** exposed premature, very- and extremely-low birth weight infants in Tygerberg hospital, Western Cape- Gut **Microbiome** and Human Milk **Oligosaccharide** composition.

#### **BIG HMO study**

#### REFERENCE NUMBER: N15/10/12

**PRINCIPAL INVESTIGATOR:** Dr. Evette Van Niekerk, Prof Ali Dhansay

**RESEARCHERS:** Klara Strydom, Thea Lodewyks

#### ADDRESS:

Division of Human Nutrition,  
Faculty of Medicine and Health Sciences,  
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Tygerberg, Stellenbosch University

**CONTACT NUMBERS:** +27 21 938 9474, +27 82 418 4906

#### 1. INVITATION

You are being invited to take part in a research project. Please take some time to read the information here, which will explain the what the project is about. Please ask the study staff any questions about any part of this project that you do not fully understand. It is very important that you are completely happy and that you clearly understand what this research is about and how you and your baby could be involved. Also, your participation is **entirely voluntary** and you are free to say that you do not want to take part in the study. If you say no, this will not affect you or your infant in any bad way whatsoever. You are also free to say at any time that you do not want to take part in the study anymore, even if you do agree to take part.

This study has been approved by the **Health Research Ethics Committee at Stellenbosch University** and will be performed according to the ethical guidelines and principles of the international Declaration of Helsinki, South African Guidelines for Good Clinical Practice and the Medical Research Council (MRC) Ethical Guidelines for Research.

BIGHMOSTUDY-ICF/VERSION 1!

## 2. WHAT IS THIS RESEARCH STUDY ALL ABOUT?

It is known that babies who are born prematurely (born early) grow differently to babies that are born at full term (born at the right age). This could mean that babies are not growing right for their age, resulting in a longer stay in hospital. Premature infants are difficult to feed. The type and quality of feeds can have an effect on how the baby grows. Growth is important for brain development and for the babies to reach general milestones (sitting, crawling, walking etc.). Preterm babies are known for having more total fat mass than babies who are born at the right age. This could mean that these premature babies stand a chance of getting or becoming: obese (very fat), diabetes (sugar) and heart problems when they are older.

Breast milk stimulates the growth of “good” germs in the gut or intestines of babies. Studies have shown that the “good” germs in breast milk can help the baby to grow better and prevent “bad” germs from making the baby sick and not grow properly. The live “good” germs that we want to look at are called Prebiotics. In this study we want to see how much of the “good” germs grow in the babies gut or intestine that are fed breast milk and if the amount of “good” germs has an effect on the overall growth of your baby.

Babies who are born very early are admitted to Ward G2 in Tygerberg Children’s Hospital. Very small premature babies who weigh less than 1200g, born to mothers who are HIV-positive or HIV-negative will take part in the study. With this study we want to find out the following:

- i. The growth and body composition (the percentage of fat, muscle, bone and water in the body) of the HIV-exposed and HIV-unexposed, premature babies.
- ii. The effect of the feeds (breastmilk, breastmilk with a supplement (FM85) and formula feed) that the babies get as food have on their growth and body composition.
- iii. The effect that the amount of time the HIV-infected mothers have been on HAART medication has on the growth and body composition of HIV-exposed, preterm and extremely low birth weight (ELBW;  $\leq 1200\text{g}$ ) babies.
- iv. To evaluate the effects of oligosaccharide composition on the babies good gut germs and the effects it has on growth and body composition.

### 2.1. How will you and your baby participate in the study?

- ✎ If your baby has a birth weight lower than  $\leq 1200\text{g}$  your baby will be admitted to Ward G2 while you will be admitted to Ward J5 or J2. We will visit you in the ward after the birth of your baby to explain the study to you and to invite you and your baby to take part in the study. We will give you a letter which explains the study and if you agree to take part, you must sign a consent form. Within a few days after the birth of your baby, you will be transferred to stay with your baby in ward G2. Once your baby is bigger, you and your baby will be transferred to ward G8 or J2 in Tygerberg Children’s Hospital where you will

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stay until your baby is ready to be transferred to another hospital where you will remain until your baby weighs more than 1800g and is ready to go home.

 Babies will be in the study for the first 28 days after birth.

## **2.2. What will happen to me if I do take part?**

Small premature babies are too weak to suckle on the breast. The mother has to express milk from her breast which is then given to the baby by means of a thin plastic tube that goes from the baby's mouth to the baby's stomach. The nurses in G2 baby ward will teach you how to express breast milk for your baby. As part of the study, we want to measure the quality of some of the good components in your breast milk. The most important one is oligosaccharide which also helps to protect your baby against infection. We will therefore need a 2ml sample of your breast milk at specific times.

The first milk sample we will need from you is on day 1 of life, then on day 7,14, 21, 28 of life. These 5 milk samples will be collected in a sterile container and frozen before being tested in the laboratory.

Information from your hospital file will be used in the study such as how your baby was delivered, what medications you received during your pregnancy, etc. All pregnant women in the Western Cape are tested for HIV during pregnancy. As it is important for us to know your HIV status, we will obtain your HIV status from your hospital folder.

## **2.3. What will happen to the baby if I give consent?**

Breast milk allows "good" bacteria to grow in your infants guts and intestines. As part of the study, we want to measure the quality of some of the "good" bacteria that grows in baby's gut and intestines by measuring the amount "good" bacteria in the stool. The "good" bacteria helps to protect your baby against infection and helps them to grow properly. We will therefore need a 1g sample of your babies stool at specific times.

The first stool sample we will need from your baby is the first stool on day 1 of life, then on day 7,14, 21, 28 of life. These 5 stool samples will be collected in a sterile container and frozen before being tested in the laboratory.

Your baby's folder will be read by the researcher every day and the results of the laboratory tests (blood tests etc.), feeds and medication the baby receives will be written down. Small babies are weighed every day to see if they are getting enough feeds and growing properly. Your baby will be weighed daily by the nursing staff. On a weekly basis the following will also be done:

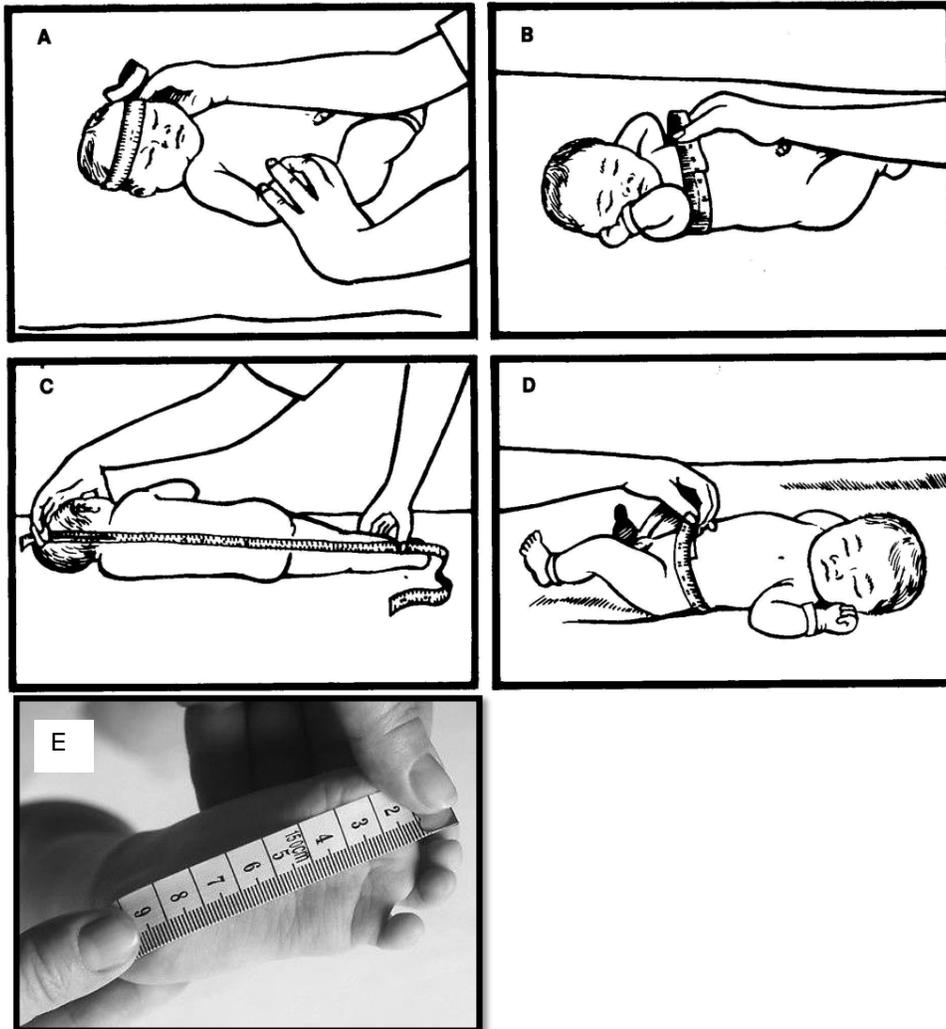
- i. Please refer to **figure 1** for the following measurements that will be taken on your baby:

**A:** Head circumference

**B and D:** Cylindrical circumferences

**C:** Length

**E:** Foot length



**Figure 1:** Measurements that will be taken of your baby

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- ii. Skinfold thickness: Only the fat layer under your skin is picked up, at different parts of the body, and it is precisely measured using a calliper to see how much fat makes up your baby's body. Refer to **figure 2**.



**Figure 2:** Skinfold thickness

### **3. WHY HAVE YOU BEEN INVITED TO PARTICIPATE?**

You have been chosen because you gave birth to a very small baby, that was born too early and was admitted to ward G2 at Tygerberg Children's Hospital (TCH).

### **4. WILL YOU BENEFIT FROM TAKING PART IN THIS RESEARCH?**

You will benefit from knowing that you were an important contributor in giving the medical team (Doctors, nurses, dietitians and researchers) important information about the growth, body composition and nutritional intake (feeds) of VLBW and ELBW premature babies.

### **5. ARE THERE ANY RISKS OR DISCOMFORTS INVOLVED IN YOUR TAKING PART IN THIS RESEARCH?**

There are no risks involved if you take part in this study. Your baby will be treated exactly the same as any of the other small babies admitted to ward G2 at Tygerberg Children's Hospital. All the measurements that will be done has been proven to be safe by other studies. Your baby might be a bit uncomfortable when moved to take the measurements but all the measurements taken are safe and the researchers will not hurt your baby. The researchers have received the right training to measure your baby.

### **6. IF YOU DO NOT AGREE TO TAKE PART, WHAT ALTERNATIVES DO YOU HAVE?**

It is your choice whether you would like you and your baby to take part in this study or not. If you decide not to take part in the study it will not affect the care given to you or your baby. Additionally you are allowed to say, at any point without giving a reason, that you do not want yourself and your baby to take part in the study. Your and your baby's routine health care will not be negatively affected.

#### **7. WHO WILL HAVE ACCESS TO YOUR MEDICAL RECORDS?**

Only the researcher, research assistant and persons directly involved with the study will have access to your and your baby's medical records. All information will always be kept confidential and private. The researcher and research assistant will treat all information collected as strictly confidential and private and no information that can identify you or your baby will be given to any person who is not directly involved with the study.

#### **8. WHAT WILL HAPPEN IN THE UNLIKELY EVENT OF SOME FORM OF INJURY OCCURRING AS A DIRECT RESULT OF YOUR TAKING PART IN THIS RESEARCH STUDY?**

It is very rare that something will go wrong. Throughout the study common methods will be used, therefore making the chances that something goes wrong, very small.

#### **9. WILL YOU BE PAID TO TAKE PART IN THIS STUDY AND ARE THERE ANY COSTS INVOLVED?**

No, you will not be paid to take part in the study. You will also not have to pay for anything, if you do take part in the study.

#### **10. IS THERE ANYTHING ELSE THAT YOU SHOULD KNOW OR DO?**

-  You can contact the principal investigator or researchers at [+27 21 938 9474](tel:+27219389474), [+27 82 418 4906](tel:+27824184906) if you have any further queries or encounter any problems.
-  You can contact the Health Research Ethics Committee at [021-938 9207](tel:0219389207) if you have any worries or complaints that have not been properly addressed by the researcher.
-  You will receive a copy of this information and consent form for your own records.

#### **11. INSURANCE**

This study does have insurance, for a period from 1<sup>st</sup> January 2016 - 31<sup>st</sup> December 2016, for the unlikely event of something going wrong during the study.

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**Declaration by participant**

By signing below, I ..... agree to take part in a research study entitled **Growth, body composition and nutritional intake of HIV exposed premature, very- and extremely-low birth weight infants in Tygerberg hospital, Western Cape..**

I declare that:

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

Signed at (place) ..... on (date) ..... 2015.

.....

**Signature of participant**

.....

**Signature of witness**

**Declaration by investigator**

I (name) ..... declare that:

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

Signed at (place) ..... on (date) ..... 2015.

.....

**Signature of investigator**

.....

**Signature of witness**

**BIG\$HMO\$TUDY-ICF/\$VERSION\$ 7!**

**Declaration by interpreter**

I (name) ..... declare that:

- I assisted the investigator (name) ..... to explain the information in this document to (name of participant) ..... using the language medium of Afrikaans/Xhosa.
- We encouraged him/her to ask questions and took adequate time to answer them.
- I conveyed a factually correct version of what was related to me.
- I am satisfied that the participant fully understands the content of this informed consent document and has had all his/her question satisfactorily answered.

Signed at (place) ..... on (date) .....(2015).

.....  
**Signature of interpreter**

.....  
**Signature of witness**

## DEELNEMERINLIGTINGSBLAD EN -TOESTEMMINGSVORM

### TITEL VAN DIE NAVORSINGSPROJEK:

**Liggaamsamestelling, Groei** en voedingsinname van **MIV** blootgestelde premature, lae geboorte gewig babas in Tygerberg Hospitaal, Wes-Kaap. Derm **Mikrobiome** en Menslike Melk **Oligosakkaried** samestelling.

### BIG HMO studie

**VERWYSINGSNOMMER: N15/10/12**

**HOOFNAVORSER:** Dr. Evette Van Niekerk, Prof Ali Dhansay

**Navorsers:** Klara Strydom, Thea Lodewyks

### ADRES:

Afdeling van Menslike Voeding,  
Fakulteit van Gesondheidswetenskappe,  
Tygerberg kampus  
Derde vloer  
Tygerberg, Stellenbosch Universiteit

**KONTAKNOMMER:** +27 21 938 9474, +27 82 418 4906

## 1. UITNODIGING

Jy word genooi om deel te neem aan 'n navorsingsprojek. Lees asseblief hierdie inligtingsblad op jou tyd deur aangesien die detail van die navorsingsprojek hierin verduidelik word. As daar enige deel van die navorsingsprojek is wat jy nie heeltemal verstaan nie, is jy welkom om die navorsingspersoneel of dokter daarvoor te vra. Dit is baie belangrik dat jy heeltemal moet verstaan waaroor die navorsingsprojek gaan en hoe jy en jou baba daarby betrokke kan wees. Jou deelname is jou keuse en dit is ook jou besluit as jy wil nie sê. Jy sal op geen manier sleg beïnvloed word as jy sou weier om deel te neem aan die studie nie. Jy mag ook op enige stadium van die navorsingsprojek onttrek, selfs al het jy nou ja gesê.

**Hierdie navorsingsprojek is deur die** Gesondheidsnavorsingsetiekkomitee (GNEK) van die Universiteit Stellenbosch **goedgekeur en sal gedoen word volgens die etiese riglyne en beginsels van die Internasionale Verklaring van Helsinki en die Etiese Riglyne vir Navorsing van die Mediese Navorsingsraad (MNR).**

## 2. WAT BEHELS HIERDIE NAVORSINGSPROJEK?

BIG HMO STUDIE-ITV/SWEERGAWES 1!

Dit is bekend dat premature babas (vroeg gebore word) anders groei as babas wat volterm gebore (gebore op die regte tyd) word. Dit kan tot gevolg hê dat hierdie babas nie reg groei vir hulle ouderdom nie en dus langer in die hospitaal moet bly. Premature babas is ook moeilik om te voed. Die tipe en kwaliteit van voedings kan 'n verskil maak aan die groei van die baba. Groei is belangrik vir brein ontwikkeling en om algemene mylpale te bereik (soos sit, kruip, stap ens). Premature babas is geneig om meer totale vet massa te hê as babas wat op die regte tyd gebore is en dit kan lei tot obesiteit (baie vet), diabetes (suiker) en ook hart probleme as hulle ouer is.

Borsmelk stimuleer die groei van "goeie" kieme in die derm of ingewande van babas. Studies het getoon dat die "goeie" kieme in borsmelk die baba kan help om beter te groei en te keer dat "slegte" kieme die baba siek maak wat kan veroorsaak dat die baba nie goed groei nie. Hierdie lewende "goeie" kieme waarna ons wil kyk word Prebiotika genoem. In hierdie studie wil ons sien die hoeveelheid van "goeie" kieme in die derm of ingewande van babas wat slegs borsmelk ontvang en ons wil ook kyk of die hoeveelheid "goeie" kieme 'n effek sal he aan die algemene groei van jou baba.

Babas wat vroeg gebore word word opgeneem in Saal G2 in Tygerberg Kinder hospitaal. Baie klein premature babas wat minder as 1200g weeg en wie se moeders MIV+ of MIV- is gaan deelneem aan hierdie studie. Met hierdie studie wil ons die volgende uitvind word:

- i. Die groei en liggaamsamestelling (persentasie vet, spier, been en water in die lyf) van MIV-blootgestelde en MIV-nie-blootgestelde premature babas.
- ii. Die effek van die voedings (borsmelk, borsmelk met supplement (FM85) en formule) wat hierdie babas kry op hulle groei en liggaamsamestelling.
- iii. Die effek van die tydperk (hoe lank) die MIV-postiewe mamma op HAART behandelings is, op die groei en liggaamsamestelling van MIV-blootgestelde, premature, BLGM (kleiner as  $\leq 1200g$ ) babas.
- iv. Die effek van oligosakkaried samestelling op die baba se goeie kieme in die derm te evalueer asook die effek wat dit het op die groei en liggaam samestelling van die baba..

### **2.1. Hoe gaan ek en my baba deelneem in die studie?**

- As jou baba 'n geboorte gewig het van minder as  $\leq 1200g$  gaan jou baba opgeneem word in saal G2 terwyl jy opgeneem gaan word in saal J5 of J2. Ons sal jou besoek in die saal nadat jou baba gebore is om die studie aan jou te verduidelik en jou en jou baba uit te nooi om aan die studie deel te neem. Ons sal vir jou 'n brief gee wat die studie verduidelik en as jy instem om deel te neem, moet jy hierdie brief teken. Binne 'n paar dae nadat jou baba gebore is sal jy

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geskuif word na saal G2 om by jou baba te wees. Sodra jou baba groter is gaan jy en jou baba geskuif word na saal G8 or J2 in die Tygerberg Kinderhospitaal. Daar sal julle bly totdat jou baba gereed is om na 'n ander hospitaal oorgeplaas te word. Julle sal gereed wees om huis toe te gaan as jou baba meer as 1800g weeg.

Babas sal deel wees van hierdie studie vir die eerste 28 dae na geboorte.

## **2.2. Wat gaan met my gebeur as ek aan die studie deel neem?**

Klein premature babas is te swak om te drink aan die bors. Die ma moet haar bors uitmelk en die melk word dan aan die baba gegee deur middel van 'n dun plastiese pyp wat strek vanaf die baba se mond na die baba se maag. Die verpleegsters in Saal G2 sal jou leer om jou borsmelk uit te melk vir jou baba. As deel van die studie, wil ons die gehalte van sommige van die goeie komponente in jou borsmelk meet. Die belangrikste een is oligosakkariede wat ook help om jou baba te beskerm teen infeksies. Ons sal dus 'n 2ml monster van jou bors melk benodig op spesifieke tye.

Die eerste melk monster word van jou benodig op dag 1 van die laktasie, dan op dag 7, 14, 21 en weer op dag 28 van die baba se lewe. Hierdie 5 melkmonsters sal geneem word in 'n steriele houer en gevries word voordat dit getoets word in die laboratorium.

Inligting uit jou hospitaal lêr gaan gebruik word soos hoe jou baba gebore is, watter medisyne jy gekry het gedurende swangerskap ens. Alle swanger vroue in die Wes-Kaap word getoets vir MIV tydens swangerskap. Omdat dit vir ons belangrik is om jou MIV status te weet, sal ons dit ook uit jou hospitaal lêr kry.

## **2.3. Wat gaan met my baba gebeur as ek toestemming gee?**

Borsmelk stimuleer die groei van "goeie" kieme in die derm of ingewande van babas.

As deel van die studie, wil ons die gehalte van sommige van die "goeie" kieme in jou baba se stoelgang meet. Die "goeie" kieme beskerm jou baba teen infeksies en help hulle om te groei. Ons sal dus 'n 1g monster van jou baba se stoelgang benodig op spesifieke tye. Die eerste stoelgangmonster wat van jou baba benodig sal wees is die heel eerste stoelgang op dag 1 van die baba lewe, dan op dag 7, 14, 21 en weer op dag 28 van die baba se lewe. Hierdie 5 stoelgangmonsters sal geneem word in 'n steriele houer en gevries word voordat dit getoets word in die laboratorium.

Jou baba se lêr gaan elke dag deur die navorser gelees word en resultate van laboratorium toetse (bloed toetse ens), voedings en die medisyne wat jou baba kry gaan neergeskryf word. Klein babas word elke dag geweeg om te sien of hulle genoeg melk kry en goed genoeg groei.

**BIG\$HMO\$STUDIE-ITV/\$WEERGAWES\$ 3!**

Jou baba gaan elke dag geweeg word deur die verpleeg personeel. Op 'n weeklikse basis gaan daar ook die volgende gedoen word:

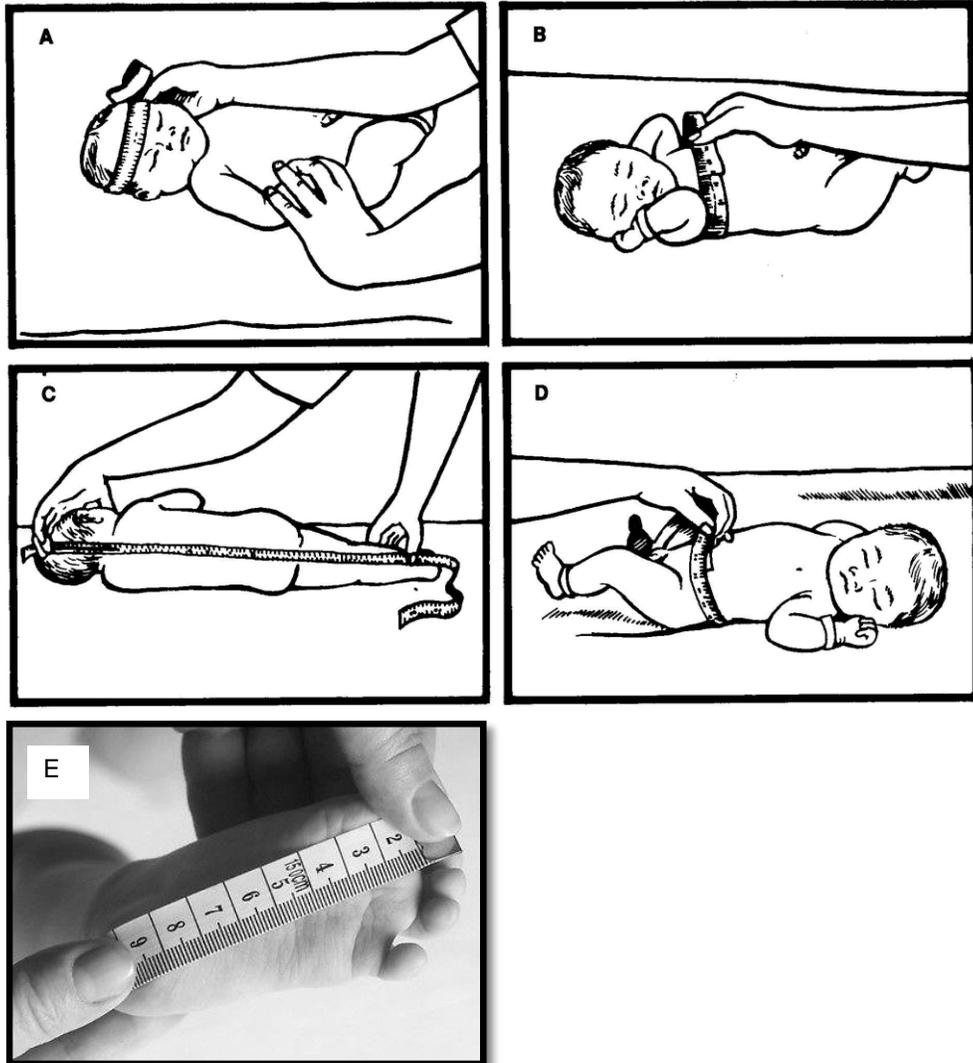
i. Kyk asseblief na **figuur 1** om te sien watter metings op jou baba gedoen gaan word:

**A:** kop omtrek

**B en D:** silindriese omtreкке (Bv. Boarm omtrek ens.)

**C:** Baba se lengte

**E:** Voet lengte



**Figuur 1:** Metings wat op jou baba gedoen gaan word

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- ii. Velvou dikte: net die vet lagie onder jou baba se vel word opgelig, op verskillende plekke op sy/haar lyf en dit word gemeet deur 'n kaliper te gebruik. Die doel van die meting is om te sien hoeveel vet jou baba se liggaam het. (Sien **figuur 2**)



**Figuur 2:** Velvou dikte

### **3. HOEKOM IS EK GEVRA OM DEEL TE NEEM?**

Jy is gekies om deel te neem aan die studie omdat jy 'n baie klein baba, wat vroeg gebore is gehad het en opgeneem is in Saal G2 in die Tygerberg Kinder Hospitaal (TKH).

### **4. SAL U VOORDEEL TREK DEUR DEEL TE NEEM AAN HIERDIE NAVORSINGSPROJEK?**

Jy gaan voordeel trek uit die wete dat jy 'n belangrike bydra gemaak deur vir die mediese span (dokters, verpleegsters, dieetkundiges en navorsers) belangrike inligting te gee oor die groei, liggaamsamestelling en voedingsinname (voedings) van baie lae gewig babas.

### **5. IS DAAR ENIGE RISIKO'S VERBONDE AAN U DEELNAME AAN HIERDIE NAVORSINGSPROJEK?**

Daar is geen risiko's verbonde as jy sou kies om aan hierdie studie deel te neem nie. Jou baba gaan presies dieselfde hanteer word as enige ander klein baba wat opgeneem word in Saal G2 in die Tygerberg Kinder Hospitaal. Al die metings wat geneem gaan word is bewys as veilig deur ander studies. Jou baba mag dalk 'n bietjie ongemaklik wees wanneer hy/sy beweeg word om die metings te neem, maar al die metings is veilig en die navorsers sal nie jou baba seermaak nie. Die navorsers het die regte opleiding gekry om jou baba te kan meet.

### **6. WATTER ALTERNATIEWE IS DAAR INDIEN U NIE INSTEM OM DEEL TE NEEM NIE?**

Dit is jou keuse of jy en jou baba wil deel neem of nie. As jy besluit om nie deel te neem nie gaan dit nie beïnvloed hoe goed daar na jou en jou baba gekyk word nie. Verder is jy welkom om enige tyd in die studie te sê, sonder 'n verduideliking, dat jy en jou baba nie meer wil deelneem nie. Jou en jou baba se gesondheidsorg gaan nie negatief beïnvloed word nie.

**BIG\$HMO\$STUDIE-ITV/\$WEERGAWES 5!**

## 7. WIE SAL TOEGANG HÊ TOT U MEDIESE REKORDS?

Net die navorser, navorsingsassistent en persone wat direk betrokke is met die studie sal toegang hê tot jou en jou baba se mediese rekords. Alle inligting sal altyd konfidensieël en privaat gehou word. Die navorser en navorsingsassistent sal alle inligting wat uit jou leër geneem is streng konfidensieël en privaat hanteer en jou of jou baba sal nie geïdentifiseer kan word op grond van die inligting nie. Die inligting sal ook nie beskikbaar wees vir enige persoon wat nie direk betrokke is met die studie nie.

## 8. WAT SAL GEBEUR IN DIE ONWAARSKYNLIKE GEVAL VAN 'N BESERING WAT MAG VOORKOM AS GEVOLG VAN U DEELNAME AAN HIERDIE NAVORSINGSPROJEK?

Die kans is baie klein dat iets verkeerd sal gaan. Deur die hele studie gaan algemene metodes gebruik word wat dus die kans dat iets verkeerd kan gaan baie klein maak. Al is die kans vir beserings so klein, is die studie en die navorsers gedek deur versekering.

## 9. SAL U BETAAL WORD VIR DEELNAME AAN DIE NAVORSINGSPROJEK EN IS DAAR ENIGE KOSTE VERBONDE AAN DEELNAME?

Jy sal nie betaal word om deel te neem aan die studie nie. Jy hoef ook nie te betaal om deel te neem nie. .

## 10. IS DAAR ENIGIETS ANDERS WAT U MOET WEET OF DOEN?

- Jy kan die hoofnavorser kontak by +27 21 938 9474, +27 82 418 4906 indien jy enige verdere vrae het of enige probleme ondervind.
- Jy kan die **Gesondheidsnavorsingsetiek administrasie** kontak by 021-938 9207 indien jy oor enige iets bekommerd is of 'n klagte het wat nie goed genoeg deur jou navorser hanteer is nie.
- Jy sal 'n afskrif van hierdie inligtings- en toestemmingsvorm ontvang vir jou eie rekords.

## 11. VERSEKERING

Hierdie studie het versekering, vir die tydperk van 1 Januarie 2016 tot 31 Desember 2016, vir die onwaarskynlike situasie wat daar iets verkeerd gaan in die studie.

### Verklaring deur deelnemer

Met die ondertekening van hierdie dokument onderneem ek, ....., om deel te neem aan 'n navorsingsprojek getiteld **Groei, liggaamsamestelling en voedingsinname van MIV blootgestelde premature, lae geboorte gewig babas in Tygerberg Hospitaal, Wes-Kaap.**

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**Ek verklaar dat:**

- Ek hierdie inligtings- en toestemmingsvorm gelees het of aan my laat voorlees het en dat dit in 'n taal geskryf is wat ek verstaan en gemaklik mee is.
- Ek geleentheid gehad het om vrae te stel en dat al my vrae goed beantwoord is.
- Ek verstaan dat deelname aan hierdie navorsingsprojek **my keuse** is en dat daar geen druk op my geplaas is om deel te neem nie.
- Ek te eniger tyd aan die navorsingsprojek mag onttrek en dat ek nie op enige wyse daardeur benadeel sal word nie.
- Ek gevra mag word om van die navorsingsprojek te onttrek voordat dit afgehandel is indien die navorser van opinie is dat dit in my beste belang is, of indien ek nie die ooreengekome navorsingsplan volg nie.

Geteken te (*plek*) ..... op (*datum*) ..... 2016.

.....  
**Handtekening van deelnemer**

.....  
**Handtekening van getuie**

**Verklaring deur navorser**

Ek (*naam*) ..... verklaar dat:

- Ek die inligting in hierdie dokument verduidelik het aan .....
- Ek hom/haar aangemoedig het om vrae te vra en voldoende tyd gebruik het om dit te beantwoord.
- Ek tevrede is dat hy/sy al die aspekte van die navorsingsprojek soos hierbo bespreek, voldoende verstaan.
- Ek 'n tolk gebruik het/nie 'n tolk gebruik het nie. (*Indien 'n tolk gebruik is, moet die tolk die onderstaande verklaring teken.*)

Geteken te (*plek*) ..... op (*datum*) ..... 2016.

**BIG\$HMO\$STUDIE-ITV/\$WEERGAWES\$ 7!**

.....  
**Handtekening van navorder**

.....  
**Handtekening van getuie**

**Verklaring deur tolk**

Ek (*naam*) ..... verklaar dat:

- Ek die navorser (*naam*) ..... bygestaan het om die inligting in hierdie dokument in Afrikaans/Xhosa aan (*naam van deelnemer*) ..... te verduidelik.
- Ons hom/haar aangemoedig het om vrae te vra en voldoende tyd gebruik het om dit te beantwoord.
- Ek 'n feitelik korrekte weergawe oorgedra het van wat aan my vertel is.
- Ek tevrede is dat die deelnemer die inhoud van hierdie dokument ten volle verstaan en dat al sy/haar vrae bevredigend beantwoord is.

Geteken te (*plek*) ..... op (*datum*) ..... 2016.

.....  
**Handtekening van tolk**

.....  
**Handtekening van getuie**

## **INCWADANA ENGOLWAZI NGOMTHATHI-NXAXHEBA KUNYE NEFOMU YEMVUMELWANO**

### **ISIHLOKO SEPROJEKTHI YOPHANDO:**

**Ukukhula, okuqulathwe ngumzimba nendlela ezitya ngayo iintsana ezizelwe phambi kwexesha ebezikwindawo enabantu abaneNtsholongwane kaGawulayo, aiintsana ezincinci kakhulu ngokwasemzimbeni ukuzalwa kwazo kwisibhedlele saseTygerberg, eNtshona Koloni – Uhlobo lweGut **Microbiome** nook kufumaneka kwiikhabhohayidreyithi ezifumaneka kuBisi loMntu (**Oligosaccharide**).**

### **Uphando lweBIG HMO**

### **INOMBOLO YONXULUMANO: N15/10/12**

**UMPHANDI OYINTLOKO:** UGqr. Evette Van Niekerk, uNjing Ali Dhansay

**RESEARCHERS:** UKlara Strydom, UThea Lodewyks

### **IDILESI:**

Icandelo loKutya kwaBantu

Isebe leeNzuluwazi ngezoNyango nezeMpilo

Ikhampasi yaseTygerberg

Umgangatho wesithathu

Iyunivesithi yaseStellenbosch, eTygerberg

**IINOMBOLO ZOQHAGAMSHELWANO:** +27 21 938 9474, +27 82 418 4906

## **1. ISIMEMO**

Uyamenywa ukuba athathe inxaxheba kwiprojekthi yophando. Nceda thatha ixesha lokufunda ulwazi oluvezwe apha, oluzakuthi luchaze iinkcukacha zale projekthi. Nceda buza nayiphina imibuzo emalunga nayiphina indawo ongayiqondiyo ngokupheleleyo kubasebenzi besi sifundo okanye kugqirha. Kubaluleke kakhulu ukuba waniliseke ngokupheleleyo yinto yokuba ucacelwe kakuhle ukuba yintoni ebangwa sesi sifundo kwaye ungabandakanyeka njani. Kwakhona, ukuthatha kwakho inxaxheba **ukwenza ngokuzithandela unganyanzelwa** kwaye ukhululekile ukuba ungarhoxa ekuthatheni inxaxheba. Ukuba uthi hayi, oku akusayi kuchaphazela ukungavumi kwakho nangayiphina indlela. Ukwakhululekile ukuba uyeke kwesi sifundo, nkqu nokokuba uyavuma ukuthatha inxaxheba.

Olu phando luvunywe ziinqobo ezisesikweni **zeKomiti yoPhando Lomntu kwiYunivesithi yaseStellenbosch** kwaye luza kwenziwa ngokwemigaqo esesikweni lophando elamkelekileyo kwiSaziso sehlabathi sika-Helsinki, iMigaqo eLungileyo yoMzantsi Afrika yokuSebenza eKliniki kunye neBhunga lezoPhando ngamaYeza (MRC) iMigaqo yeNqobo yezoPhando.

## 2. SIMALUNGA NANTONI ESI SIFUNDO SOPHANDO?

Kuyazeka ukuba iintsana ezizalwa phambi kwexesha zikhula ngendlela eyahlukileyo kwiintsana ezizalwa ngexesha elifanelekileyo. Oku kungathetha ukuba iintsana azikhuli ngendlela efanele iminyaka yazo, oko kubangele ukuba zihlale ixesha elide esibhedlele. Iimveku eziselwe phambi kwexesha kunzima ukuzondla. Uhlobo nomgangatho woko zikutyayo kungayichaphazela indlela indlela ezikhula ngayo iintsana. Ukukhula kubalulekile ukuze nengqondo ikhule kwaye zikwazi iintsana ukufikelele kwiindlela zokukhula gabalala (ukuhlala, ukukhasa, ukuhamba njlnj). Iintsana zaphambi kwexesha zaziwa ngokuba namafutsha amaninzi kuneentsana ezizalwa ngexesha elifanelekileyo. Oku kungathetha ukuba ezi ntsana zizelwe phambi kwexesha zingatyebe ngaphaya kokuba kufanelekile (zityebe kakhulu), zibenesifo seswekile neengxaki zentliziyo xa zindala.

Ubisi lwebele luvuselela ukukhula kweentsholongwane “ezilungileyo” kumetyiso okanye kumathumbu osana. Uphando lubonakalise ukuba iintsholongwane “ezilungileyo” ezikubisi lwebele zingalunceda usana lukhule ngcono kwaye lukhusela iintsholongwane “ezingalunganga” ukuba zingalugulisi usana naxa lungakhuli ngokufanelekileyo. Iintsholongwane “ezilungileyo” eziphilayo esifuna ukuzijonga zibizwa ngokuba ziiPrebiotics. Kolu phando sifuna ukujonga ukuba zikhula kangakanani na iintsholongwane “ezilungileyo” kumetyiso okanye kumathumbu eentsana ezifumana ubisi lwebele nokuba ubungakanani beentsholongwane “ezilungileyo” buneempembelelo ekukhuleni jikelele kosana lwakho.

Iintsana ezizalwa msinya kakhulu ziye zilaliswe kwaWadi G2 kwiSibhedlele saBantwana eTygerberg. Iintsana ezincinci kakhulu (ezizelwe phambi kwexesha) zinobukhulu obungaphantsi kwe-1200g, zizalwa ngoomama abaneNtsholongwane kaGawulayo okanye abangenayo iNtsholongwane kaGawulayo ziza kuthatha inxaxheba kolu phononongo. Ngolu phononongo sifuna ukufumanisa oku kulandelayo:

- i. Ukukhula noko kukhoyo emzimbeni (ipesenti yamafutha, izihlunu, amathambo namanzi asemzimbeni) kwiintsana ezizelwe phambi kwexesha zabo baneNtsholongwane kaGawulayo nabangenayo.
- ii. Iimpembelelo zokutya (ubisi lwebele, ubisi lwebele oluhamba nezinye izongezeleli zondlo (FM85) nobisi lwebhotile) okutyiwa ziintsana ezibangela ukukhula noko kusemizimbeni yazo.
- iii. Iimpembelelo zexesha oomama abachatshazelwe yiNtsholongwane kaGawulayo besebenzisa amayeza e-HAART ekukhuleni noko kusemzimbeni wosana oluchaphazeleke yiNtsholongwane kaGawulayo, ukuzalwa phambi kwexesha, neentsana ezincinci kakhulu emzimbeni xa zizalwa (ELBW; zingaphantsi kwe-1200g).
- iv. Ukuvavanya iimpembelelo zoko kukhoyo kwiikhabhohayidreyithi (oligosaccharide) ezikwiintsholongwane “ezilungileyo” ezikumetyiso wosana neempembelelo zoko ekukhuleni kwalo nakwizinto ezikhoyo kumzimba walo.

### **3. Niza kuyithatha njani inxaxheba wena nosana lwakho kolu phononongo?**

- ✍ Ukuba usana lwakho linobunzima bomzimba ongaphantsi kwe-1200g usana lwakho luza kulaliswa kwaWadi G2 ngeli xesha uza kulaliswa kwaWadi J5 okanye J2. Siza kuya kuwe ewadini emva kokubeleka kwakho usana lwakho sikucacisele ngophononongo kwaye sikucele wena nosana lwakho ukuba nithathe inxaxheba kuphononongo. Siza kukunika ileta ekucacisela ngophononongo kwaye ukuba uyavuma ukuthatha inxaxheba, kufuneka utyikitye ifomu yesivumelwano. Kwiintsuku nje ezimbalwa emva kokuzalwa kosana lwakho, uza kuthunyelwa uyohlala nosana lwakho kwawadi G2. Xa usana lwakho lulukhulu, wena nosana lwakho niza kusiwa kwawadi G8 okanye J2 kwiSibhedlele saBantwana eTygerberg apho uza kuhlala lude usana lwakho lukulungele ukusiwa kwesinye isibhedlele apho uza kuhlala khona lude usana lwakho lubenobukhulu obungaphezu kwe-1800g kwaye lulungele ukugoduka.
- ✍ lintsana ziza kuba kolu phononongo kwiintsuku ezingama-28 emva kokuba zizelwe.

### **4. Kuza kwenzeka ntoni kum ukuba ndithatha inxaxheba?**

lintsana ezincinci ezizelwe phambi kwexesha zibuthathaka kakhulu ukuba zinganxanxa zifunxe ibele. Umama kufuneka alicudise ibele lakhe akhuphe ubisi oluye lunikwe usana ngombhobho weplastiki obhityileyo osuka kumlomo wosana usiya kwisisu sosana. Oonesi abakwiwadi labantwana i-G2 baza kukufundisa ukuba ulucudisa njani ubisi ebeleni usenzela usana. Njengenxalenye yophando, sifuna ukuthelekisa umgangatho wokunye okulungileyo okufumaneka kubisi lwakho lwebele. Eyona ibalulekileyo yi-oligosaccharride ekwanceda ukukhusela usana lwakho lungosuleleki zizifo. Ngoko ke siza kufuna isampuli ye-2ml kubisi lwakho lwebele ngamaxesha athile.

Isampuli yokuqala yobisi esiza kuyifuna kuwe iza kuba yeyosuku loku-1, yosuku lwesi-7. Lwe-14, lwama-21, lwama-28 luzelwe. Ezi sampuli zi-5 zobisi ziza kuqokelelwa zifakwe kwinkonkxa enezicoci necocekileyo ifakwe kwisikhenkcezisi phambi kokuba luhlolwe elebhu.

linkcukacha ezifumaneka kwifayile yasesibhedlele ziza kusetyenziswa kuphononongo ezifana nokuba ubeleke njani, ngawaphi amayeza owafumeneyo ngoku ubukhulelwe, njlnjl. Bonke ababhinqileyo abakhulelweyo eNtshona Koloni bayahlolwa ukuba abanayo na iNtsholongwane kaGawulayo, siza kuyifumana imeko yakho kaGawulayo kwifayile yakho yasesibhedlele.

### **5. Kuza kwenzeka kusana xa ndinika imvume yam?**

Ubisi lwebele luvumela iintsholongwane “ezilungileyo” zikhule kumetyiso okanye kumathumbu osana lwakho. Njengenxalenye yophando, sifuna ukuthelekisa umgangatho wezinye iintsholongwane “ezilungileyo ezikhulayo kumetyiso okanye kumathumbu osana lwakho ngokujonga umlinganiselo wobungakanani beentsholongwane “ezilungileyo” kwilindle lakhe. Iintsholongwane

“ezilungileyo” zanceda ukukhusela usana lwakho ekosulelekeni zizifo kuzancede zikhule ngendlela efanelekileyo. Ngoko ke siza kufuna isampuli ye-1g yelindle losana lwakho ngamaxesha athile. Isampuli yelindle yokuqala esiza kuyifuna kusana lwakho lilindle lokuqala losuku lokuqala lwesi-7. Lwe-14, lwama-21, lwama-28 luzelwe. Ezi sampuli zi-5 zobisi ziza kuqokelelwa zifakwe kwinkonkxa enezicoci necocekileyo ifakwe kwisikhenkcezisi phambi kokuba luhlolwe elebhu.

Ifayile yosana lwakho iza kufundwa ngumphandi yonke imihla kwaye iziphumo zohlolo lwaselebhu (uhlolo lwegazi njlnjl.), ukutya namayeza afunyanwa lusana lwakho aza kubhalwa phantsi. Iintsana ezincinci ziza kubekwa esikalini yonke imihla kujongwe ukuba ingaba zifumana ukutya okwaneleyo na kwaye zikhula ngokufanelekileyo na. usana lwakho luza kubekwa esikalini ngamanesi. Rhoqo ngeveki kuza kwenziwa oku kulandelayo:

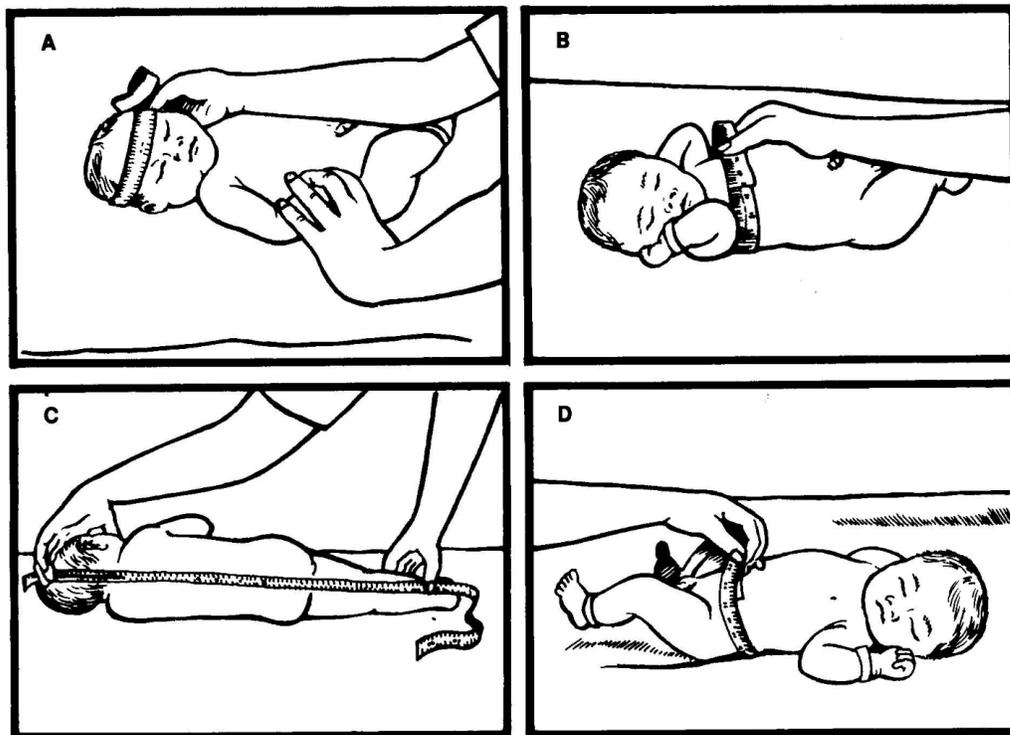
- i. Nceda ujonge **iSazobe 1** kule milinganiselo ilandelayo eza kujongwa kusana lwakho:

**A:** Ububanzi bentloko

**U-B no-D:** Umlinganiselo ojonga ubude nobubanzi

**C:** Ubude

**E:** Ubude bonyawo



**Isazobe 1:** Umlinganiselo oza kuthathwa kusana lwakho

- ii. Ubukhulu kububanzi besikhumba: Ngumaleko wamanqathe kuphela phantsi kofele ojongwayo, kwiindawo ezahlukeneyo emzimbeni, kwayo oko kujongwa ngqo umlinganiselo

kusetyenziswa isixhobo esibizwa ngokuba yi-calliper ukujonga ubungakanani bamanqathe akhoyo emzimbeni wosana lwakho. Jonga **kwisazobe 2**.



**Isazobe 2:** Ubukhulu kububanzi besikhumba

#### **6. KUTHENI UMENYIWE UKUBA UTHATHE INXAXHEBA?**

Uye wakhethwa kuba ubeleke usana oluncinci kakhulu, oluzelwe phambi kwexesha kakhulu lwaze lwalaliswa kwawadi G2 kwiSibhedlele saBantwana saseTygerberg (TCH).

#### **7. INGABA UZA KUZUZA EKUTHATHENI INXAXHEBA KOLU PHANDO?**

Uza kuxhamla ngokwazi ukuba ube negalelo elibalulekileyo ngokunika iqela labonyango (ooGqirha, amanesi, iingcali ngokutya nabaphandi) iinkcukacha ezibalulekileyo malunga nokukhula, okuqulathwe ngumzimba nendlela ezitya ngayo iintsana ezizelwe phambi kwexesha zeVLBW ne-ELBW.

#### **8. INGABA ZIKHO IINGOZI EZIBANDAKANYEKAYO EKUTHATHENI KWAKHO INXAXHEBA KOLU PHANDO?**

Akukho mngcipheko okhoyo ngokuthatha kwakho inxaxheba kolu phononongo. Usana lwakho luza kuphatheka ngendlela enye efanayo neyezinye iintsana ezilaliswa kwawadi G2 kwiSibhedlele saBantwana saseTygerberg. Konke ukuthathwa komlinganiselo nobunzima okuza kwenziwa kuqinisekisiwe ukuba kukhusselekile lolunye uphononongo. Usana lwakho lungangaphatheki kakuhle xa luyothatha imilinganiselo kodwa yonke imilinganiselo ethathwayo ikhuselekile kwaye abaphandi soze balwenzakalise usana lwakho. Abaphandi bafumene uqeqesho olufanelekileyo lokuthatha umlinganiselo kusana lwakho.

#### **9. UKUBA AWUVUMI UKUTHATHA INXAXHEBA, LOLUPHI OLUNYE UNYANGO ONALO?**

Nguwe onokuzikhethela ukuba ingaba wena nomntwana wakho niyafuna na ukuthatha inxaxheba kolu phononongo. Ukuba ugqiba ukuthatha inxaxheba kuphononongo oko akuzokuchaphazela wena nosana lwakho. Okunye, uvumelekile, nanini na nokuba awunasizathu, ukuba awufuni wena nomntwana wakho ukuthatha inxaxheba kuphononongo. Indlela oqhele ukukhathalelwa ngayo kwezempilo wena nosana lwakho ayizi kuchaphazeleka kakubi.

**10. NGUBANI UZA KUFUMANA INGXELO YAKHO YAMAYEZA?**

Ngumphandi kuphela, abancedisi kuphando nabantu ababandakanyeka ngqo kolu phononongo abazokwazi ukuzifumana iingxelo zakho nezosana lwakho. Zonke iinkcukacha ziza kusoloko zigcinwe ziyimfihlo bucala. Abaphandi nabancedisa kuphando baza kuzithatha zonke iinkcukacha ezifunyenweyo njengeziyimfihlo kwaye akukho zinkcukacha ezinokubangela waziwe ukuba ungubani wena okanye umntwana wakho aza kunikwa nabani na ongabandakanyekiyo ngendlela ethe ngqo kolu phononongo.

**11. KUZA KWENZEKA NTONI KWIMEKO YESIGANEKO ESINGALINDEKANGA SOKWENZAKALA NGENXA YOKUTHATHA KWAKHO INXAXHEBA KWESI SIFUNDO SOPHANDO?**

Kunqabe kakhulu ukuba kubekho into engahambi kakuhle. Kulo lonke uphononongo kuza kusetyenziswa iindlela eziqhelekileyo, ngoko ke oko kuza kwenza amathuba amancinci okuba kungakho into embi enokwenzeka.

**12. INGABA UZA KUHLAWULWA NGOKUTHATHA INXAXHEBA KWESI SIFUNDO KWAYE INGABA KUKHO IINDLEKO EZIBANDAKANYEKAYO?**

Hayi, awuzi kuhlululwa ngokuthatha kwakho inxaxheba kolu phononongo. Nawe akukho nto kuza kufuneka uyihlawule, ukuba uthatha inxaxheba kolu phononongo.

**13. INGABA IKHO ENYE INTO EKUMELE UYAZI OKANYE UYENZE?**

-  Ungaqhagamshelana nomphandi oyintloko okanye abenza uphando ku [+27 21 938 9474](tel:+27219389474), [+27 82 418 4906](tel:+27824184906) ukuba unayo nayiphi na into ofuna ukuyiqonda okanye ufumana nayiphi na ingxaki.
-  Ungaqhagamshelana neKomiti yoPhando Lomntu kwa-021-938 9207 ukuba unenkxalabo okanye izikhalazo ezingasonjululwanga kakuhle ngugqirha wakho wesifundo.
-  Uza kufumana ikopi yezi nkcukacha neyefomu yesivumelwano ukuba uzigcinele.

**14. I-INSHORENSI**

Olu phononongo lunayo i-inshorensi esusela ngomhla woku-1 ngoJanuwari ka-2016 – kowama-31 ngoDisemba ka-2016, xa kunokwenzeka isehlo esingaqhelekanga sento engahambanga kakuhle ngeli xesha kuqhutywa olu phononongo.

**Isifungo somthathi-nxaxheba**

Ngokuytyikitya ngezantsi, Mna ..... ndiyavuma ukuthatha inxaxheba kwisifundo sophando semfuzo esibizwa ngokuba **Ukukhula, okuqulathwe ngumzimba nendlela ezitya ngayo iintsana ezizelwe phambi kwexesha ebezikwindawo enabantu abaneNtsholongwane kaGawulayo, aiintsana ezincinci kakhulu ngokwasemzimbeni ukuzalwa kwazo kwisibhedlele saseTygerberg, eNtshona Koloni.**

Ndazisa ukuba:

- Ndilufundile okanye ndalufunda olu lwazi kunye nefomu yemvumelwano kwaye ibhalwe ngolwimi endiliciko nendikhululekileyo kulo
- Bendinalo ithuba lokuba ndibuze imibuzo kwaye yonke imibuzo yam iphendulwe ngokwanelisayo.
- Ndiyakuqonda ukuba ukuthatha inxaxheba kolu phando kube **kukuzithandela kwam** kwaye andikhange ndinyanzelwe ukuba ndithathe inxaxheba.
- Ndingakhetha ukusishiya isifundo naninina kwaye andisayi kohlwaywa okanye uqal' ugwetywe nangayiphi indlela.
- Usenokucelwa ukuba usishiye isifundo phambi kokuba sipele, ukuba ugqirha wesifundo okanye umphandi ukubona kuyinzuzo kuwe, okanye ukuba andisilandeli isicwangciso sesifundo, ekuvunyelenwe ngaso.

Kutyikitywe e-(indawo) ..... ngo-(usuku) ..... 2016.

.....  
**Umtyikityo womthathi-nxaxheba**

.....  
**Umtyikityo wengqina**

**Isifungo somphandi**

Mna (*igama*) ..... ndiyafunga ukuba:

- Ndilucacisile ulwazi olu kweli xwebhu ku-.....
- Ndimkhuthazile ukuba abuze imibuzo kwaye athathe ixesha elifanelekileyo ukuba ayiphendule.

· Ndiyaneliseka kukuba uyakuqonda ngokwanelisayo konke okumalunga nophando okuxoxwe ngasentla.

· Ndisebenzise/andisebenzisanga toliki. (*Ukuba itoliki isetyenzisiwe kumele ityikitye isaziso ngezantsi.*)

Kutyikitywe e-(indawo) ..... ngo-(usuku) ..... 2016.

.....  
**Umtyikityo womphandi**

.....  
**Umtyikityo wengqina**

**Isifungo setoliki**

Mna (*igama*) ..... ndazisa ukuba:

· Ndicende umphandi (*igama*) ..... Ekucaciseni ulwazi olu lapha kweli xwebhu ku-(*igama lomthathi-nxaxheba*) ..... ndisebenzisa ulwimi lwesiAfrikaans/lwesiXhosa.

· Simkhuthazile ukuba abuze imibuzo kwaye athathe ixesha elifanelekileyo ukuba ayiphendule.

· Ndimxelele eyona nto iyiyo malunga nokunxulumene nam.

· Ndiyaneliseka kukuba umthathinxaxheba ukuqonda ngokupheleleyo okuqulathwe loluxwebhu lwemvumelwano eyazisiweyo kwaye nemibuzo yakhe yonke iphendulwe ngokwanelisayo.

Kutyikitywe e-(indawo) ..... ngo-(usuku) ..... 2016.

.....  
**Umtyikityo wetoliki**

.....  
**Umtyikityo wengqina**

## ADDENDUM B: DATA COLLECTION FORMS

		PATIENT INFORMATION		
PARTICIPANT NO AND CODE		DATE OF ENTRY INTO STUDY		
Infant's sticker		Mother's sticker		
MATERNAL INFORMATION		CONSENT FORM SIGNED: Y/N	MULTIPLE BIRTHS: Y/N	
HIV status:	<input type="checkbox"/> Pos =1 <input type="checkbox"/> Neg=2			
CD4 cell count:	<input type="text"/>			
PMTCT:	<input type="checkbox"/> HAART =1; <input type="checkbox"/> TREATMENT IN LABOUR= 2; <input type="checkbox"/> NO TREATMENT= 3; <input type="checkbox"/> NA=4			
Duration PMTCT Med:	YEARS <input type="text"/> MONTHS <input type="text"/> DAYS <input type="text"/>			
TB:	<input type="checkbox"/> YES=1; <input type="checkbox"/> NO=2			
TB treatment:	<input type="checkbox"/> YES=1; <input type="checkbox"/> NO=2; <input type="checkbox"/> Not Applicable=NA			
VDRL + :	<input type="checkbox"/> YES=1; <input type="checkbox"/> NO=2			
Syphilis treated:	<input type="checkbox"/> YES=1; <input type="checkbox"/> NO=2; <input type="checkbox"/> Not Applicable=NA	Treatment completed:	<input type="checkbox"/> YES=1; <input type="checkbox"/> NO=2	
Age:	<input type="text"/>			
Race:	<input type="checkbox"/> BLACK=1; <input type="checkbox"/> COLOURED=2; <input type="checkbox"/> WHITE=3; <input type="checkbox"/> OTHER=4	If other - Specify: <input type="text"/>		
Employment	<input type="checkbox"/> YES=1; <input type="checkbox"/> NO=2			
Drug use/abuse	<input type="checkbox"/> YES=1; <input type="checkbox"/> NO=2			
Alcohol use	<input type="checkbox"/> YES=1; <input type="checkbox"/> NO=2			
Smoking	<input type="checkbox"/> YES=1; <input type="checkbox"/> NO=2			
Gravida	<input type="text"/>			
Para	<input type="text"/>			
Mortality	<input type="text"/>			
Mode of delivery:	<input type="checkbox"/> NVD=1; <input type="checkbox"/> C-SECTION=2			
PROM:	<input type="checkbox"/> YES=1; <input type="checkbox"/> NO=2			
Antepartum haemorrhage:	<input type="checkbox"/> YES=1; <input type="checkbox"/> NO=2			
Abruption placenta:	<input type="checkbox"/> YES=1; <input type="checkbox"/> NO=2			
Other (please state):	<input type="checkbox"/> YES=1; <input type="checkbox"/> NO=2			
Diabetes:	<input type="checkbox"/> Type1= 1; <input type="checkbox"/> Type 2= 2; <input type="checkbox"/> Gestational=3; <input type="checkbox"/> None=4			
Hypertension/ pre-eclampsia:	<input type="checkbox"/> YES=1; <input type="checkbox"/> NO=2			
Breech	<input type="checkbox"/> YES=1; <input type="checkbox"/> NO=2			
Other (please state):	<input type="text"/>			
Maternal antibiotics:	<input type="checkbox"/> YES=1; <input type="checkbox"/> NO=2	Specify:		<input type="text"/>
State antibiotics:	<input type="text"/>			
Maternal steroids:	<input type="checkbox"/> YES=1; <input type="checkbox"/> NO=2			
Other medication- specify:	<input type="text"/>			
INFANT INFORMATION				
GENDER:	<input type="checkbox"/> Male= 1; <input type="checkbox"/> Female=2			
DOB:	<input type="text"/>			
APGAR:	<input type="text"/> /1min <input type="text"/> /5min <input type="text"/> /10min			
Birth weight:(grams)	<input type="text"/>			
Early ultra sound	<input type="text"/>			
GA- dates:	<input type="text"/>			
Foot length	<input type="text"/>	LENGTH: (cm)	<input type="text"/>	
VDRL + :	<input type="checkbox"/> YES=1; <input type="checkbox"/> NO=2	HEAD CIRCUMFERENCE:(cm)	<input type="text"/>	
Syphilis treatment:	<input type="checkbox"/> YES=1; <input type="checkbox"/> NO=2; <input type="checkbox"/> Not Applicable=NA			
Respiratory:	<input type="checkbox"/> None=1; <input type="checkbox"/> RDS/ HMD=2; <input type="checkbox"/> Wet lung=3; <input type="checkbox"/> Pneumonia=4; <input type="checkbox"/> Other=5			
Other - specify:	<input type="text"/>			
nCPAP:	<input type="checkbox"/> YES=1; <input type="checkbox"/> NO=2			
Cardiac echo:	<input type="checkbox"/> Normal=1; <input type="checkbox"/> PDA=2; <input type="checkbox"/> Other=3; <input type="checkbox"/> Not done= 4			
Cranial ultrasound:	<input type="checkbox"/> YES=1; <input type="checkbox"/> NO=2	Other -Specify: <input type="text"/>		
Cranial ultrasound results:	<input type="checkbox"/> Normal=1; <input type="checkbox"/> IVH 1/2=2; <input type="checkbox"/> IVH 2/3=3			
Chest X-ray	<input type="checkbox"/> YES=1; <input type="checkbox"/> NO=2			
Jaundice req Phototherapy:	<input type="checkbox"/> YES=1; <input type="checkbox"/> NO=2	Total days: <input type="text"/>		
ARVs	<input type="checkbox"/> YES=1; <input type="checkbox"/> NO=2; <input type="checkbox"/> NOT APPLICABLE=3			
Nevirapine:	<input type="checkbox"/> YES=1; <input type="checkbox"/> NO=2			
AZT:	<input type="checkbox"/> YES=1; <input type="checkbox"/> NO=2	Dose:	<input type="text"/>	
ARV Treatment period:	<input type="text"/>	Dose:	<input type="text"/>	
CRP:	<input type="checkbox"/> YES=1; <input type="checkbox"/> NO=2			
FBC at birth:	<input type="checkbox"/> YES=1; <input type="checkbox"/> NO=2			
Blood culture	<input type="checkbox"/> YES=1; <input type="checkbox"/> NO=2	FBC later:	<input type="checkbox"/> YES=1; <input type="checkbox"/> NO=2	
Blood gas at birth:	<input type="checkbox"/> YES=1; <input type="checkbox"/> NO=2	Blood culture later:	<input type="checkbox"/> YES=1; <input type="checkbox"/> NO=2	
PCR BIRTH RESULTS	<input type="checkbox"/> Pos =1 <input type="checkbox"/> Neg=2 <input type="checkbox"/> NA=3	COMPLETE INFECTION SCREEN	<input type="checkbox"/> YES=1; <input type="checkbox"/> NO=2	
		COMPLETE INFECTION SCREEN	<input type="checkbox"/> YES=1; <input type="checkbox"/> NO=2	
		COMPLETE INFECTION SCREEN	<input type="checkbox"/> YES=1; <input type="checkbox"/> NO=2	

DAILY MONITORING FORM

Participant n:

Birth	Day	Exit reason (codes)	INTAKE AND OUTPUT													FEED						NEC DIAGNOSIS			CLINICAL	RECEIVED PHOTO	BLOOD TRANSF	MEDICATION									
			Fluid intake							Supplementation						Output			FEED						NEC DIAGNOSIS			CLINICAL	RECEIVED PHOTO	BLOOD TRANSF	MEDICATION						
			Feeding code (BRAL-1, ENTERAL-2, NPO-3, IV-4, TPN-5)	Breast milk Amount	Formula feed	Neonatal type	Saline	TPN	Other	Blood product type	Blood product amount	TOTAL volume per day	PNBS	MCT oil	Hydralin	MILK	Fe	Other	Urine (apples)	Stool Frequency	Stool Consistency	Intolerance	NPO Suspicious Intolerance	Vomiting	Vomiting Frequency	Aspirates	Aspirates	Aspirates Frequency	Drainage	NEC SUSPECT	Abdominal X-ray	BELLS STAGE (1,2,3)	Abnormal clinical signs	RECEIVED Phototherapy	RECEIVED Blood product type	Medication type	
				ml	ml	ml	ml	ml	ml	ml	ml	Gram	ml	Y/N	Y/N	Y/N	Y/N	Y/N		CODE	Y/N	Y/N	CODE	x times	CODE	ml	x times	ml	Y/N	Y/N	Y/N	CODE	Y/N	Y/N	Code		
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Participant no: \_\_\_\_\_

DAY OF LIFE	Date (dd/mm/yy)	CPAP	Weight (0.001kg)	Head circumference (d)	Supine length (s)	Foot length	SKINFOLD THICKNESS				CIRCUMFERENCES					LENGTH MEASUREMENTS		
							Tricep skinfold thickness (TSF/TS) (s)	Tricep skinfold thickness (TSF/TS) (s)	Subscapular skinfold thickness (SSSF/SB)	Subscapular skinfold thickness (SSSF/SB)	Chest Circumference	Mid point of the arm	Mid upper arm circumference (MUAC)	Mid thigh circumference	Mid calf circumference	Crown-ump length (C)	Upper arm length (a)	Lower arm length (a)
		Y/N	Gram	cm	cm	cm	cm	mm	cm	mm	cm	cm	cm	cm	cm	cm	cm	cm
Birth																		
DAY 2																		
DAY 3																		
DAY 4																		
DAY 5																		
DAY 6																		
DAY 7																		
DAY 8																		
DAY 9																		
DAY 10																		
DAY 11																		
DAY 12																		
DAY 13																		
DAY 14																		
DAY 15																		
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DAY 17																		
DAY 18																		
DAY 19																		
DAY 20																		
DAY 21																		
DAY 22																		
DAY 23																		
DAY 24																		
DAY 25																		
DAY 26																		
DAY 27																		
DAY 28																		

Patient identification

CULTURES,ROUTINE(BLOODS,BLOODGAS,)INFECTION)SCREENING

		INFECTION)SCREENING											SEPSIS	
Day	Date	CRP	CULTURE)SCREEN		FBC						PCR	Confirmed		
		Result	Pos/Neg	Organism	Hct Result	Hb Result	WBC Result	Neutrophil Result	Lymph Result	Plateletcount Result	Pos/Neg	Code		
Birth														
2														
3														
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## ADDENDUM C: ETHICS APPROVAL



UNIVERSITEIT•STELLENBOSCH•UNIVERSITY  
jou kennisvenoot • your knowledge partner

### Approval Notice

#### Response to Modifications- (New Application)

14-Dec-2015  
Van Niekerk, Evette E

**Ethics Reference #:** N15/10/102

**Title:** Growth, body composition and nutritional intake of HIV exposed preterm, very- and extremely-low birth weight infants in Tygerberg hospital, Western Cape.

Dear Dr. Evette Van Niekerk,

The **Response to Modifications - (New Application)** received on **10-Dec-2015**, was reviewed by members of **Health Research Ethics Committee 1** via Expedited review procedures on **14-Dec-2015** and was approved.

Please note the following information about your approved research protocol:

Protocol Approval Period: **14-Dec-2015 -13-Dec-2016**

Please remember to use your **protocol number (N15/10/102)** on any documents or correspondence with the HREC concerning your research protocol.

Please note that the HREC has the prerogative and authority to ask further questions, seek additional information, require further modifications, or monitor the conduct of your research and the consent process.

#### After Ethical Review:

Please note a template of the progress report is obtainable on [www.sun.ac.za/rds](http://www.sun.ac.za/rds) and should be submitted to the Committee before the year has expired. The Committee will then consider the continuation of the project for a further year (if necessary). Annually a number of projects may be selected randomly for an external audit.

Translation of the consent document to the language applicable to the study participants should be submitted.

Federal Wide Assurance Number: 00001372  
Institutional Review Board (IRB) Number: IRB0005239

The Health Research Ethics Committee complies with the SA National Health Act No.61 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 Part 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes 2004 (Department of Health).

#### **Provincial and City of Cape Town Approval**

Please note that for research at a primary or secondary healthcare facility permission must still be obtained from the relevant authorities (Western Cape Department of Health and/or City Health) to conduct the research as stated in the protocol. Contact persons are Ms Claudette Abrahams at Western Cape Department of Health ([healthres@pgwc.gov.za](mailto:healthres@pgwc.gov.za) Tel: +27 21 483 9907) and Dr Helene Visser at City Health ([Helene.Visser@capetown.gov.za](mailto:Helene.Visser@capetown.gov.za) Tel: +27 21 400 3981). Research that will be conducted at any tertiary academic institution requires approval from the relevant hospital manager. Ethics approval is required BEFORE approval can be obtained from these health authorities.

We wish you the best as you conduct your research.  
For standard HREC forms and documents please visit: [www.sun.ac.za/rds](http://www.sun.ac.za/rds)

If you have any questions or need further assistance, please contact the HREC office at 0219389657.

#### **Included Documents:**

Declaration K Strydom  
Data collection sheet  
CV K Strydom

CV E van Niekerk  
20151211 MOD Consent English  
20151211 MOD Consent Afrikaans  
Protocol  
Payment form  
Protocol Synopsis  
20151211 MOD Protocol  
Cover letter  
20151211 MOD Cover letter  
Consent form (Afrikaans)  
Declaration E van Niekerk  
Declaration T Lodewyks  
Consent form (English)  
Application form  
CV MA Dhansay  
Checklist  
Declaration MA Dhansay  
CV T Lodewyks  
Confirmation of Liability and PI insurance

Sincerely,

Franklin Weber  
HREC Coordinator  
Health Research Ethics Committee 1

## Investigator Responsibilities

### Protection of Human Research Participants

Some of the responsibilities investigators have when conducting research involving human participants are listed below:

1. Conducting the Research. You are responsible for making sure that the research is conducted according to the HREC approved research protocol. You are also responsible for the actions of all your co-investigators and research staff involved with this research.
2. Participant Enrolment. You may not recruit or enrol participants prior to the HREC approval date or after the expiration date of HREC approval. All recruitment materials for any form of media must be approved by the HREC prior to their use. If you need to recruit more participants than was noted in your HREC approval letter, you must submit an amendment requesting an increase in the number of participants.
3. Informed Consent. You are responsible for obtaining and documenting effective informed consent using **only** the HREC-approved consent documents, and for ensuring that no human participants are involved in research prior to obtaining their informed consent. Please give all participants copies of the signed informed consent documents. Keep the originals in your secured research files for at least fifteen (15) years.
4. Continuing Review. The HREC must review and approve all HREC-approved research protocols at intervals appropriate to the degree of risk but not less than once per year. There is **no grace period**. Prior to the date on which the HREC approval of the research expires, **it is your responsibility to submit the continuing review report in a timely fashion to ensure a lapse in HREC approval does not occur**. If HREC approval of your research lapses, you must stop new participant enrolment, and contact the HREC office immediately.
5. Amendments and Changes. If you wish to amend or change any aspect of your research (such as research design, interventions or procedures, number of participants, participant population, informed consent document, instruments, surveys or recruiting material), you must submit the amendment to the HREC for review using the current Amendment Form. You **may not initiate** any amendments or changes to your research without first obtaining written HREC review and approval. The **only exception** is when it is necessary to eliminate apparent immediate hazards to participants and the HREC should be immediately informed of this necessity.
6. Adverse or Unanticipated Events. Any serious adverse events, participant complaints, and all unanticipated problems that involve risks to participants or others, as well as any research-related injuries, occurring at this institution or at other performance sites must be reported to the HREC within **five (5) days** of discovery of the incident. You must also report any instances of serious or continuing problems, or non-compliance with the HREC's requirements for protecting human research participants. The only exception to this policy is that the death of a research participant must be reported in accordance with the Stellenbosch University Health Research Ethics Committee Standard Operating Procedures [www.sun025.sun.ac.za/portal/page/portal/Health\\_Sciences/English/Centres%20and%20Institutions/Research\\_Development\\_Support/Ethics/Application\\_package](http://www.sun025.sun.ac.za/portal/page/portal/Health_Sciences/English/Centres%20and%20Institutions/Research_Development_Support/Ethics/Application_package). All reportable events should be submitted to the HREC using the Serious Adverse Event Report Form.
7. Research Record Keeping. You must keep the following research-related records, at a minimum, in a secure location for a minimum of fifteen years: the HREC approved research protocol and all amendments; all informed consent documents; recruiting materials; continuing review reports; adverse or unanticipated events; and all correspondence from the HREC.
8. Reports to the MCC and Sponsor. When you submit the required annual report to the MCC or you submit required reports to your sponsor, you must provide a copy of that report to the HREC. You may submit the report at the time of continuing HREC review.
9. Provision of Emergency Medical Care. When a physician provides emergency medical care to a participant without prior HREC review and approval, to the extent permitted by law, such activities will not be recognised as research nor will the data obtained by any such activities should it be used in support of research.
10. Final reports. When you have completed (no further participant enrolment, interactions, interventions or data analysis) or stopped work on your research, you must submit a Final Report to the HREC.
11. On-Site Evaluations, MCC Inspections, or Audits. If you are notified that your research will be reviewed or audited by the MCC, the sponsor, any other external agency or any internal group, you must inform the HREC immediately of the impending audit/evaluation.

## ADDENDUM D: INSURANCE



Marsh (Pty) Limited  
50 Dorp Street  
Stellenbosch, 7600  
P.O. Box 414  
Stellenbosch  
7599  
+27 21 834 1346  
[www.marsh-africa.com](http://www.marsh-africa.com)

06<sup>th</sup> October 2015

### TO WHOM IT MAY CONCERN

#### STELLENBOSCH UNIVERSITY: CONFIRMATION OF INSURANCE.

#### **Growth, body composition and nutritional intake of HIV exposed preterm, very- and extremely-low birth weight infants in Tygerberg hospital, Western Cape**

This serves to confirm that the following cover has been arranged for Stellenbosch University and others:

1. Primary General Liability (Broad form) insurance policy number 1000/28439 underwritten by Stalker Hutchison Admiral for a limit of R5 000 000.
2. Umbrella Liability insurance policy no 1000/22890 underwritten by Stalker Hutchison Admiral for a limit of R150 000 000.
3. Total Liability limit – R155 000 000
4. Professional Indemnity insurance policy number P01380 underwritten by Stalker Hutchison and Admiral for a limit of ZAR 150 000 000.
5. The cover mentioned above is extended to include North American extension.
6. Period of insurance: 1 January 2015 to 31 December 2015

Subject to the terms, conditions and exclusions of the policy wordings.

We trust that you will find the above to be in order. Please do not hesitate to contact the writer should you have any queries.

Kind regards

Jackie Beer  
Senior Broker

An authorised financial services provider  
FSB/FSP Licence no.: 8414  
Registration no.: 1999/000348/07  
Directors: IB Skosana (Non-Executive Chairman), JJ Erwee (CEO), BR Blake (Vice Chairman)\*, F Abrahams,  
R Ebrahim, S Montsi, M Pienaar, MG Sokkie (\*British)

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