UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Growth, feeding practices, and haemoglobin levels in 6 to 12 month old infants expose and unexposed to maternal HIV status in a peri-urban area in Gauteng Province, South Africa

Submitted in partial fulfilment of the requirements for the degree Doctor of Philosophy (PhD) in Nutrition

By

Phumudzo Tshiambara

in the

Department of Consumer and Food Sciences

Faculty of Natural and Agricultural Sciences

University of Pretoria

South Africa

February 2024

DECLARATION

I hereby declare that this thesis, entitled *Growth, feeding practices and haemoglobin levels in 6- to 12-month-old infants, exposed and unexposed to maternal HIV status, in a peri-urban area in Gauteng Province, South Africa,* submitted at the University of Pretoria for the award Doctor of Philosophy (PhD) degree in Nutrition is my own work and has not been submitted by me for a degree at any other University or Institution of Higher Education.

Phumudzo Tshiambara

February 2024

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Growth, feeding practices and haemoglobin levels in 6- to 12-month-old infants exposed and unexposed to maternal HIV status in a peri-urban area in Gauteng Province, South Africa

By

Phumudzo Tshiambara

Supervisor:	Dr. AMP Hoffman
Co-Supervisors:	Dr. MH Legodi
	Prof UD Feucht
Department:	Department of Consumer and Food Sciences
Faculty:	Natural and Agricultural Sciences
Degree:	PhD (Nutrition)

ABSTRACT

Introduction: Undernutrition is a significant public health issue, primarily caused by inappropriate feeding practices that negatively impact infant growth and development. In Sub-Saharan Africa, South Africa has the highest prevalence of human immunodeficiency virus (HIV). Antiretroviral therapy (ART) programmes reduce the infection rate, but leave infants exposed to maternal HIV infection but uninfected (HEU), and more than one million HEU infants are born annually. HEU infants are at a higher risk of mortality and morbidity than HIV-unexposed-uninfected (HUU) infants, leading to poor growth and increased risk of stunting, underweight and anaemia. Limited information exist on the growth, feeding practices and haemoglobin levels of 6-12 months old infants.

Aim: To compare the growth, feeding practices, and haemoglobin levels of HEU and HUU infants aged 6–12-months, and determine the relationship between growth, feeding practices and haemoglobin levels in these infants, stratified by maternal HIV status.

Methods: This cross-sectional study with repeated measurements was embedded in the Siyakhula Study, where data collection started in October 2018 until August 2023. Infants' anthropometric measurements (weight, length, head circumference and mid-upper-arm circumference), feeding practices (complementary- and breastfeeding, food frequently consumed, and dietary intake), and haemoglobin level (a marker for anaemia) were collected using calibrated scales, structured questionnaires and the HemoCue® device by trained research assistants using a local language. Hand expressed breastmilk was analysed for the nutrient composition of copper, iron, manganese, selenium, and zinc using the inductively coupled plasma mass spectroscopy method.

Results: The study included 181 infants (86 HEU; 95 HUU), with 58% males. High percentages of low birth weight was found in HEU than HUU infants (22% vs. 13%; $p<0.001$). Length-for-age Z-scores (LAZ) ($p<0.05$), weight-for-age Z-scores (WAZ) ($p<0.05$), and mid-upper-arm circumference-for-age Z-scores (MUACAZ) ($p<0.05$) were lower in HEU versus HUU infants at 6 and 9 months. Lower weight-for-length Z-scores (WLZ) (−0.2±1.2 vs. 0.2±1.2; $p=0.020$) was observed at 12 months in HEU infants. The HEU and HUU infants were introduced to complementary foods too early, and lower breastfeeding rates were found in HEU than HUU infants at 9 (36% vs. 57%; $p=0.013$) and 12 months (25% vs. 48%; $p=0.005$). Only

11% of HEU and 6% of HUU achieved a minimum dietary-diverse diet and flesh foods consumption was significantly higher in HEU (24%) than HUU (11%) at 12 months. Dietary fat intakes were low in HEU and HUU infants, with iron, calcium, and vitamin A intakes higher in HEU than HUU infants at 12 months. Haemoglobin levels and anaemia was similar in HEU and HUU infants but more percentages of anaemia were found in HEU than HUU infants at 6 (27% vs. 18%) and 9 months (33% vs. 29%). Haemoglobin levels positively correlated with WAZ (p=0.039), LAZ (p=0.007), and MUACAZ (p=0.039) at 9 months, and at 12 months WAZ (p=0.018) and WLZ (p=0.041) in HEU infants.

Conclusion: The HEU infants had suboptimal growth and inappropriate feeding practices including lower breastfeeding rates than HUU infants. The HEU infants had higher dietary intakes, dietary diversity, and flesh consumption than HUU infants. Infant growth and feeding habits are impacted by maternal HIV infection but better infant feeding counselling and implementation need to be prioritized for all South African mothers, irrespective of the HIV status.

Keywords: complementary feeding, dietary intake, haemoglobin levels, HIV exposure, growth, infants, nutrition, South Africa, undernutrition

RESEARCH OUTPUTS

Journal article

Tshiambara P.; Hoffman, M.; Legodi, H.; Botha, T.; Mulol, H.; Pisa, P.; Feucht, U. Comparison of Feeding Practices and Growth of Urbanized African Infants Aged 6–12 Months Old by Maternal HIV Status in Gauteng Province, South Africa. Nutrients 2023, 15(6), 1500; https://doi.org/10.3390/nu15061500

Conference presentations

Oral presentations

15th International Virtual Conference of the South African Association of Family Ecology and Consumer Science, 10-12 May 2022

Growth patterns over 12 months in HIV-exposed-unexposed infants in Gauteng Province, South Africa

Authors: **P Tshiambara**, M Hoffman, MH Legodi, UD Feucht

chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://saafecs.co.za/wp-content/uploads/2022/05/Book-of-Abstracts-SAAFECS-Conference-2022.pdf

South African Paediatric Association Research Day, 16 September 2022

Feeding practices for infants born to mothers living with- and without HIV residing in Tshwane, South Africa

Authors: **P Tshiambara**, M Hoffman, MH Legodi, UD Feucht

https://journals.co.za/doi/epdf/10.10520/ejc-m_sajch_v16_n3_a10

Child Health Priorities Conference, 24-26 November 2022

Anthropometric indices and nutritional classifications of HIV-exposed-uninfected compared to HIV-unexposed-uninfected infants in Tshwane District, South Africa

Authors: **P Tshiambara**, M Hoffman, MH Legodi, UD Feucht

https://drive.google.com/file/d/1Z09TXVVcF77X3DmX-gNlbD3wq9UfTj17/view

Poster presentations

University of Pretoria, Faculty of Health Sciences Research Day, 24 August 2022

Feeding practices for infants born to mothers living with- and without HIV residing in Tshwane, South Africa

Authors: **P Tshiambara**, M Hoffman, MH Legodi, UD Feucht

https://drive.google.com/drive/folders/10Utx7XwmJLcbCJapEyw5-AZnfN3N5KNP

Grow great summit, 3 November 2022

Anthropometric indices and nutritional classifications of HIV-exposed-uninfected compared to HIV-unexposed-uninfected infants in Tshwane District, South Africa.

Authors: **P Tshiambara**, M Hoffman, MH Legodi, UD Feucht

DEDICATION

This thesis is dedicated to:

My father (Ndiambani Alfred Mamphwe), late mother (Livhuwani Joyce Nngwekhulu), aunt (Getty Tshianeo Maphangula); my daughters Orinea and Oluga Tshiambara, and my husband, Fhatuwani Tshiambara; my siblings and cousins (Tshilidzi, Zwothe, Livhu, Ele, Joy, Mashudu); and the Sandton Assemblies of God family.

For their love, patience and spiritual support

and

The Almighty God, for His Grace

ACKNOWLEDGEMENTS

I am forever grateful and thankful to my lead Supervisor, Dr AMP Hoffman, for her guidance, unfailing support, invaluable suggestions, commentary and all the conscious efforts during supervision.

Dr MH Legodi; my co-supervisor, thank you for your guidance, support and valuable suggestions in my PhD journey, thank you for being my "three-in-one", wiping my tears, and praying for me.

I am grateful to my co-supervisor and principal investigator of the Siyakhula Study, Prof UD Feucht. Your feedback always made me think as a scientist. Thank you for your stellar work ethic, for being a sound board for my PhD and for providing opportunities to present my progress in the Siyakhula meetings.

My mentor Prof FM Mulaudzi, for your guidance and support.

Dr J Muchiri, thank you for your support, and the constant check-in messages and prayers.

Dr H Mulol, for being there, training and guiding me with the management of Siyakhula data.

Dr C Tosh for editing of research article manuscripts 1, 2 and 3, and Dr M Janse van Rensburg for editing Chapters 1, 2, 6 and 7.

I am very appreciative of the support received from the National Research Foundation (NRF), new Generation of Academics Programme (nGAP), and the University of Pretoria's University Capacity Development Programme (UCDP) for financing the study.

I am also grateful to all the academic staff, administrative staff, and my fellow postgraduate colleagues at the Department of Consumer and Food Sciences and Department of Human Nutrition (Profs Gyebi Duodu & Elna Buys, Johanna Malatji, Shanece, Mothusi, Dr Claire Martin, Juna Botha, Rodney, Gontse), at the University of Pretoria for their assistance and support. Thank you to all the mothers and the participants who participated in the study.

I also thank my father, husband, children, siblings, and friends for their encouragement, love, and spiritual support during this journey. I acknowledge my late mother for the being the HIV champion and councillor, your hard work inspired me to study maternal and child health.

Above all, I give praise and honour to the Almighty God for being my source of inspiration and strength during this period of study.

Isaiah 43:19

"See I am doing a new thing! Now it springs up; do you not perceive it? I am making a way in the wilderness and streams in the wasteland"

TABLE OF CONTENTS

DECLARATION ii
ABSTRACT iv
RESEARCH OUTPUTS vi
DEDICATION viii
ACKNOWLEDGEMENTS ix
TABLE OF CONTENTS xi
LIST OF TABLES xvi
LIST OF FIGURES xvii
ACRONYMS AND ABBREVIATIONS xix
CHAPTER 1: INTRODUCTION 1
1.1. BACKGROUND 1
1.2. PROBLEM STATEMENT 5
1.3. AIM, HYPOTHESIS AND OBJECTIVES 6
1.3.1. Aim 6
1.3.2. Hypothesis 7
1.3.3. Objectives 7
1.3.4. Outline of the study 8
1.4 SIGNIFICANCE OF THE STUDY 8
1.5. STRUCTURE OF THE DISSERTATION 9
1.6. CONCLUSION 10
1.7. REFERENCES 11
CHAPTER 2: LITERATURE REVIEW 17
2.1. INTRODUCTION 17
2.2. INFANT FEEDING PRACTICES AND GROWTH 20
2.2.1. Complementary feeding and undernutrition 20
2.2.2. Breastfeeding: an intervention to prevent undernutrition 22

2.2.3. Breastmilk composition of MLWH and MnLWH 25
2.2.4. Dietary intake of infants 27
2.2.5. Food frequently consumed by infants 29
2.2.6. The importance of introducing correct appropriate complementary foods 29
2.3. HAEMOGLOBIN AND GROWTH 30
2.3.1. Classification of anaemia (haemoglobin levels) 30
2.3.2. Prevalence of anaemia 31
2.3.3 Consequences and risk factors of anaemia 32
2.3.4. Importance of nutrition in anaemia prevention 33
2.4. HUMAN IMMUNODEFICIENCY VIRUS AND ACQUIRED IMMUNODEFICIENCY SYNDROME 33
2.4.1. HIV prevalence worldwide, Sub-Saharan Africa and South Africa 35
2.4.2. HIV vertical transmission prevention and exposed infants 35
2.4.3. The importance of nutrition in HEU infants 39
2.5. UNDERNUTRITION IN INFANTS AGED 6–12 MONTHS 40
2.5.1. Stunting (length-for-age Z-scores) 41
2.5.2. Underweight (weight-for-age Z-scores) 42
2.5.3. Wasting (weight-for-length Z-score) 42
2.5.4. Head circumference and acute malnutrition 42
2.5.5. The importance of good nutrition in the prevention of undernutrition 43
2.6. OTHER FACTORS CONTRIBUTING TO GROWTH OF INFANTS 44
2.6.1. Socio-Economic Status 44
2.6.2. Body mass index of mothers and infants' growth 45
2.6.3. Maternal maid-upper arm circumference (MUAC) and infants growth 46
2.7. SUMMARY 47
2.8. CONCLUSION 47
2.9. REFERENCES 48

CHAPTER 3: ARTICLE 1 (Manuscript 1) 58
3.1. INTRODUCTION 59
3.2 METHODS 61
3.2.1. Study design and setting 61
3.2.2. Data collection 62
3.2.3. Data processing and statistical analysis 63
3.2.4. Ethical consideration 63
3.3. RESULTS 63
3.3.1. Description of the study population 63
3.3.2. Birth characteristics of the infants 65
3.3.3. Feeding practices 66
3.3.4. Growth of HEU vs. HUU infants 71
3.4. DISCUSSION 74
3.5. CONCLUSIONS 76
3.6. REFERENCES 77
CHAPTER 4: ARTICLE 2 (Manuscript 2) 84
4.1. INTRODUCTION 85
4.3. RESULTS 89
4.5. DISCUSSION 94
4.6. CONCLUSION 99
4.7. REFERENCES 103
CHAPTER 5: ARTICLE 3 (Manuscript 3) 110
5.1. INTRODUCTION 111
5.2. METHODS AND MATERIALS 113
5.2.1. Study design, setting and participants 113
5.2.2. Data collection 114
5.2.3 Data processing and statistical analysis 116

5.2.4. Ethical considerations 116
5.3. RESULTS 117
5.4. DISCUSSION 125
5.5. CONCLUSION 129
5.6. REFERENCES 135
CHAPTER 6: GENERAL SUMMARY 143
6.1. INTRODUCTION 143
6.2. MAIN RESEARCH FINDINGS 146
6.3. STRENGTHS AND LIMITATIONS 148
6.3.1. Strengths 148
6.3.2. Limitations 148
6.4. PUBLIC HEALTH IMPLICATIONS AND FUTURE RESEARCH 149
6.5. CONCLUSION 150
6.6. RECOMMENDATIONS 150
6.7. REFERENCES 151
7. APPENDICES 156
APPENDIX A: Siyakhula study poster presentation by the PI 156
APPENDIX B: INFORMED CONSENT 157
APPENDIX C: MATERNAL AND INFANT POSTPARTUM QUESTIONNAIRE 166
APPENDIX D: INFANT FOLLOW-UP FORM 183
APPENDIX E: 24 HOUR RECALL 192
APPENDIX F: FOOD FREQUENCY QUESTIONNAIRE 194
APPENDIX G: BREASTMILK COLLECTION CHART 198
APPENDIX H: SIYAKHULA STUDY APPROVAL CERTIFICATES 199
APPENDIX I: SUB-STUDY/PHD STATISTICAL SUPPORT LETTER 203
APPENDIX J: SUB-STUDY/PHD APPROVAL CERTIFICATES 204
APPENDIX K: ARTICLE 1 209

APPENDIX K: POSTER PRESENTATION FOR RESEARCH DAY 222

APPENDIX L: POSTER FOR THE GROW GREAT SUMMIT 2022 PRESENTATIONS 223

APPENDIX M: *NUTRIENTS* AUTHOR GUIDELINES 224

APPENDIX N: *SOUTH AFRICAN JOURNAL OF CLINICAL NUTRITION* AUTHOR GUIDELINES 225

APPENDIX O: *MATERNAL & CHILD NUTRITION* AUTHOR GUIDELINES.....226

APPENDIX P: LETTER FROM LANGUAGE EDITOR.....227

LIST OF TABLES

Table 2.1. Breastfeeding and growth outcomes of HEU infants in SSA (studies) 24
Table 2.2: Breast milk composition ranges (mean ± SD).....27
Table 2.3. Anaemia cut-offs of infants from 6 months using haemoglobin levels from different studies 31
Table 2.4. Growth and health outcomes of HEU infants in Africa (studies).....37
Table 2.5. BMI classification of adults from the ages of 19-years and older.....45
Table 2.6. Adults MUAC classification.....46
Table 3.1. Socio-demographic characteristics of the study mothers according to HIV status……...…64
Table 3.2. Gestational age, sex, and anthropometric measurements and indices of HIV-exposed-uninfected and HIV-unexposed-uninfected infants at birth……..……65
Table 3.3. Feeding practices of HEU and HUU infants before 6 months based on maternal recall……..…..66
Table 3.4. Usual intake of food items by 6 and 12 months old HEU compared to HUU infants determined by quantified food frequency questionnaire (%)…….…….…….69
Table 3.5. Anthropometric measurements, Z-score indices, and nutritional classifications of the infants between 6–12 months of life by HIV exposure status……..……...71
Table 4.1. Maternal characteristics stratified by maternal HIV status…….…….……..89
Table 4.2. Anthropometric measurements, Z-score indices and nutritional classifications of infants at 6 and 12 months of life by HIV exposure status…….……91
Table 4.3. Food group consumption (any intake) and dietary diversity score of HEU and HUU infants at 6 and 12 months……..……..…...93
Table 4.4. Trace elements (mg/L) in human breastmilk nutrient composition in MLWH and MnLWH at 6 and 12 months postpartum……..……....93

Table 4.5. Dietary intake of HEU and HUU infants according to breastfeeding practices at 6 and 12 months, to account for the unmeasured breastmilk intake in breastfed infants, presented as median (IQR)……..101
Table 5.1. Maternal characteristics stratified by maternal HIV status……..117
Table 5.2. Characteristics of HIV-exposed-uninfected and HIV-unexposed-uninfected infants at birth……..…….118
Table 5.3. Haemoglobin levels and anaemia status of mothers and infants at 6, 9, and 12 months postpartum, stratified by maternal HIV status……..…….120
Table 5.4 Maternal anthropometric measurements at 6, 9, and 12 months postpartum, stratified by maternal HIV status……..121
Table 5.5. Correlation between infant haemoglobin levels, Z-scores indices, and confounding factors in HEU and HUU infants at 6, 9, and 12 months……..…….123
Table 5.6. Anthropometric measurements, Z-score indices, and nutritional classifications of the infants between 6–12 months of life by HIV exposure status…….……131
Table 5.7. Maternal haemoglobin levels, anaemia status, and anthropometric measurements at antenatal care visits and delivery, stratified by maternal HIV status……..……133
Table 6.1. Summary of key findings of this study.....1444

LIST OF FIGURES

Figure 1.1. Outline of the study 8
Figure 2.1 Schematic overview of the literature review 17
Figure 2.3. Factor affecting human breastmilk composition 27
Figure 2.4: Conceptual framework of HIV and ART exposure.....34
Figure 2.5: Factors affecting growth of infants (adapted from WHO, 2016) 40
Figure 2.6 Malnutrition classification using MUAC in children under-5 years 43
Figure 3.1. Infant breastfeeding practices by HIV exposure status in the first 12 months of life……....67

Figure 5.1. Sub-study flow diagram of participants at 6, 9, and 12 months……...……...…….……114
Figure 5.2. Infant breastfeeding practices (any breastfeeding) by HIV exposure status at 6, 9, and 12 months of life……..…….……134

ACRONYMS AND ABBREVIATIONS

AI	Adequate intakes
AIDS	Acquired immunodeficiency syndrome
ART	Anti-retroviral therapy
AZT	Zidovudine
BMI	Body mass index
CHEU	children-HIV-exposed-uninfected
CHUU	children-HIV-unexposed-uninfected
DRI	Dietary Reference Intakes
EBF	Exclusive breastfeeding or exclusively breastfeeding
EAR	Estimated average requirement
g/dL	Grams per decilitre
HC	Head circumference
HCAZ	Head circumference-for-age Z-scores
HCZ	Head circumference Z-scores
HEU	HIV-exposed-uninfected
HIV	Human immunodeficiency virus
HUU	HIV-unexposed-uninfected
HVTP	HIV Vertical Transmission Prevention
ICP-MS	Inductively coupled plasma mass spectroscopy
IYCF	Infant and Young Child Feeding
kcal	kilocalories
LAZ	Length-for-age Z-scores
LMICs	Low- and middle-income countries
mcg	micrograms
mg	milligrams
mg/kg	milligrams per kilogram
mg/L	milligrams per litre
mL	millilitres
MLWH	Mothers living with HIV
MnLWH	Mothers not living with HIV
MUAC	Mid-upper-arm circumference
MUACAZ	Mid-upper-arm circumference-for age Z-scores

nGAP	new Generation of Academics Programme
NGOs	Non-governmental organizations
NRF	National Research Foundation,
NVP	Nevirapine
PLWH	People living with HIV
RDA	Recommended daily allowance
RTHB	Road to Health Booklet
SA	South Africa
SAFOODS	South African Food Data System
SAM	Severe acute malnutrition
SAMRC	South African Medical Research Council
SD	Standard deviation
SGDs	Sustainable Development Goals
SPSS	Statistical Package for the Social Sciences
SSA	Sub-Saharan Africa
TB	Tuberculosis
UNAIDS	Joint United Nations Programme on HIV/AIDS
UNICEF	United Nations International Children's Emergency Fund
vs	versus
WAZ	Weight-for-age Z-scores
WHO	World Health Organization
WLZ	Weight-for-length Z-scores

CHAPTER 1: INTRODUCTION

1.1. BACKGROUND

One in 26 children in Sub-Saharan Africa (SSA) dies before reaching their fifth birthday and 80% of the global children under-5 deaths are from SSA (UNICEF, 2023). In 2021, around 13 800 children under the age of 5 years old died daily, an unacceptably high number of mostly preventable child deaths, especially in Africa (UNICEF, 2023a). Diseases that are linked to poverty, that include diarrhoea, HIV/AIDS, tuberculosis (TB), respiratory illnesses, measles, malaria, and worm infestations, add to high mortality rates in Sub-Saharan Africa (SSA) (World Health Organization (WHO), 2020a). Undernutrition is responsible for 45% of mortality in children under the age of five in low- and middle-income countries (LMICs), where the prevalence of human immunodeficiency virus (HIV) is also very high (UNICEF, 2023b; WHO, 2020a).

In the year 2022, 39 million people were living with HIV globally (UNAIDS, 2023). Women and girls accounted for 53%, and 1.5 million children aged from 0–14 years were living with HIV globally in 2022 (UNAIDS, 2023). In the same year, 77 % of new infections in SSA were in women of reproductive age (UNAIDS, 2023). Furthermore, only 42% of SSA districts had specialised prevention program for women of reproductive age in the year 2021 (UNAIDS, 2023). In South Africa, 8.45 million (13.9%) of the population were living with HIV in 2022, with 19.6% of adults between the ages of 15–49 years living with HIV (Stats, 2022). Low and MacDonell, (2019), reported that 13.05% of people living with HIV were residing in Gauteng Province. HIV can be transmitted to foetuses and children through vertical transmission during pregnancy, delivery, and breastfeeding (UNICEF, 2016).

Despite the successful rollout of antiretroviral therapy (ART) in South, Africa 85 796 HIV-related death were reported in the year 2022 (Stats, 2022). In South Africa alone it was estimated that there were 3.7 million orphans due to HIV related death and these orphaned infants or children experienced extreme poverty and homelessness (Breckenridge, Black-Hughes, Rautenbach & McKinley, 2019; Evans, Jones & Prendergast, 2016). The vertical transmission of HIV, which accounted for 90% of infections in children, has been reduced markedly through the use of ART in pregnant women and their newborns (Obumneme-Anyim, Ibeziako, Emodi, Ikefuna & Oguonu, 2016). Ninety-six percent of pregnant women received

ART for the HIV vertical transmission prevention (HVTP) in 2021 in South Africa. The successful global implementation of the HVTP programme reduced the transmission rates of HIV from 25–30% to 2–5%, leaving infants to be exposed to maternal HIV infection (Slogrove, Johnson & Powis, 2019). Hence, in SSA, over one million HIV-exposed-uninfected (HEU) infants are born annually (Sugandhi, Rodrigues, Kim, Ahmed, Amzel, Tolle *et al*., 2013; UNAIDS, 2018). HIV infection and or exposure is the main determinant of undernutrition in children under-5-years of age in Africa, leading to multiple nutritional deficiencies affecting the growth and development of children (Ricci, Asare, Carboo, Conradie, Dolman & Lombard, 2019).

Nutrition is vital for the growth and development of children, and 45% of deaths under-5 years of age are related to nutrition factors, such as lack of breastfeeding, early introduction of solid foods, poor nutrient intake (WHO, 2020b), increased nutrient requirements, and decreased food intake (Ricci *et al*., 2019). In the year 2019, 1.5 million infants died due to preventable conditions globally, mostly avoidable by proper growth and monitoring services, adequate nutrition, and safe water and food quality (WHO, 2020b).

Infants' nutritional needs increase due to rapid growth and development, as from 6–12 months the infant's birthweight triples, length doubles, and total body fat also increases (Mahan & Raymond, 2016). The second half of infancy is critical, as meeting the nutrient needs, either in breastmilk or introduced solid food, is a challenge (either introduced too early or inappropriately, and not safe) (WHO, 2021a). For the first six months of life, human milk supplies all of the nutrients required (Mahan & Raymond, 2016). From 6 months of age, the mature sucking movement has developed and stomach capacity has increased to 200 millilitres, allowing them to consume more food at the same time, and, thus, infants can be introduced to complementary foods (Mahan & Raymond, 2016). As infants transition from exclusive breastfeeding to the complementary feeding stage, a balanced diet is important for infants from 6 months of age onwards in order to meet the nutrient requirements for their growing bodies (Fabusoro & Mejia, 2021;WHO, 2010).

Infants from 6 months have high dietary needs to sustain growth and development, yet breastfed infants often ingest just a limited amount of foods other than breast milk (Mahan & Raymond, 2016). As a result, complementary foods must have a high nutritional density, defined as the amount of each nutrient per 100 kilocalories (kcal) of food. Iron and zinc are the most troublesome minerals during the supplemental feeding period, owing to low amounts in

human milk relative to demands (Dewey & Brown, 2003; Dewey & Adu-Afarwuah, 2008; Dewey, 2013; Mahan & Raymond, 2016).

Many changes affect dietary and nutrient intake throughout the first two years of life, which are defined by rapid physical and social development (Mahan & Raymond, 2016). Most dietary requirements rise between 6–12 months of age, for example, protein: 11 grams per day; calcium: 260 milligrams per day; water: 0.8 litre per day; and fluoride: 0.7 milligrams per day (Mahan & Raymond, 2016). By the time complementary foods are introduced, infants from six months are vulnerable to undernutrition, as most complementary foods are bulky, poor in calorie density, lack minerals, and contain higher phytates (WHO, 2021a), thus compromising the bioavailability of micronutrients, particularly iron, which may lead to anaemia (Mahan & Raymond, 2016). Undernutrition is exacerbated by insufficient feeding practices (WHO, 2021a).

The WHO (2016) infant and young child feeding (IYCF) policy recommends that all infants should be exclusively breastfed for the first six months of life, even in the context of maternal HIV infection, provided ART is provided. With continued breastfeeding and the introduction of safe, diverse, nutrient-dense complementary foods from 6 months up to 24 months and beyond (WHO, 2016).

Breastfeeding improves the HIV-free survival rate in LMICs and ART significantly reduces the risk of vertical HIV transmission and also improves the mother's health (WHO, 2016). Breastfeeding is protective against all-cause morbidity and mortality for all children, and breastmilk contains the anti-bodies and complete nutrition required for proper growth and development (Allen & Hector, 2005; Binns, Lee & Low, 2016; Lyons, Ryan, Dempsey, Ross & Stanton, 2020). Shorter duration or no breastfeeding by mothers living with HIV increases mortality related to common childhood illnesses (Goga, Doherty, Jackson, Sanders, Colvin, Chopra *et al.*, 2012; Kaldenbach, 2018). Although ART has been rolled out well in South Africa, and the benefits of breastfeeding outweighing the risks, some mothers still fear to breastfeed their infants due lack of knowledge on vertical transmission of HIV to their infants (Rossouw, Cornell, Cotton & Esser, 2016; West, Schwartz, Yende, Schwartz, Parmley, Gadarowski *et al*., 2019).

The HEU infants should also be exclusively breastfeed for the first six months of life, as the risk of mother-to-child transmission rates are lower than with mixed feeding (Coutsoudis,

Pillay, Kuhn, Spooner, Tsai, Coovadia *et al*., , 2001; Goga *et al*., 2012). However, the composition of breastmilk, especially trace minerals including iron and zinc, is understudied in South Africa. A Nigerian study found the trace minerals, such as iron and zinc, to be lower in mothers living with HIV (MLWH) (Fouché, van Niekerk & du Plessis, 2016), with a South African study suggesting that MLWH may safely breastfeed their infants without compromising their fat mass (Mulol & Coutsoudis, 2016), as the human breastmilk composition is affected by the body composition of the mothers.

The HEU infants are at a higher risk of mortality and vulnerable to infections, such as lower respiratory infections, malaria, diarrhoea, ear and skin infections (Evans, Jones & Prendergast, 2016a; Saloojee, De Maayer, Garenne & Kahn, 2007). Due to higher risk of severe infections, HEU infants are at a higher risk of dying in the first years if life as compared to HIV-unexposed-uninfected (HUU) infants (Sugandhi *et al*., 2013). Multifaceted factors, such as biological, maternal, social, behaviour, and health systems, may explains the higher risk of mortality in HEU infants (Johnson, Dorrington & Moolla, 2017).

The HEU infants have a higher risk of low birth weight (Kidzeru, Hesseling, Passmore, Myer, Gamieldien, Tchakoute *et al*., 2014; Rollins, Ndirangu, Bland, Coutsoudis, Coovadia & Newell, 2013) and stunting (Prendergast, Chasekwa, Evans, Mutasa, Mbuya, Stoltzfus *et al*., 2019; Ram, Gupte, Nayak, Kinikar, Khandave, Shankar *et al*., 2012) from 6 months and are vulnerable to growth faltering, micronutrient deficiencies (such as anaemia) and infectious illnesses (Teklemariam, Mitiku & Mesfin, 2015).

Anaemia is a global public health concern affecting children under 5 years old (Turawa, Awotiwon, Dhansay, Cois, Labadarios, Bradshaw *et al*., 2021) and is an indicator of poor nutrition in children (WHO, 2021b). Poor dietary intake of iron may lead to anaemia (WHO, 2020b) resulting from low levels of haemoglobin in the blood (Sundararajan & Rabe, 2021). The prevalence of anaemia in children under 5 is high, with global rates at almost 40%; 60% in Africa and 44% in South Africa reported in the year 2019 (WHO, 2021b). Infants from 6 months of age are at a higher risk of anaemia associated with undernutrition, due to increased demands of nutrients, including iron (WHO, 2020b). Iron is vital for the production of haemoglobin, responsible for transporting oxygen to the body (Joo, Kim, Kim, Lee & Kim, 2016).

Anaemia can lead to poor birth outcomes, as iron demands increase during pregnancy, especially in the third trimester, also impacting the transfer of iron from the mother to the foetus (O'Brien, Zavaleta, Abrams & Caulfield, 2003). Low haemoglobin levels restrict the transportation of oxygen in the blood, reduce physical and mental capacity (WHO, 2020b), increase the risk of infections (Santos, Gonzalez, Albuquerque, Arruda, Diniz, Figueroa *et al*., 2011), and lead to poor nutrition and growth in children (WHO, 2021a). Anaemia has multiple causes, including infections such as HIV, diarrhoea, undernutrition, prematurity, and low birth weight (Allali, Brousse, Sacri, Chalumeau & de Montalembert, 2017; Da Silva, Fawzi, Cardoso & ENFAC Working Group, 2018; Sanou & Ngnie-Teta, 2012; Siekmans, Receveur & Haddad, 2014). Although the MLWH may be taking ART, the risk anaemia is still increased (Dryden-Peterson, Shapiro, Hughes, Powis, Ogwu, Moffat *et al*., 2011), contributing to poor health outcomes and may lead to psychosocial- and economic challenges that affect the overall well-being of HEU infants (Moraleda, de Deus, Serna-Bolea, Renom, Quintó, Macete *et al*., 2014; Sugandhi *et al*., 2013).

1.2. PROBLEM STATEMENT

Undernutrition greatly affects children under 5 years old. Nutritional needs in infants increase from six months of age onwards, in that breastmilk alone is not sufficient to meet the daily nutrient requirement for growth and development (Mahan, Raymond, 2016; WHO, 2020a). The presence of HIV in the mother's body reduces breastmilk composition of trace elements including iron and zinc (Rahamon, Onifade & Arinola, 2018). Despite the benefits of breastfeeding and recommendations of infant feeding while living with HIV, some mothers still fear vertical transmission of HIV to their infants through breastfeeding (Rossouw *et al*., 2016; West *et al*., 2019).

Early introduction of complementary foods is a major problem in Africa and is common in South Africa. Low consumption of iron-rich foods, low socio-economic status (Faber, Laubscher, Berti, 2016; Mugware, Motadi & Mushaphi, 2022) and diseases such as HIV are risk factors associated with undernutrition and anaemia, affecting the growth of HEU infants (UNICEF, 2019). HIV is the main determinant of undernutrition in Africa (Ricci *et al*., 2019), where the highest growing population of HEU infants is found (Prendergast & Evans, 2023). HEU are at greater risk of poor feeding practice in LMICs, as mothers may be too unwell to prepare nutrient dense meals for their infants (Gladstone, Chandna, Kandawasvika, Ntozini, Majo, Tavengwa *et al*., 2019; WHO, 2010).

There are limited studies focusing on growth in the second half of infancy in relation to feeding practices and haemoglobin levels of HEU infants. Existing studies employed the cross-sectional study design, or included HIV infected infants, or no comparison group with similar numbers of infants in the groups. The poor growth in HEU infants is well studied, but it remains unclear which nutritional factors contribute to their suboptimal growth. Further investigation is warranted, especially during the complementary feeding stage. In view of the higher prevalence of undernutrition in HEU infants, this doctoral research study aims to determine and compare the growth, in relation to feeding practices and haemoglobin levels, of 6–12-month-old infants exposed- and unexposed to maternal HIV infection.

More prospective cohort studies are needed to determine the growth, feeding practices and haemoglobin levels in mediating poor outcomes of HEU infants. In order to ensure the provision of appropriate care to HEU infants, understanding of their growth and monitoring HEU infants is vital, as this may help to improve the clinical course and quality of life of these infants. In addition, understanding the impact of infant exposure to maternal HIV infection is needed and, thus, interventions may be targeted to the long-term health needs of these infants (Sugandhi *et al*., 2013). Discovering deeper insights into the factors affecting growth, such as the immediate and underlying causes of undernutrion, feeding practices, and anaemia in HEU infants, calls for intervention studies focusing on nutrition and education regarding appropriate infant complementary feeding for mothers or caregivers.

1.3. AIM, HYPOTHESIS AND OBJECTIVES

1.3.1. Aim

This research is embedded in a prospective longitudinal descriptive cohort study, namely the Siyakhula study, that aimed to understand how the in-utero and early postnatal environments, altered by maternal HIV-positive status, influence infants' growth trajectories and cognitive development, and alter their immune development and function, irrespective of the infants' HIV status.

The overall aim of this PhD research was to compare the growth of infants, aged 6–12 months and exposed to maternal HIV infection, in relation to feeding practices and haemoglobin levels, in an urban area in Gauteng Province, South Africa.

1.3.2. Hypothesis

Within the context of near-universal antiretroviral treatment access for pregnant women, in the urban area of the Tshwane District, Gauteng Province, South Africa, the following hypotheses apply:

Hypothesis 1: There is no difference in the growth of HIV-exposed and HIV-unexposed infants.

HEU infants are at a higher risk of undernutrition due to improper feeding practices, nutrient intake and other illnesses (Goga *et al*., 2012; Prendergast *et al*., 2019; Rossouw *et al*., 2016).

Hypothesis 2: There is no difference in the feeding practices and the composition of breastmilk fed to HEU and HUU infants aged 6 and 12 months.

Inappropriate feeding practices were found in pregnant MLWH (Goga *et al*., 2012; Jones, Sherman & Varga, 2005; Rollins *et al*., 2013). There are differences in human breastmilk composition of mothers living with- and not living with HIV (Fouché *et al*., 2016), with current information lacking on trace minerals concentration during the complementary feeding phase (Rahamon, Arinola & Akiibinu, 2013).

Hypothesis 3: There is no difference in terms of haemoglobin levels in infants aged 6–12 months according to maternal HIV exposure.

Infants' anaemia, defined by haemoglobin less than 11g/dL, was found to be higher in HEU infants due to their mothers' education level and infants' nutritional status (Wu, Li, Li, Loo, Yang, Wang *et al*., 2018). There are significant changes in the haematological indices of infants of mothers living with HIV at birth (Obumneme-Anyim *et al*., 2016).

1.3.3. Objectives

1. To compare the feeding practices and growth of HIV-exposed-unexposed and HIV-unexposed uninfected infants between 6–12 months of age in Tshwane District, Gauteng Province, South Africa.
2. To compare and determine the dietary intake and growth of HIV-exposed-unexposed and HIV-unexposed uninfected infants between 6–12 months of age.
3. To determine the micronutrient composition of breastmilk fed to HIV-exposed-unexposed and HIV-unexposed uninfected infants at 6 and 12 months.

4. To determine the correlation between haemoglobin in South African infants aged 6–12 months who are exposed to maternal HIV infection.

1.3.4. Outline of the study

The outline of the study is illustrated in Figure 1.1., with grey-coloured areas representing the objective measurements for determining and comparing the growth, feeding practices and haemoglobin levels of in 6–12-month-old urban infants.

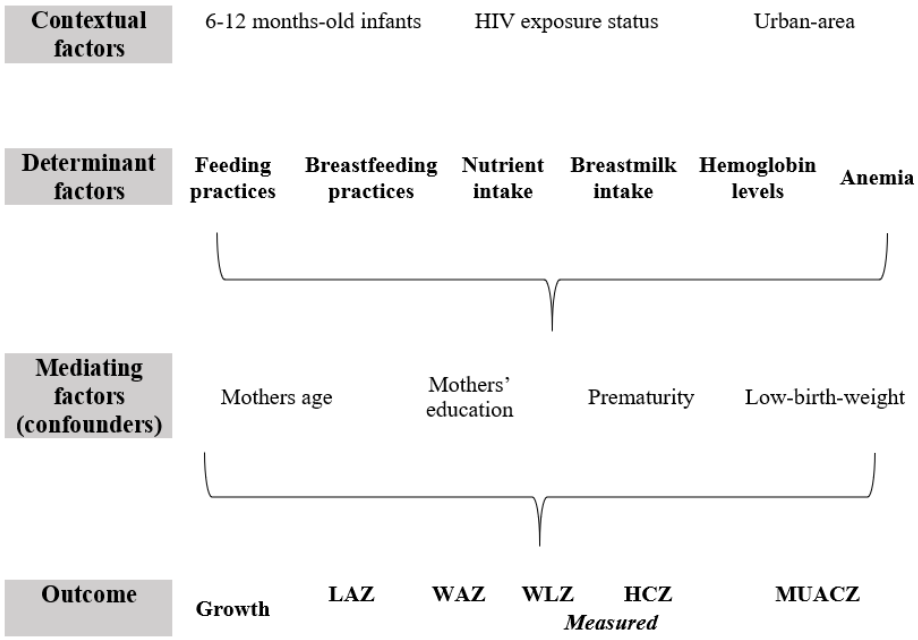


Figure 1.1. Outline of the study

Abbreviations: HEU: HIV-exposed-uninfected; HUU: HIV-unexposed-uninfected; WAZ: weight-for-age Z-score; LAZ: length-for-weight Z-score; HCZ: head circumference-for-age Z-score; MUACZ: mid-upper-arm circumference-for-age Z-score.

1.4 SIGNIFICANCE OF THE STUDY

This study provides baseline information for future studies concerning growth of 6–12-month-old infants (with increased nutritional needs due to growth and development) in relation to feeding practices and haemoglobin levels in the context of maternal HIV infection (where there is an increased risk of morbidity and high mortality).

This is the first comparative multi-cross-sectional analysis in Gauteng Province, South Africa, a high HIV-prevalence area, with similar sample size in HEU and HUU infants, investigating the growth, feeding practices, and haemoglobin levels of these infants at 6, 9 and 12 months.

Understanding the growth and monitoring of HEU infants will help in understanding and dealing with the immediate and underlying causes of undernutrition and calls for intervention to address the causes of malnutrition in infants. Comparing the growth, feeding practices, and haemoglobin levels between HEU and HUU infants at 6–12 months may contribute to the development of interventions tailored to HEU infants, such as nutrition education.

Measuring and comparing the growth, feeding practices, and haemoglobin levels between HEU and HUU infants at 6–12 months will contribute to an understanding of the problem of undernutrition in an urban area in South Africa, where the double burden of malnutrition exists in the black population.

This PhD will inform nutrition policy interventions aimed at educating mothers and caregivers about appropriate complementary feeding in order to promote optimal growth, even in the context of HIV.

Investigating the feeding practices, including the food frequently consumed, nutrient intake, and breastmilk composition is important as it is associated with the growth of HEU and HUU infants. In addition, this study provides information for future intervention studies aiming at improving the growth of HEU infants.

1.5. STRUCTURE OF THE DISSERTATION

Chapter 1 deals with the introduction and problem statement of this study in terms of HIV, nutrition status, feeding practices and haemoglobin levels. The aim, hypotheses, objectives, and the significance of the study are also presented in this chapter.

Chapter 2 deals with a discussion of the reviewed literature on growth, nutritional status, feeding practices and haemoglobin levels in HEU and HUU infants residing in a limited-resource setting.

Chapter 3 Article 1: Comparison of Feeding Practices and Growth of Urbanized African Infants Aged 6–12 Months Old by Maternal HIV Status in Gauteng Province, South Africa

Chapter 4 Article 2: Dietary intake and growth of infants in the complementary feeding phase by maternal HIV status in an urban setting in Gauteng Province, South Africa.

Chapter 5 Article 3: Haemoglobin levels and growth in South African infants, aged 6–12 months, exposed to maternal HIV infection

Chapter 6 presents a general discussion, summary, strengths and limitations, implications, conclusion and recommendations of the study.

References are listed at the end of each chapter in the thesis, and Appendices are listed at the end of the thesis.

1.6. CONCLUSION

This chapter introduced the topic and problem statement, and presented the research aims and objectives, as well as the significance of the study. The following chapter presents available literature in relation to growth, feeding practices and haemoglobin levels of HEU and HUU infants.

1.7. REFERENCES

Allali, S., Brousse, V., Sacri, A.-S., Chalumeau, M. and De Montalembert, M. 2017. Anemia in children: Prevalence, causes, diagnostic work-up, and long-term consequences. *Expert review of hematology*. 10(11)**:**1023-8.

Allen, J. and Hector, D. 2005. Benefits of breastfeeding. *New South Wales public health bulletin*. 16(4)**:**42-6.

Baumgartner, J. and Barth-Jaeggi, T. 2015. Iron interventions in children from low-income and middle-income populations: Benefits and risks. *Current opinion in clinical nutrition and metabolic care*. 18(3)**:**289-94.

Binns, C., Lee, M. and Low, W.Y. 2016. The long-term public health benefits of breastfeeding. *Asia Pacific Journal of Public Health*. 28(1)**:**7-14.

Breckenridge, T.A., Black-Hughes, C., Rautenbach, J. and Mckinley, M. 2019. Hiv/aids orphans in south africa: Ngo interventions supporting transitions to alternative care. *International Social Work*. 62(2)**:**502-17.

Coutsoudis, A., Pillay, K., Kuhn, L., Spooner, E., Tsai, W.-Y., Coovadia, H.M. and South African Vitamin A Study Group. 2001. Method of feeding and transmission of HIV-1 from mothers to children by 15 months of age: Prospective cohort study from Durban, South Africa. *AIDS*. 15(3)**:**379-87.

Da Silva, L.L.S., Fawzi, W.W., Cardoso, M.A. and Group, E.W. 2018. Factors associated with anemia in young children in Brazil. *PloS One*. 13(9)**:**e0204504.

Dewey, K.G. 2013. The challenge of meeting nutrient needs of infants and young children during the period of complementary feeding: An evolutionary perspective. *The Journal of nutrition*. 143(12)**:**2050-4.

Dewey, K.G. and Brown, K.H. 2003. Update on technical issues concerning complementary feeding of young children in developing countries and implications for intervention programs. *Food and nutrition bulletin*. 24(1)**:**5-28.

Dewey, K.G. and Adu-Afarwuah, S. 2008. Systematic review of the efficacy and effectiveness of complementary feeding interventions in developing countries. *Maternal & child nutrition*. 424-85.

Dryden-Peterson, S., Shapiro, R.L., Hughes, M.D., Powis, K., Ogwu, A., Moffat, C., et al. 2011. Increased risk of severe infant anemia following exposure to maternal HAART, Botswana. *Journal of Acquired Immune Deficiency Syndromes* (1999). 56(5)**:**428.

Evans, C., Jones, C.E. and Prendergast, A.J. 2016a. Hiv-exposed, uninfected infants: New global challenges in the era of paediatric HIV elimination. *The Lancet Infectious Diseases*. 16(6)**:**e92-e107.

Evans, C., Jones, C.E. and Prendergast, A.J. 2016b. Hiv-exposed, uninfected infants: New global challenges in the era of paediatric hiv elimination. *The Lancet Infectious Diseases*. 16(6)**:**e92-e107.

Faber, M., Laubscher, R. and Berti, C. 2016. Poor dietary diversity and low nutrient density of the complementary diet for 6- to 24-month-old children in urban and rural Kwazulu-Natal, South Africa. *Maternal & Child Nutrition*. 12(3)**:**528-45.

Fabusoro, O.K. and Mejia, L.A. 2021. Nutrition in hiv-infected infants and children: Current knowledge, existing challenges, and new dietary management opportunities. *Advances in nutrition*. 12(4)**:**1424-37.

Fouché, C., Van Niekerk, E. and Du Plessis, L.M. 2016. Differences in breast milk composition of hiv-infected and hiv-uninfected mothers of premature infants: Effects of antiretroviral therapy. *Breastfeeding Medicine*. 11(9)**:**455-60.

Gladstone, M.J., Chandna, J., Kandawasvika, G., Ntozini, R., Majo, F.D., Tavengwa, N.V., et al. 2019. Independent and combined effects of improved water, sanitation, and hygiene (wash) and improved complementary feeding on early neurodevelopment among children born to HIV-negative mothers in rural zimbabwe: Substudy of a cluster-randomized trial. *PLoS medicine*. 16(3)**:**e1002766.

Goga, A.E., Doherty, T., Jackson, D.J., Sanders, D., Colvin, M., Chopra, M. and Kuhn, L. 2012. Infant feeding practices at routine PMTCT sites, south africa: Results of a prospective observational study amongst HIV exposed and unexposed infants-birth to 9 months. *International Breastfeeding Journal*. 7(1)**:**4.

Johnson, L.F., Dorrington, R.E. and Moolla, H. 2017. Progress towards the 2020 targets for hiv diagnosis and antiretroviral treatment in south africa. *Southern African journal of HIV medicine*. 18(1).

Jones, S., Sherman, G. and Varga, C. 2005. Exploring socio-economic conditions and poor follow-up rates of HIV-exposed infants in Johannesburg, South Africa. *AIDS Care*. 17(4)**:**466-70.

Joo, E.Y., Kim, K.Y., Kim, D.H., Lee, J.-E. and Kim, S.K. 2016. Iron deficiency anemia in infants and toddlers. *Blood research*. 51(4)**:**268.

Kaldenbach, S., 2018. *Infant growth in the KwaZulu-Natal province, South Africa: Results from the KwaZulu-Natal initiative for breastfeeding support (KIBS) survey II at 13-15 weeks and 6 months of age* (Master's thesis, The University of Bergen).

Kidzeru, E.B., Hesseling, A.C., Passmore, J.-a.S., Myer, L., Gamieldien, H., Tchakoute, C.T., Gray, C.M., Sodora, D.L and Jaspan, H.B. 2014. In-utero exposure to maternal HIV infection alters t-cell immune responses to vaccination in HIV-uninfected infants. *AIDS*. 28(10)**:**1421.

Low, M. and MacDonell, S., 2019. Graphs that tell the story of HIV in South Africa's provinces.

Lyons, K.E., Ryan, C.A., Dempsey, E.M., Ross, R.P. and Stanton, C. 2020. Breast milk, a source of beneficial microbes and associated benefits for infant health. *Nutrients*. 12(4)**:**1039.

Mahan, L.K. and Raymond, J.L. 2016. *Krause's food & the nutrition care process-e-book*. Elsevier Health Sciences.

Moraleda, C., De Deus, N., Serna-Bolea, C., Renom, M., Quintó, L., Macete, E., Menéndez, C. & Naniche, D. 2014. Impact of HIV exposure on health outcomes in HIV-negative infants born to HIV-positive mothers in sub-saharan africa. *Journal of Acquired Immune Deficiency Syndromes*. 65(2)**:**182-9.

Mugware, A., Motadi, S.A. and Mushaphi, L.F. 2022. Feeding practices and micronutrient status of children aged 0–36 months in thulamela municipality, Limpopo Province. *Health SA Gesondheid 27(0), a1973.*

Mulol, H. and Coutsoudis, A. 2016. Breastmilk output in a disadvantaged community with high HIV prevalence as determined by the deuterium oxide dose-to-mother technique. *Breastfeeding Medicine*. 11(2)**:**64-9.

O'Brien, K.O., Zavaleta, N., Abrams, S.A. and Caulfield, L.E. 2003. Maternal iron status influences iron transfer to the fetus during the third trimester of pregnancy. *The American Journal of Clinical Nutrition*. 77(4)**:**924-30.

Obumneme-Anyim, I., Ibeziako, N., Emodi, I., Ikefuna, A. and Oguonu, T. 2016. Hematological indices at birth of infants of HIV-positive mothers participating in a prevention of mother-to-child transmission program. *Journal of tropical pediatrics*. 62(1)**:**3-9.

Prendergast, A.J. and Evans, C. 2023. Children who are HIV-exposed and uninfected: Evidence for action. *AIDS*. 37(2)**:**205-15.

Prendergast, A.J., Chasekwa, B., Evans, C., Mutasa, K., Mbuya, M.N.N., Stoltzfus, R.J., Smith, L.E., Majo, F.D., Tavengwa, N.V., Mutasa, B., Mangwadu, G.T., Chasokela, C.M., Chigumira, A., Moulton, L.H., Ntozini, R. and Humphrey, J.H. 2019. Independent and combined effects of improved water, sanitation, and hygiene, and improved complementary feeding, on stunting and anaemia among hiv-exposed children in rural zimbabwe: A cluster-randomised controlled trial. *The Lancet Child & Adolescent Health*. 3(2)**:**77-90.

Rahamon, S.K., Arinola, G.O. and Akiibinu, M.O., 2013. Total antioxidant potential and essential trace metals in the breast milk and plasma of Nigerian Human Immunodeficiency Virus-infected lactating mothers. *Journal of Research in Medical Sciences: The Official Journal of Isfahan University of Medical Sciences*, 18(1).

Ram, M., Gupte, N., Nayak, U., Kinikar, A.A., Khandave, M., Shankar, A.V., Sastry, J., Bollinger, R.C., Gupta, A. and SWEN India and BJMC-JHU Clinical Trials Study Team, 2012. Growth patterns among HIV-exposed infants receiving nevirapine prophylaxis in Pune, India. *BMC infectious diseases*. 12.

Ricci, C., Asare, H., Carboo, J., Conradie, C., Dolman, R.C. and Lombard, M. 2019. Determinants of undernutrition prevalence in children aged 0–59 months in sub-saharan africa between 2000 and 2015. A report from the world bank database. P*ublic health nutrition*. 22(9).

Rollins, N.C., Ndirangu, J., Bland, R.M., Coutsoudis, A., Coovadia, H.M. and Newell, M.L. 2013. Exclusive breastfeeding, diarrhoeal morbidity and all-cause mortality in infants of hiv-infected and hiv uninfected mothers: An intervention cohort study in Kwazulu Natal, South Africa. *PloS One*. 8(12).

Rossouw, M.E., Cornell, M., Cotton, M.F. and Esser, M.M. 2016. Feeding practices and nutritional status of HIV-exposed and HIV-unexposed infants in the Western Cape. *Southern African journal of HIV medicine*. 17(1).

Saloojee, H., De Maayer, T., Garenne, M.L. and Kahn, K. 2007. What's new? Investigating risk factors for severe childhood malnutrition in a high HIV prevalence south african setting1. *Scandinavian Journal of Public Health.* 35(69_suppl).

Sanou, D. and Ngnie-Teta, I. 2012. Risk factors for anemia in preschool children in Sub-Saharan Africa.

Santos, R.F.D., Gonzalez, E.S.C., Albuquerque, E.C.D., Arruda, I.K.G.D., Diniz, A.D.S., Figueroa, J.N. and Pereira, A.P.C. 2011. Prevalence of anemia in under five-year-old

children in a children's hospital in Recife, Brazil. *Revista brasileira de hematologia e hemoterapia*. 33100-4.

Siekmans, K., Receveur, O. and Haddad, S. 2014. Can an integrated approach reduce child vulnerability to anaemia? Evidence from three african countries. *PloS One*. 9(3).

Slogrove, A.L., Johnson, L.F. and Powis, K.M., 2019. Population-level mortality associated with HIV exposure in HIV-uninfected infants in Botswana and South Africa: a model-based evaluation. *Journal of tropical pediatrics*, 65(4), pp.373-379..

Stats, S. 2022. *Mid-year population estimates 2022*. Stats SA: Pretoria, South Africa.

Sugandhi, N., Rodrigues, J., Kim, M., Ahmed, S., Amzel, A., Tolle, M., et al. 2013. Hiv exposed infants: Rethinking care for a lifelong condition. *AIDS* (London, England). 27(0 2).

Sundararajan, S. and Rabe, H. 2021. Prevention of iron deficiency anemia in infants and toddlers. *Pediatric research*. 89(1).

Teklemariam, Z., Mitiku, H. and Mesfin, F., 2015. Prevalence of anemia and nutritional status among HIV-positive children receiving antiretroviral therapy in Harar, eastern Ethiopa. *HIV/AIDS-Research and Palliative Care.*

Turawa, E., Awotiwon, O., Dhansay, M.A., Cois, A., Labadarios, D., Bradshaw, D. and Pillay-Van Wyk, V. 2021. Prevalence of anaemia, iron deficiency, and iron deficiency anaemia in women of reproductive age and children under 5 years of age in south africa (1997–2021): A systematic review. *International Journal of Environmental Research and Public Health*. 18(23)**:**12799.

UNAIDS. Global HIV & AIDS statistics — Fact sheet; UNAIDS: Geneva, Switzerland, 2022. Available online: https://www. unaids. org/en/resources/fact-sheet (accessed on 23 August 2023).

UNICEF. *Adopting Optimal Feeding Practices Is Fundamental to a Child's Survival, Growth and Development, but too Few Children Benefit*; UNICEF: New York, NY, USA, 2016.

UNICEF. *Under-five mortality: UNICEF Data: Monitoring the situation of children and women.* UNICEF: New York, NY, USA, 2023a. Available on: https://data.unicef.org/topic/child-survival/under-five-mortality/

UNICEF. *Malnutrition rates remain alarming: Stunting is declining too slowly while wasting still impacts the lives of far too many young children.* UNICEF: New York, NY, USA, 2023b. Available from: https://data.unicef.org/topic/nutrition/malnutrition/

West, N.S., Schwartz, S.R., Yende, N., Schwartz, S.J., Parmley, L., Gadarowski, M.B., Mutunga, L., Bassett, J. and Van Rie, A., 2019. Infant feeding by South African mothers living with HIV: implications for future training of health care workers and the need for consistent counseling. *International Breastfeeding Journal*, 14(1), pp.1-7.

Woldesenbet, S., Lombard, C., Manda, S., Kufa, T., Ayalew, K. and Cheyip, M. 2021. *The 2019 national antenatal sentinel HIV survey, South Africa, national department of health.* The 2019 National Antenatal Sentinel HIV Survey, South Africa, National Department of Health.

World Health Organization. *Antiretroviral therapy of HIV infection in infants and children: Towards universal access: Recommendations for a public health approach-2010 revision*. World Health Organization. Geneva, Switzerland, 2010.

World Health Organization. *Guideline: updates on HIV and infant feeding: the duration of breastfeeding, and support from health services to improve feeding practices among mothers living with HIV*. Geneva, Switzerland, 2016.

World Health Organization. *Guideline: Updates on HIV and infant feeding: The duration of breastfeeding, and support from health services to improve feeding practices among mothers living with HIV*. Geneva, Switzerland, 2020a.

World Health Organization. *UNICEF/WHO/The World Bank Group Joint Child Malnutrition Estimates: Levels and Trends in Child Malnutrition: Key Findings of the 2020 Edition*; World Health Organization: Geneva, Switzerland, 2020b.

World Health Organization. Edition Global Anaemia Estimates in Women of Reproductive Age, by Pregnancy Status, and in Children Aged 6–59 Months. Geneva, Switzerland, 2021.

World Health Organization. *Indicators for assessing infant and young child feeding practices: definitions and measurement methods.* Geneva, Switzerland, 2021.

Wu, J., Li, J., Li, Y., Loo, K.K., Yang, H., Wang, Q., Duan, R., Xiao, X., Song, X., Yang, S. and Sun, L., 2018. Neurodevelopmental outcomes in young children born to HIV-positive mothers in rural Yunnan, China. *Pediatrics International*, 60(7), pp.618-625.

CHAPTER 2: LITERATURE REVIEW

2.1. INTRODUCTION

Chapter 1 provided the introduction and the research problem of this PhD study. Chapter 2 builds a theoretical foundation for the research by reviewing relevant literature to identify the key issues. Besides the discussion of the feeding practices in the form of complementary feeding, dietary intake, food frequently consumed; haemoglobin levels, anaemia; prevalence of HIV; undernutrition in the form of stunting, underweight, and wasting; and in infants, this chapter will further identify the main methodologies and research techniques that were used, as well as distinguish what was done in this research. The chapter includes the introduction, body, and summary of the literature reviewed, and a schematic overview of this literature review is presented in Figure 2.1.

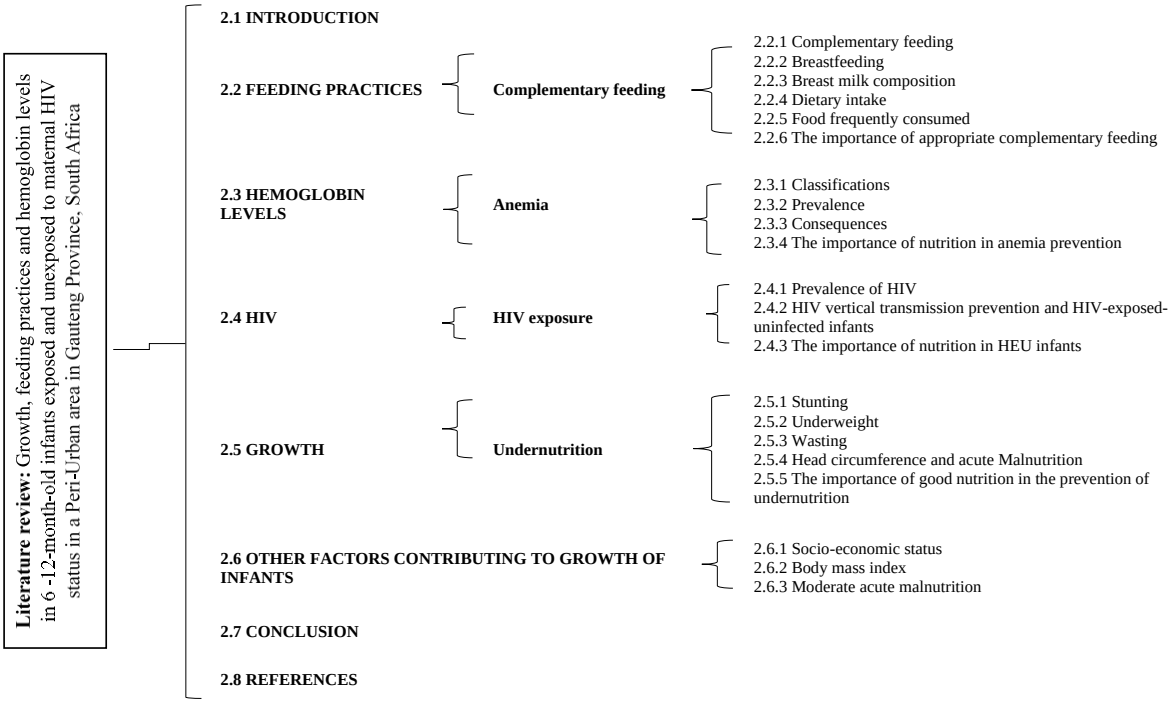


Figure 2.1 Schematic overview of the literature review

The third goal of the 17 Sustainable Development Goals (SDGs) focuses on good health and well-being, and its three targets aim at ensuring healthy lives and promoting the well-being of people of all age groups. The goal is to decrease maternal mortality to less than 70 per 100,000 live births, eliminate preventable infants and young children, and eradicate acquired immunodeficiency syndrome epidemics by 2030 (Nakai, 2018; UNICEF, 2016).

In the year 2019, South Africa had more than 200 deaths per 100 000 population related to maternal, perinatal and nutritional conditions such as lower respiratory infections, diarrhoeal diseases, TB, protein-energy malnutrition and HIV/AIDS in infants (World Health Organization, 2022). South Africa experiences a burden of malnutrition (underweight, stunting and wasting) in children under five years of age, which may lead to growth faltering, infections and death (Mandela, 2020). The hidden hunger or micronutrient deficiencies contribute to poor growth and development, poor immunity and tissue development, poor health, and risk of death in these children (UNICEF, 2018).

Due to the high nutritional needs of children under 2 years of age, the amount of food ingested needs to be closely monitored if they are still breastfeeding, in order to sustain growth and development (Dewey, 2013). Even with the continued benefits of breastfeeding in this age group, iron and zinc are still the most troublesome minerals during the complementary feeding period, as they are in low amounts in human breastmilk relative to requirement (Dewey, 2013; Dewey & Brown, 2003; Mahan & Raymond, 2016). Complementary foods must have a high nutritional density and for each nutrient amounting to per 100 kcal (kilocalories) of food (Dewey & Brown, 2003).

Appropriate feeding practices, including breastfeeding, have the potential to reduce malnutrition and common infection-related deaths in infants and children (Blanton, Barratt, Charbonneau, Ahmed & Gordon, 2016). Mothers' knowledge about feeding practices influence the choices that they opt for in infant- and child feeding (Chaponda, Goon & Hoque, 2017; Chezem, Friesen & Boettcher, 2003; Deeney & Harris-Fry, 2020; Fadnes, Engebretsen, Wamani, Semiyaga, Tylleskar & Tumwine, 2009; Goga, Doherty, Jackson, Sanders, Colvin, Chopra & Kuhn, 2012). HIV status of the mothers impacts on their infant feeding choices with regards to breastfeeding (Matji, Wittenberg, Makin, Jeffery, MacIntyre & Forsyth, 2009; West, Schwartz, Yende, Schwartz, Parmley, Gadarowski *et al*., Rie, 2019).

According to the UNAIDS (2023) HIV is one of the worlds among the ten leading causes of death, with Sub-Saharan Africa (SSA) being ranked number two accounting for 260 000 related AIDS death. Furthermore, South Africa has the greatest number of deaths associated with HIV in the SSA region, the third cause of maternal mortality and it has significant impact on pregnancy outcomes (Stats SA, 2022). HIV is one of the primary causes of death in children under 5-years of age, impacting on long-term health (National Department of Health, 2019;

WHO, 2019). These deaths have since reduced significantly to the introduction of the HIV vertical transmission prevention (HVTP) programme (UNAIDS, 2018). As a result, more infants are born HIV-exposed but uninfected (HEU) (UNAIDS, 2018). More than one million HEU children are born each year in SSA (UNAIDS, 2018).

HEU infants have greater nutritional needs, as they are more vulnerable to infections and micronutrient deficiencies, especially iron (Chalashika, Essex, Mellor, Swift & Langley-Evans, 2017; Evans, Jones & Prendergast, 2016; Evans, Chasekwa, Ntozini, Majo, Mutasa, Tavengwa, Mutasa *et al*., 2020; Omoni, Ntozini, Evans, Prendergast, Moulton, Christian *et al*., 2017; Wedderburn, Evans, Yeung, Gibb, Donald & Prendergast, 2019). The growth, feeding practices, nutritional needs and haemoglobin levels of these infants differ from those who are HIV-unexposed and uninfected (HUU).

The feeding practices of infants as they transition from exclusive breastfeeding to the introduction of complementary foods have the potential of compromising their growth and development (WHO, 2020). From 6 months of age, infants are at increased risk of anaemia, as iron levels in breastmilk start to decrease (Mahan & Raymond, 2016). Infants with low haemoglobin levels are at risk of stunting, as this is a marker of anaemia (WHO, 2021). Stunted growth has immediate and long-term consequences, such as increased morbidity and mortality, poor child development, compromised learning capacity, increased link to infections and increased risk to non-communicable-diseases (NCDs) later in life (WHO, 2020).

Current national policy, in line with the WHO guidelines, recommend exclusive breastfeeding for infants HEU and HUU for 0–6 months, and the introduction of complementary foods together with breastfeeding from 6 months up to 24 months (WHO, 2021). The implementation of these guidelines is not without challenges in infants starting from 6 months of age, with most mothers introducing inappropriate complementary foods that compromise the daily macro- and micronutrient requirements of the infants (Faber, 2007, Faber, Laubscher & Berti, 2016).

There is limited research focusing on the relationship between growth, feeding practices, and haemoglobin levels in 6–12 months HEU and HUU infants. This phenomenon has not yet been explored in an urban population with high HIV prevalence in the South African context (Ejigu, Magnus, Sundby & Magnus, 2020; Isanaka, Duggan & Fawzi, 2009; Lane, Widen, Collins & Young, 2020; Lane, Bobrow, Ndatimana, Ndayisaba & Adair, 2019b; Matsungo, Kruger, Faber, Rothman & Smuts, 2017; Morden, Technau, Giddy, Maxwell, Keiser & Davies, 2016).

The factors contributing to growth of the infants will be presented in the sub-headings to follow.

2.2. INFANT FEEDING PRACTICES AND GROWTH

Adequate nutrition is important for growth, health and development to full potential during infancy. Poor nutrition increases the risk of illnesses and is a major cause of death in children under 5-years. Optimal feeding is deemed important in the first thousand days as it ensures appropriate growth (WHO, 2020, 2021). Infants use large amounts of energy and nutrients, in proportion to their body size, to keep all their metabolic processes going (Whitney & Rolfes, 2018).

All MLWH and MnLWH or women with an unknown HIV status should receive four antenatal counselling sessions on infant feeding based on exclusive breastfeeding, appropriate complementary feeding, expressing, and storage of breastmilk at each ante-natal care visit (WHO, 2016, 2019).

2.2.1. Complementary feeding and undernutrition

Complementary feeding is a process of introducing solid foods and liquids other than breast milk or formula that are age-appropriate, nutrient dense, safe and diverse to an infant's diet, and plays a crucial role in the growth and development of infants. WHO recommends complementary foods for infants, including protein-rich foods, poultry, meat, fish, eggs, vitamin A-rich vegetables, and fortified foods (WHO, 2021). Low-income households find it difficult to buy commercially available fortified infant foods, which forces them to supplement their diets with regular home-cooked plant-based meals, which may be lacking in nutritional value (Abate, Nigat, Demelash, Emiru, Tibebu, Tiruneh *et al*., 2022).

Inadequate feeding practices are one of the underlying causes of undernutrition, therefore correct complementary feeding is important for optimal growth and development from 6 months of age, even in the context of HIV. At 6 months, appropriate, safe and nutritional adequate complementary foods should be introduced with continuous breastfeeding for two years if mothers are HIV negative. If mothers are HIV positive or have an unknown HIV status, they should continue to breastfeed only until 12 months with introduction of solids foods at 6 months (Faber *et al*., 2016; WHO, 2016).

According to UNICEF (2019a), poor diets have be shown to be the drivers of malnutrition in infants and children globally, as 44% of 6–24-month-old children are not receiving fruits or vegetables, and, furthermore, 59% are not receiving eggs, dairy, fish or meat. One in five children aged 6–23 months from rural areas are not receiving the minimum recommended diverse diet for healthy growth and brain development. Moreover, millions of children, especially in low-and middle-income countries (LMICs) are receiving too little of what is needed and too much of what is not needed; poor diets are now the main risk factor for the global burden of disease (UNICEF, 2019). Poor complementary feeding practices are widespread, complementary foods often are introduced too early or too late and are often nutritionally inadequate and unsafe (UNICEF, 2019).

In order to meet nutritional needs, infants should frequently be fed a variety of foods (UNICEF, 2016). The appropriate number of feeds depends on the energy density of local foods, and how much of the food is consumed at each feeding (WHO, 2021). Due to rapid growth and developments of 6-month-old infants, breastmilk is no longer sufficient to meet the nutritional needs of the growing infants. Therefore at 6 months, solid food should be introduced to the infants in addition to breastmilk, which is referred to as complementary feeding (WHO, 2021). From the age of 6 months, infants' dietary intakes are adjusted in order to meet energy recommended dietary allowance (RDA) that may be due to sensitivity to hunger and satiety cues. The gastric capacity of the infant also increases from 10 millilitres (mL) at birth to 200 mL by 12 months (Dewey & Adu-Afarwuah, 2008; WHO; 2021).

A challenge is found from 6–12 months, where the solid foods provided are less nutrient rich, causing infants to fail to exceed their required daily intake, making them more susceptible to infection and disease (Mugware, Motadi & Mushaphi, 2022). This is observed more in LMICs where poverty, and high morbidity and mortality rates of under-5 exist (Ricci *et al*., 2019).

Due to lack of regular and detailed data on the quality and quantity of foods consumed by South African young children (Hall, Sambu, Berry, Giese, Almeleh & Rosa, 2017), especially 6–12 months where complementary foods are introduced and breastmilk no longer contains enough nutrients to meet the infants' requirements, this study aims to determine the food frequently consumed, dietary intake of the infants at home by using a single 24-hour recall and food frequency questionnaire. A South African childhood review showed that 18% of infants were introduced to complementary food earlier than 6 months (Hall *et al*., 2017). With the introduction of solids foods as early as three months (Faber, Laubscher & Berti, 2016;

Mugware *et al*., 2022; Tshiamabra *et al*., 2023). Moreover, the solid foods introduced are micronutrient inadequate leading to poor diversity score and possibly iron and zinc deficiency (Dewey, 2013; Faber, 2005; Mahan & Raymond, 2016; WHO, 2021).

2.2.2. Breastfeeding: an intervention to prevent undernutrition

Good nutrition is important for normal growth and development, and has a positive effect on early childhood development in infants (Gladstone *et al*., 2019). The WHO recommends that that all infants should be exclusively breastfed (EBF) for the first 6 months of life, regardless of the mother's HIV status (Coovadia, Rollins, Bland, Little, Coutsoudis, Bennish *et al.*, 2007; Goga *et al*., 2012; WHO, 2019).

Exclusive breastfeeding means that the infants receive only breastmilk from the mother for the first 6 months of life, no additional water or solids given, and medicine is only given when provided by a healthcare worker. Sadly, the rates of exclusive breastfeeding in the Southern African region is as low as 32% (WHO, 2021). In the rural areas of Kwa-Zulu Natal in South Africa, breastfeeding had a positive effect on the growth outcomes of HEU infants and also lowered the risk of diarrhoea (Kindra, Coutsoudis, Esposito & Esterhuizen, 2012).

MLWH and who are on lifelong ART, whose infants are uninfected or having an unknown status, are advised to exclusively breastfeed. The infants should receive ART from birth until 6-weeks of age, or longer, depending on risk profile (WHO, 2016). At 6 months, mothers should introduce safe, appropriate complementary foods and continue to breastfeed their infants until 12 months and beyond. While MLWH and are not on lifelong ART whose infants are uninfected or haven unknown status should exclusively breastfeed their infants for 6 months and introduce complementary foods with continued breastfeeding for 12 months and beyond. When the mother wants to cease breastfeeding, the mother and the infant or only the infant whose mother wants to cease breastfeeding should receive ART and continue taking ART for one week after all breastfeeding has ceased (Department of Health, 2011; WHO, 2016).

Breastfeeding improves the HIV-free survival rate in LMICs (WHO, 2021). Giving ART to breastfeeding MLWH significantly reduces the risk of transmission through breastfeeding and also improves the mothers health (WHO, 2010). Moreover, breastfeeding is protective against all-cause morbidity and mortality for all children (Coutsoudis, Goga, Rollins & Coovadia, 2002; Goga *et al*., 2012). MLWH and exclusively breastfed their infants have a lower risk of transmitting HIV to their infants than mixed feeding. Besides the benefits of breastfeeding, if

ART therapy is not initiated timely and adhered to, the risk of vertical transmission is increased (Khanal, Scott, Lee, Karkee & Binns, 2015; Rollins, Ndirangu, Bland, Coutsoudis, Coovadia & Newell, 2013). Shorter duration or no breastfeeding by MLWH increases mortality of common childhood illnesses (Goga *et al*., 2012; Hailemariam, Adeba & Sufa, 2015; Kaldenbach, 2018).

Breastfeeding benefits both mothers and their children nationwide (Du Plessis *et al*., 2016), and it has the potential to prevent about 13% of deaths in South Africa (Du Plessis *et al*., 2016; Goga *et al*., 2012; Maccow, 2013) Despite all the benefits of breastfeeding, the breastfeeding rates in LMICs are still low, with only 37% of infants exclusively breastfed for the first 6 months of life, as one in five infants are exclusively breastfed before 6 months globally (UNICEF, 2019), regardless of HIV status (Rollins *et al.*, 2016). Exclusive breastfeeding rates for HEU infants were lower, at 54% (Ballard & Morrow, 2013; Butts, Hedderley, Herath, Paturi, Glyn-Jones, Wiens *et al*., 2018; Grote, Verduci, Scaglioni, Vecchi, Contarini, Giovannini *et al*., 2016). Another study in the North West Province found that 71% of infants at age 6 months were still being breastfed, but the rates decreased at 12 months (56%) (Swanepoel, 2018). The decrease in breastmilk intake after 6 months may be explained by the introduction of complementary foods, or mothers going back to work, amongst other reasons.

Maternal health status may also account for differences in breastfeeding practices between MLWH and MnLWH (Goga *et al*., 2012; Khanal et al., 2015). Maternal HIV status significantly impacts feeding practices (Chisti, Salam, Smith, Ahmed, Ashraf, Bardhan *et al*., 2011; Goga *et al*., 2012; Hailemariam *et al*., 2015). A Ugandan study showed that more MLWH ceasing breastfeeding before 12 months as compared to MnLWH (Fadnes *et al*., 2009).

Limited information exists on the growth and feeding practices of HEU infants, and available studies are presented and summarised in Table 2.2 with lack or breastfeeding reported in MLWH. MLWH in Uganda make poorer infant feeding decisions due to fear of HIV transmission through breastfeeding, largely due to their poverty and lack of education (Fadnes *et a*l., 2009; Jones, Sherman & Varga, 2005; Rollins *et al*., 2013). The risk of higher morbidity and mortality is still high in HEU infants with the counselling provided (Evans *et al*., 2020).

Table 2.1. Breastfeeding and growth outcomes of HEU infants in SSA (studies)

Authors, country & years	**Title**	**Objective**	**Study design Participants and sample size**	**Results**	**Limitations**	**Conclusions**
Le Roux, Abrams, Donald, Brittain., Phillips., Nguyen., Zerbe., Kroon. and Myer (2019); Kenya	Growth trajectories of breastfed HIV-exposed uninfected and HIV-unexposed children under conditions of universal maternal antiretroviral therapy: a prospective study	-To study and compare the growth of breastfed HEU children born to women who initiated ART during pregnancy	-Prospective cohort study -1087 mothers and newborn babies -Breastfed children until approximately 12 months postpartum.	-717 mother-infant pair data available -Breastfeeding was shorter among HEU than HUU infants -HEU children had consistently lower mean WAZ, LAZ scores than HUU infants -HEU were more stunted and overweight	-Lack of birth data: HC and length -Loss of follow-ups -Findings limited to infants not conceived on ART	-Compared with HU children, HEU children have small deficits in early growth trajectories -Large proportions of both HEU and HU children were overweight by 12 months
Kindra et al. (2012); South Africa	Breastfeeding in HIV Exposed Infants Significantly Improves Child Health: A Prospective Study	-To examine the impact of feeding mode on health and nutritional changes in mothers and their infants over a 9-month period.	-Prospective cohort study	-138 mother-infant pair -75 were in the formula feeding group -63 in the breastfeeding group	-Not reported	-Breastfeeding was associated with a positive impact on child growth and development and lower incidence of diarrhoeal disease on HEU infants.
Lang'at, Ogada, Steenbeek, MacDonald, Ochola, Bor and Odinga (2018); Kenya	Infant feeding practices among HIV-exposed infants less than 6 months of age in Bomet County, Kenya: an in-depth qualitative study of feeding choices	-To identify barriers to optimal feeding among HIV-exposed infants 0–5 months of age attending a mission hospital in Bomet County, Kenya.	-Cross-sectional qualitative study -Four focus group with 9 mothers/caregivers of HIV-exposed infants aged 0–5 months -Interviews were also held with healthcare worker	-Factors influencing the infant feeding choices were: • financial constraints, • Cultural beliefs and practices, and HIV-related stigma. • In addition, conflicting knowledge among mothers/caregivers and healthcare workers	-Lack of direct observation of infant feeding practices -The study was conducted in one rural area only	-Barriers can be modified. -Training emphasising the correct WHO recommendations

2.2.3. Breastmilk composition of MLWH and MnLWH

Human breastmilk contains essential nutrients that are significant to infant health and survival, growth, and development (Ballard & Morrow, 2013). Breastmilk is the most nutritious food for infants comprising of 87% water, 1% protein, 4% fat, 7% carbohydrate and 1% other nutrients (Andreas, Kampmann & Le-Doare, 2015; Boquien, 2018). Breastmilk is a dynamic source of many specific- and non-specific immune factors associated with maternal and infant health and infant nutrition (Pedersen, Wilkinson, Andreasen, Kinung'hi, Urassa, Michael *et al*., 2016).

Maternal, infant, and physiological factors influence human milk composition, as shown in Figure 2.5. Common factors include mother's age, nutritional status, and infants' nutritional status. Regardless of HIV status, body composition affects breastmilk composition (Boix-Amorós, Collado, Van't Land, Calvert, Le Doare, Garssen *et al*., 2019). A South African study found that MLWH with high breastmilk production allows mothers to breastfeed infants without compromising fat-free mass (Mulol & Coutsoudis, 2016).

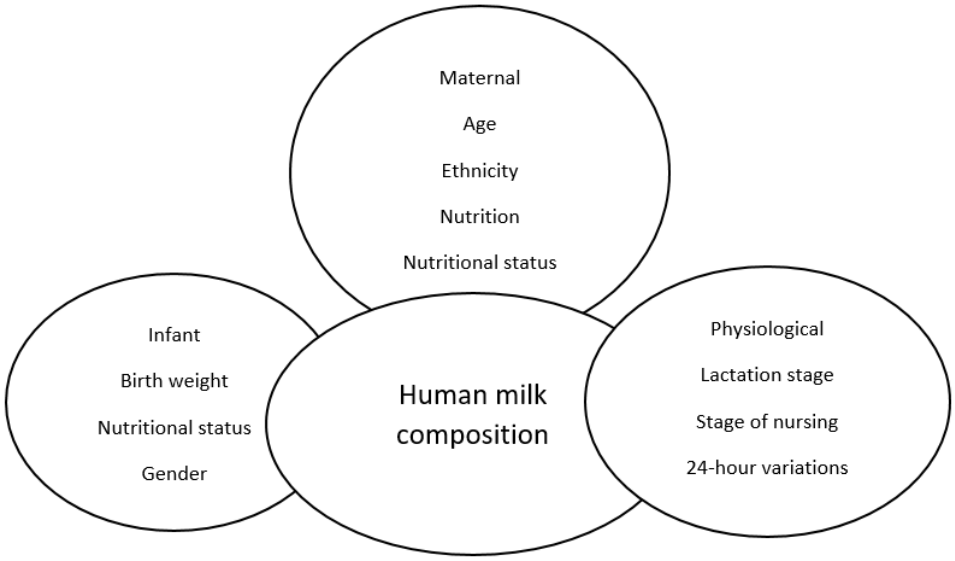


Figure 2.3. Factor affecting human breastmilk composition

A systematic review by Keikha, Bahreynian, Saleki and Kelishadi (2017) including 59 observational- and 43 interventional studies, found mothers' dietary intake to be associated with the breastmilk composition. However, some studies reported no correlation with the breastmilk composition, and A New Zealand study found no significant correlation between mothers' dietary nutrients (Butts et al., 2018; Bzikowska-Jura, Czerwonogrodzka-Senczyna, Oledzka, Szostak-Wegierek, Weker & Wesolowska, 2018). This highlights the importance of

investigating the composition of breastmilk in MLWH and MnLWH in a high HIV-prevalence setting with a similar comparison group (Evans *et al*., 2020; Slogrove *et al*., 2016) to understand the factors contributing to poor growth in HEU infants.

Human breastmilk is vital for infant immune system development and reducing allergic phenotypes (Boix-Amorós *et al*., 2019). Mature breastmilk contains immunological components for baby health and nutrition. Trace elements are needed in small amounts, but play a major role in the growth and development of the infants, as the infant's diet mainly comes from breastmilk in the first 6 months of life (Mahan & Raymond, 2016; Pedersen *et al*., 2016).

The composition of micronutrients in breastmilk is lower in MLWH than MnLWH due to the presence of HIV in the body which reduces breastmilk composition (Rahamon, Onifade, Arinola, 2018). A small Nigerian study found low levels of essential trace minerals in breastmilk of MLWH (n=20) and MnLWH (n=30), with notable differences in copper (67.7±5.0 vs 71.1±5.5; p=0.033) and iron (66.2±6.3 vs 71.2±6.5; p=0.011) levels in MLWH and MnLWH (Rahamon, Arinola, Akiibinu, 2013). This finding was also observed in a South African study who found the breastmilk composition of copper, iron, riboflavin, vitamin B6, vitamin C, and folate lower in MLWH than MnLWH (Fouché, van Niekerk & du Plessis, 2016). Zinc composition, however, was similar in MLWH and MnLWH (Fouché *et al*., 2016; Rahamon *et al*., 2013; Rahamon *et al*., 2018).

There are many different methods of analysing breastmilk composition. However, the preferred method for analysis depends on the micronutrient of interest and its (active) forms found in milk (Hampel, Dror & Allen, 2018; Lönnerdal, 2017). The inductively coupled plasma mass spectroscopy (ICP-MS) method has been used and described as a suitable method in analysing human breastmilk (Hampel *et al*., 2018). Iron, zinc, manganese, selenium, and copper are needed in small amount in the body, but their function is vital for the growth and development of postnatal outcomes and the ranges in human breastmilk are summarised in Table 2.3. Mineral analyses such as iron, zinc have evolved over time from the colorimetric approaches to more sophisticated techniques such as the ICP-MS (Hampel *et al*., 2018).

Table 2.2: Breast milk composition ranges (mean ± SD)

Trace elements (references)	**Normal ranges of breastmilk composition** (mg/L)
Iron (Friel & Qasem, Cai, 2018)	0.2–0.4
Zinc (Rios-Leyvraz & Yao, 2023)	1–3
Manganese (Frisbie, Mitchell, Roudeau, Domart, Carmona & Ortega, 2019)	0.1–0.3
Copper (Khaghani, Ezzatpanah, Mazhari, Givianrad, Mirmiranpour & Sadrabadi, 2010)	0.1–0.3
Selenium (Zachara & Pilecki, 2000)	0.1–0.2

Iron is an essential nutrient that exists in low quality in human breastmilk, but it declines with time as the infant grows. Although its quality is low, it is more bioavailable to the infant, as about 50% of iron is absorbed by the infant, unlike cow's milk with only about 10% bioavailability (Lönnerdal, 2017). The infants first 6 months of life is a crucial time in meeting iron needs, as breastmilk contains very little iron content (0.4 mg/L). In order to support the infant's rapid growth and development, additional iron intake is required, as milk products (both human milk and formula milk) do not contain sufficient iron to adequately supply needs after 6 months of age (Whitney & Rolfes, 2018). Lower levels of iron may affect the growth of the infants, as it is important for red blood formation and muscle mass. On the other hand, zinc is an anti-inflammatory and antioxidant agent that is important for good appetite, growth improvements and good hair for infants (Mahan & Raymond, 2016).

2.2.4. Dietary intake of infants

A single 24-hour recall structured questionnaire is reliable, as it is quick and easy to administer with low respondent burden, and, based on previous studies (Cape, Faber & Benadé, 2007; Faber, 2007; Faber *et al*., 2017), it may be used in research settings. Estimated average requirements (EAR) are the minimum nutrient intakes needed to meet half of a population's needs (Institution of Medicine, 2000; Patel & Rouster, 2020). These are used to plan nutritionally adequate diets and assess individual nutrient intakes. Recommended dietary allowance (RDA) is the daily nutrient and calorie intake necessary for good health, calculated for various ages and genders (Meyers & Suitor, 2007). In cases where EAR or RDA are insufficient, adequate intakes (AI) is the acceptable range. However, strategies for estimating insufficient intakes and planning complementary feeding for infants are limited, particularly in

LMICs where fortified foods may be required (Meyers & Suitor, 2007; Smuts, Dhansay, Faber, van Stuijvenberg, Swanevelder, Gross *et al*., 2005; Swanepoel, Havemann-Nel, Rothman, Laubscher, Matsungo, Smuts *et al*., 2019).

In order to achieve the minimal dietary diversity, the WHO recommends the intake of at least five of the eight food groups, including breastmilk; grains, white/pale starchy roots, tubers and plantains; beans, peas, lentils, nuts and seeds; dairy products; flesh foods; eggs; vitamin A-rich fruits and vegetables; and other fruits and vegetables (WHO, 2021). South African infants' diet mainly comprises of maize meal and commercial products, and mostly fail to meet the dietary diversity score (Faber *et al*., 2016; Swanepoel *et al*., 2019; Tshiambara *et al*., 2023). In South Africa, more than 94% of the infants aged 6–11 months in both rural and urban areas did not meet the minimum dietary diversity score (Faber *et al*., 2016). However, in Ethiopia, only 42% of MLWH with HEU children aged 6–24 months did not achieve the minimum dietary diversity score (Yisak, Ambaw, Walle, Alebachew & Ewunetei, 2020).

Macronutrients are needed in large amounts in the body, and play a crucial role in growth and development of infants (Mahan & Raymond, 2016). The intake of carbohydrate is high in LMICs, and a South African study reported a high intake and doubling the dietary reference intakes (DRI) of 6–12-month-olds (Faber, 2007). However, HEU children had a low carbohydrate intake in Malawi (Parker, Tembo, Adair, Chasela, Piwoz, Jamieson *et al*., 2013). High protein intake has been reported in South African and Rwandan infants (Faber *et al*., 2016a; Lane, Bobrow, Ndatimana, Ndayisaba, Adair, 2019a) and this may be due to the consumption of breastmilk substitutes containing high percentage of casein, with a high risk of allergy, overfeeding and poor absorption (Hernell, 2011; Stuebe, 2009). Fat intake is low in LMICs, as has been reported in breastfed Malawian HEU and South African infants (Faber *et al*., 2016; Parker *et al*., 2013; Swanepoel *et al*., 2019). The low intake of fat is a concern, as dietary fat plays a crucial role in the growth and development of infants by providing energy, absorption of fat-soluble vitamins, and brain development (Mahan & Raymond, 2016; Parker *et al*., 2013). Higher intakes of carbohydrates and protein may lead to overweight and obesity early in life, which can lead to non-communicable diseases later in life (Mahan & Raymond, 2016).

Vitamin A intake is lower in LMICs than high-income countries, with low intakes found in Malawian HEU children and South African infants (Faber *et al*., 2016; Parker *et al*., 2013).

Consumption of vitamin A-rich foods should be encouraged in our setting, as the rich food sources of vitamin A are inexpensive and widely available (Faber, 2007).

Calcium is essential for the development of strong bones and teeth, and supporting the immune system, with lack of calcium intake from 6 months during growth spurts known to have detrimental effects on bone development, resulting in rickets and stunting (Mahan & Raymond, 2016). Low calcium intake was found in Rwandan infants (Lane *et al*., 2019) and Malawian HEU children (Parker *et al*., 2013). Low intakes of iron was reported in HEU breastfed children in Malawi (Parker *et al*., 2013). Due to the increased needs of iron in the body for optimal growth and development, low iron concentration in breastmilk and low consumption of flesh food products, failing to meets nutritional needs may lead to anaemia (Mahan & Raymond, 2016).

It is important to determine the dietary intake of breastfed and non-breastfed in HEU and HUU infants as data on the actual intake of breastmilk of infants is limited.

2.2.5. Food frequently consumed by infants

Food frequency questionnaires have previously been used and described elsewhere (Faber et al., 2016a; Theron, Amissah, Kleynhans, Albertse, MacIntyre, 2007). This tool is quick and easy to administer, but may lead to recall bias as it asks mothers to think about what the infants had consumed in the past 7 days. In KwaZulu-Natal Province of South Africa, a study found soft maize meal porridge (84%), potato (72%), yoghurt (35%), eggs (44%), meat (16%), and vitamin A-rich foods (below 50%) and miscellaneous products such as chips (< 65%) were commonly consumed foods by infants 6–11 months old (Faber et al., 2016a). In HEU and HUU infants, the commonly consumed food was soft porridge, maize meal, miscellaneous products and cereals, with animal foods, fruits and vegetables rarely consumed (Tshiambara et al., 2023).

2.2.6. The importance of introducing correct appropriate complementary foods

After the first 6 months of life, breast milk might not provide all of the nutrients required for an infant's growth (Mahan & Raymond, 2016) and complementary feeding helps infants meet their nutrient needs (Dewey, 2013). Proper nutrition during the complementary feeding stage is important for cognitive development, and omega-3 fatty acids found in foods such as fish and eggs are essential for brain development (Mahan & Raymond, 2016). Infants iron stores

diminishes from 6 months and complementary foods rich in iron, including fortified cereals and animal products, are important for increasing haemoglobin levels and preventing anaemia (Dewey & Adu-Afarwuah, 2008). Appropriate complementary foods assist in the development of oral- and motor skills, including chewing, swallowing, and self-feeding, which are essential for development (Dewey, 2001). The exposure from the variety of diets helps infants develop different food preferences, contributing to healthier eating habits later in life. A variety of food, including fruits, vegetables and yogurt in the infant's diet, is important (Gibson, Ferguson & Lehrfeld, 1998), and complementary foods help infants to gain appropriate weight and growth for their age and prevent malnutrition (WHO, 2021).

2.3. HAEMOGLOBIN AND GROWTH

During the first few months of life, infants experience a physiological decline in their blood volume and haemoglobin concentration, and an active shift from foetal haemoglobin to an adult type haemoglobin. Biochemistry is a method used to assess an individual's nutritional status, often conducted in hospitals and laboratories. It involves analysing factors such as total cholesterol, serum triglycerides, serum glucose, and haemoglobin (Mahan, Raymond, 2016). At birth, full-term healthy infants have an iron content of 75 mg/kg, high blood volume, and haemoglobin concentration proportional to their body weight (Lönnerdal, 2017). However, during the first few months, infants experience a decline in blood volume and haemoglobin concentration, and a shift from foetal- to adult type haemoglobin. Iron is also an essential component of haemoglobin, the oxygen-carrying pigment for red blood cells. Insufficient haemoglobin results in insufficient oxygen supply to cells (Lönnerdal, 2017; Qasem & Friel, 2015). Iron is a crucial component of haemoglobin, the oxygen-carrying pigment in red blood cells. Insufficient haemoglobin results in insufficient oxygen supply to cells (Mahan & Raymond, 2016; Motadi, Mbhenyane, Mbhatsani, Mabapa & Mamabolo, 2015).

2.3.1. Classification of anaemia (haemoglobin levels)

The normal haemoglobin levels for infants between the ages of 6–12 months is 13.5 g/dL (Irwin & Kirchner, 2001; Teklemariam, Mitiku & Mesfin, 2015; WHO, 2021). Infants aged 6–12 months are classified as anaemic when the haemoglobin level is < 11g/dl (WHO, 2021). Foetuses and infants tend to have higher average haemoglobin levels than adults, as there is an increased need for oxygen levels in the womb, for red blood cells to transport the oxygen, and for faster growth and development, more than in adults (Mahan & Raymond, 2016). Table 2.4

summarises the cut-off for haemoglobin levels in classifying anaemia in infants aged 6 months and all agree that haemoglobin levels below 11 g/dL are classified as anaemia.

Table 2.3. Anaemia cut-offs of infants from 6 months using haemoglobin levels from different studies

Authors and country		**Cut-offs**	**Age (months/years)**
Nambiema, Robert and Yaya (2019), Togo		<11 g/dL	6–59 months
Li, Liang, Liang, Shi and Han (2019), Beijing		<110 g/L	6 months
Huo, Sun, Fang, Chang, Zhao, Fu, Wang, Huang, Wang and Begin (2015), China		<11g/dL	6–59 months
Allali, Brousse, Sacri, Chalumeau and de Montalembert (2017)		<110 g/L	6 months
Provan, Singer and Baglin (2009)		<101 g/L	12 months
World Health Organization (2021c)	Pregnant women	<11 g/dL	(≥15 years)
	Children months of age	<11 g/dL	6–59 months
	Non-pregnant women	<12 g/dL	(≥15 years)

2.3.2. Prevalence of anaemia

Children in LMICs experience higher prevalence of anaemia. Anaemia is a major public health problem in developing countries and a direct cause of childhood mortality and morbidity (WHO, 2011). Mothers' haemoglobin levels may influence infants' haemoglobin levels (Ntenda, Nkoka, Bass & Senghore, 2018), with mothers with lower haemoglobin levels more likely to give birth to infants with lower haemoglobin levels (<11 g/dL).

The South African Childhood Review revealed that about 11% of children under-5 are anaemic. However, the sample size was too small to analyse for provincial level (Hall et al., 2017). A study conducted in the North West Province found 36.5% infants aged 6 months were anaemic with a haemoglobin <11g/dL (Matsungo, Kruger, Faber, Rothman & Smuts, 2017). Anaemia was also one of the contributing factors of stunting as 6-month-old infants who were anaemic were also stunted (45.3%) in the same Province (Matsungo *et al*., 2017). Moreover, 50 % of the women in LMICs were anaemic (WHO, 2017). A strong association exists between maternal anaemia and childhood anaemia according to a study concluded in four Southern African countries of 6–23-month-old children (Ntenda *et al.*, 2018).

Children in the 6–24 months age group are the most severely affected by anaemia in South Africa, which may be compromised by poor quality diet, poor selection of foods, or any dietary extremes. Iron in infants is crucial for better cognition development. According to the UNICEF (2018), impaired cognitive ability, reduced school performance and work performance result due to poor nutrition in the first 1000 days of life, which then leads to stunted growth. Furthermore, positive cognition predicts better educational outcomes (Smith, Zizzo, Amzel, Wiant, Pezzulo, Konopka, Golin & Vrazo, 2018). A comparative study in China found that HEU infants and children from 6-30 months had lower neurodevelopment than the HUU children (Wu, Li, Li, Loo, Yang, Wang *et al*., 2018).

Positive cognition has been used to predict better educational outcomes (Smith *et al*., 2018). It was demonstrated by a study conducted in Cape Town that children living with HIV, who started ART early in life, have better short-term neurodevelopmental outcomes than infants for whom treatment is deferred (Cortina, Jack, Pearson, Kahn, Tollman, Hlungwani, *et al.*, 2019; Wu *et al*., 2018). Child development is negatively impacted by HIV, with children that are infected and affected by HIV performing worse than their peers in cognitive assessments (Sherr, Hensels, Tomlinson, Skeen & Macedo, 2018).

2.3.3 Consequences and risk factors of anaemia

Anaemia can be caused by a diet poor in terms of iron-rich foods, or a high consumption of food that prevents the absorption of iron. Two types of iron are absorbed into the body differently and come from different sources. Heme iron is found in meat and meat products, but calcium hinders the absorption of heme iron. Non-heme iron is found in variety of foods, such as fruits and vegetables, and the absorption is affected by the amount of iron in the body (National Department of Health, 2019). Acute illness, opportunistic infections, and drugs may cause anaemia in infants. HIV is the main determinants of undernutrition in Africa, which then leads to multiple deficiencies and malnutrition (Ricci *et al*., 2019). As the morbidity of HEU infants is high, their risk of failure to thrive is also high. However, the prophylaxis in the form of ART treatment improves weight, growth and development of HEU infants, whilst improving their survival.

Anaemia is a frequent complication of HIV infection and is commonly found in HEU infants. The HVTP programme reduces child infection rates, but may increase the risk of anaemia in

infants. Higher risk was found in infants breastfed than formula fed in Botswana (Slogrove *et al.*, 2018).

HEU infants are at a higher risk for neurodevelopmental delay. Anaemia has been observed to be more prevalent in HEU than HUU infants (Teklemariam *et al*., 2015). A high prevalence of anaemia and growth failure among the HIV-infected children was found in Ethiopia, (Teklemariam *et al*., 2015) which may be due to the inadequate complementary feeding practices in 6–59-months-old children. Furthermore, the main predictors of anaemia in children during 6–59 months was inadequate complementary foods (Da Silva *et al*., 2018).

2.3.4. Importance of nutrition in anaemia prevention

Good nutrition is critical for the first 1000 days, especially in preventing undernutrition and anaemia (Mahan & Raymond, 2016; WHO, 2021). Foetal haemoglobin depends on the mother's haemoglobin, but after birth the infant produces its own haemoglobin, hence, exclusive breastfeeding is important for the first 6 months in order to supply the body with haemoglobin from breastmilk (WHO, 2020). After 6 months, iron requirements increase and breastmilk alone does not provide the needed amount, thus, the introduction of age-appropriate, safe and diverse food starts from 6-months with continuation of breastfeeding (Dewey, 2013; WHO, 2021).

2.4. HUMAN IMMUNODEFICIENCY VIRUS AND ACQUIRED IMMUNODEFICIENCY SYNDROME

The literature suggests that HIV exposure without infection can affect child growth and development through two pathways: indirectly through augmenting universal risk factors for poor development, and directly through HIV-specific mechanisms like HIV virions, immune activation, and antiretroviral therapy (ART) toxicity (Wedderburn *et al*., 2019). A conceptual framework has been developed to hypothesise the pathways through which HIV/AIDS and ART exposure affect child growth and development, with lines in red and blue representing HIV-specific pathways and universal pathways, respectively (Wedderburn *et al*., 2019). The conceptual framework was adjusted to fit this study's focus.

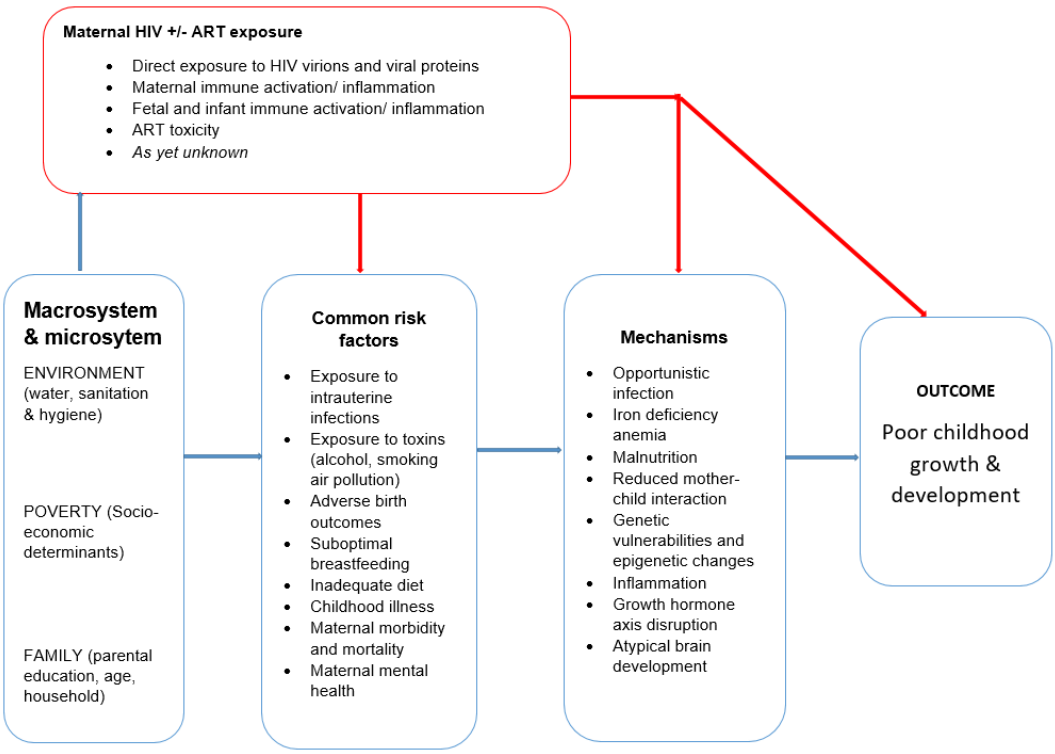


Figure 2.4: Conceptual framework of HIV and ART exposure (adapted from Wedderburn et al., 2019).

According to the WHO, HIV is a virus that attacks cells that help the body fight infection, making a person more vulnerable to other infections and diseases. It is spread by contact with certain bodily fluids of a person with HIV. If left untreated, HIV can lead to the disease AIDS (UNAIDS, 2018). AIDS is the late stage of HIV infection that occurs when the body's immune system is badly damaged because of the virus. However, by taking ART, people with HIV/AIDS can live long and healthy lives and prevent transmitting HIV, including from mother to child (Desalu, 2017; Martin, Chinnock, Perales, Lee, Lee, Wu *et al*., 2017). HIV/AIDS is a multifaceted biological, behavioural, and social phenomenon with significant morbidity and mortality implications. Its prevention and treatment using ART requires a complex strategy based on specific guidelines. According to the National Department of Health (2019) a person with HIV is considered to have progressed to AIDS when:

- the number of their CD4 cells falls below 200 cells per cubic millimetre of blood (200 cells/mm3). (In someone with a healthy immune system, CD4 counts are between 500 and 1,600 cells/mm3.) OR
- they develop one or more opportunistic infection, regardless of their CD4 count.

2.4.1. HIV prevalence worldwide, Sub-Saharan Africa and South Africa

The prevalence of people living with HIV (PLWH) keeps increasing globally. In 2020, 1.7 million people were newly infected with HIV (UNAIDS, 2022b; UNIAIDS, 2020). According to the Joint United Nations Programme on HIV/AIDS (UNAIDS) (UNAIDS, 2022a), 38.4 million people were living with HIV globally in the year 2021, even though HIV infection rates have been reduced by 40%. Women and girls accounted for 54% and 1.8 million were children, with 1.7 million children between 0–14 years of age living with HIV globally in 2021 (UNAIDS, 2022a).

In South Africa, 8.45 million (13.9%) of the population were living with HIV in 2022, with 19.6% of adults between the ages of 15-49-years living with HIV (Stats, 2022). In 2021, 63% of new infections in SSA were from women of reproductive age (UNAIDS, 2022a). In 2019, 30% of South African pregnant women seeking care in the public health sector were living with HIV (Woldesenbet, Lombard, Manda, Kufa, Ayalew, Cheyip, 2021). In the Eastern and Southern part of Africa, 95% of pregnant women were accessing ART medicines to prevent-mother-to-child transmission. However, more than one million children are born who are exposed but uninfected to maternal HIV infection in the SSA regions (UNIAIDS, 2020).

2.4.2. HIV vertical transmission prevention and exposed infants

The high prevalence of HIV/AIDS in women in has a direct impact on the number of infants born with- or exposed to HIV. This is because it can spread through vertical transmission during pregnancy, delivery and breastfeeding (Evans et al., 2016a; UNICEF, 2016). South Africa, like many other countries, has HIV vertical transmission prevention (HVTP) and ART programmes, which were initiated in the early 2000s, reducing the rates of vertical transmission. These programmes have resulted in a reduction of vertical transmission from between 25–30% to 2–5% (Burton, Giddy, Stinson, 2015; Study, 2005). The VT rates were further reduced from 3.6% in 2011 to 1.3% in 2017 (Department of Health, 2014; Musakwa, Feeley, Magwete, Patz, McNamara, Sanne *et al*., 2020). Successful implementation of the HVTP programme has, therefore, seen the emergence of HEU infants born to mothers living with HIV (MLWH) (Evans et al., 2016a; Slogrove, Johnson & Powis, 2018). Hence, in SSA over one million HEU infants are born annually (Sugandhi *et al*., 2013; UNAIDS, 2018).

The ART program aims to achieve and sustain viral suppression while reducing morbidity and death from HIV and improving the quality of life for people living with HIV (National

Department of Health, 2019). Despite the successful rollout of ART in South Africa, 85 796 HIV-related deaths were reported in the year 2022 (Stats, 2022).

Studies suggested that HEU infants and children have an increased risk of severe infections and mortality compared to HUU infants (Evans et al., 2016a; Filteau, 2009; Gladstone, Chandna, Kandawasvika, Ntozini, Majo, Tavengwa *et al*., 2019; Saloojee, De Maayer, Garenne & Kahn, 2007). HEU infants have a higher incidence of severe infections, hospitalisations and death, and lower immune responses to some vaccines compared to HUU (Jalbert, Williamson, Kroehl, Johnson, Cutland, Madhi *et al*., 2019; Yeganeh, Watts, Xu, Kerin, Joao, Pilotto, Theron *et al*., 2018). The excess morbidity and mortality are largely due to respiratory viral pathogens and *S. pneumoniae*, with lower maternal antibodies against these pathogens. According to Evans et al (2016) and Wedderburn et al. (2019), the high morbidity results in poor growth and development outcomes, as sick mothers may not be able to provide adequate care, or their children may even eventually be orphaned.

Orphaned infants often experience extreme poverty and homelessness, leading to psychosocial- and economic challenges that affect the overall well-being of the children (Breckenridge, Black-Hughes, Rautenbach, McKinley, 2019; Evans, Jones, Prendergast, 2016b), with over 3.7 million estimated AIDS orphans. These two factors, therefore, contribute significantly to poor health outcomes and may lead to psychosocial and economic challenges that affect the overall well-being of the children (Sugandhi et al., 2013). Literature has shown that the HEU infants suffer from high morbidity and mortality rates, as well as poor growth outcomes. However, it is not clear what contributes to the poor growth of HEU infants, whether it is the feeding practices, haemoglobin levels, or composition of breastmilk fed to these infants (Horwood, Haskins, Vermaak, Phakathi, Subbaye, Doherty, 2010). Studies conducted on the growth and health outcomes of HEU infants in Africa are summarised and presented in Table 2.4 where the prevalence of stunting, underweight and wasting is high in HEU infants as compared to HUU infants.

Table 2.4. Growth and health outcomes of HEU infants in Africa (studies)

Authors	Title	Objective	Participants	Study design	Results	Conclusion
Ejigu et al. (2020), Ethiopia	Differences in Growth of HIV-exposed uninfected Infants in Ethiopia according to timing of in-utero antiretroviral therapy exposure	To compare growth of HEU-infants according to timing and type of ART exposure	-HIV-infected mothers and HEU-infants in Addis Ababa, Ethiopia -Between February 2013 and October 2016	-Retrospective cohort study	-624 HEU-infants -Risk of stunting was 51.9 per 100 person-years -Risk of underweight was 26.7 per 100 person-years	In HEU-infants, exposure to ART from conception was associated with decreased growth during early infancy and higher incidence of stunting compared with treatment exposure later in pregnancy.
Evans et al. (2016c), Zimbabwe	HIV-exposed uninfected infants in Zimbabwe: insights into health outcomes in the pre-antiretroviral therapy era	To propose a conceptual framework to explain the increased risk of infectious morbidity, mortality, and growth failure among HEU infants, hypothesising that immune activation and inflammation are key drivers of both infection susceptibility and growth failure	-14 110 mother–infant pairs -1–2 years of follow-up	-Randomised control trial -Vitamin A between 1997 and 2000, before the availability of antiretroviral therapy for HIV prophylaxis or treatment in Zimbabwe	-Immune activation and inflammation may be key drivers of both infection susceptibility and growth failure in HEU infants.	-Infants recruited to the ZVITAMBO trial have contributed to understanding of the HEU population. -Future studies should draw on these and other results in order to determine the causes of infection susceptibility and growth failure, and determine the impact of ART and cotrimoxazole on outcomes of this vulnerable group of infants.
Rosala-Hallas, Bartlett and Filteau (2017), Zambia	Growth of HIV-exposed uninfected, compared with HIV-unexposed, Zambian children: a longitudinal analysis from infancy to school age	To determine whether the early and later growth, measured as weight-for-age, height-for-age and body mass index (BMI)-for-age Z-scores, of HIV unexposed-uninfected (HUU) and HEU children differ.	-207 HUU and 200 HEU infants from the Breastfeeding and Postpartum Health (BFPH) study, and -580 HUU and 165 HEU from the Chilenje Infant Growth, Nutrition and Infection Study (CIGNIS) -had anthropometric measurements taken during infancy and again when school-aged, -at which time 66 BFPH children and 326 CIGNIS children	-Longitudinal analysis to compare growth	-HEU children had lower weight-for-age, length-for-age and BMI-for-age Z-scores during early growth, and -these differences still existed when children were school-aged.	-HEU children have poorer early growth than HUU children, which persists into later growth. -Interventions to improve growth of HEU children need to target pregnant women and infants.

Authors	Title	Objective	Participants	Study design	Results	Conclusion
Slogrove, Goetghebuer, Cotton, Singer and Bettinger (2016), South Africa	Pattern of infectious Morbidity in HIV-exposed uninfected infants and children	To appraise the existing clinical evidence of the pattern of HEU infant infectious morbidity to aid understanding of the potential mechanism of susceptibility.	n/a	-Systematic review	-Only 3 of 22 eligible identified studies were designed to primarily compare HEU and HUU infants for infectious morbidity. -Fourteen were conducted prior to 2009.	-Llarger prospective studies with appropriate HU infant comparison groups are necessary. - Collaboration among researchers.
Morden et al. (2016), South Africa	Growth of HIV-Exposed Uninfected Infants in the First 6 Months of Life in South Africa: The IeDEA-SA Collaboration	To describe the characteristics of South African HEU infants, investigate factors impacting birth weight, and assess their growth within the first 28 weeks of life.	-2621 HEU infants from McCord Hospital (MH) in KwaZulu-Natal Province and Rahima Moosa Mother and Child Hospital (RMMCH), Gauteng Province	-Retrospective cohort clinical data review -Data were collected between 2007 and 2013	-Exclusive breastfeeding=0.5%, -Breastfeeding=7.9%, -Mixed feeding=0.08% -Formula feeding= 89.2% -Lower birth WAZ for CD4 <200 cells/µl compared CD4 500 cells/µl mothers	-Less severe maternal disease and the use of antiretrovirals positively impacts birth weight in this cohort of South African HEU infants. -Formula feeding was common with breastfed infants experiencing marginally slower longitudinal growth.
Lane et al. (2020), Rwanda	Determinants of growth in HIV-exposed and HIV-uninfected infants in the Kabeho Study	-To characterise infant growth trajectories in a cohort of HEU infants, and to identify factors associated with healthy growth.	-Pregnant mothers -Infants 0–2 years old	-Longitudinal cohort study	-HEU infants had moderate linear growth faltering, but only modest faltering in weight, resulting in lower mean WLZ and LAZ. -Low neonatal WAZ and a high rate of illness increased the likelihood that infants were in the lightest WLZ	-Mean growth trajectories of these HIV-exposed and HIV-uninfected infants show that they continue to experience meaningfully lower LAZ and elevated WLZ than the WHO standard. -This pattern is not seen among other infants in low-resource settings.
Ram et al. (2012), India	Growth patterns among HIV-exposed infants receiving nevirapine prophylaxis in Pune, India	To compare the growth patterns of HIV-infected and HIV-exposed but uninfected infants and account for timing of HIV infection, and identify risk factors for stunting, underweight and wasting.	HEU and HUU infants	-Randomise control trial	HIV-infected and HEU infants, baseline prevalence of stunting (48% vs. 46%), underweight (27% vs. 26%) and wasting (7% vs. 11%) was similar ($p>0.29$), but by 12 months stunting and underweight but not wasting were significantly higher in HIV-infected infants (80% vs. 56%, 52% vs. 29%, $p< 0.0001$; 5% vs. 6%, $p=0.65$, respectively).	-Baseline stunting and underweight was high in both HIV-infected and uninfected infants. -Growth indices diverged early.

2.4.3. The importance of nutrition in HEU infants

Good nutrition allows children to survive, grow, develop, learn, play, participate and contribute, while malnutrition robs children of their futures and leaves young lives hanging in the balance (WHO, 2020). The rates of malnutrition remain disturbing, as stunting declines too slowly, and wasting continues to affect a majority of children under five years of age (WHO, 2020). In the year 2019, 1.5 million infants died globally due to conditions that can be prevented by growth and monitoring services, adequate nutrition, and safe water and food quality (WHO, 2020). The leading cause of death in under 5-year-old children in South Africa from the years 1997-2012 were identified as HIV/AIDS (19%), diarrhoea (16%) and pneumonia (12%), all of which can be prevented (Nannan, Groenewald, Pillay-van Wyk, Nicol & Msemburi, 2019).

HIV is an independent risk factor for poor nutritional outcomes (Kimani-Murage, Norris, Pettifor, Tollman, Klipstein-Grobusch, Gómez-Olivé, *et al.,* 2011) and is of the major determinants of child undernutrition in Africa (Ricci *et al*., 2019). HIV-infection may lead to multiple nutritional deficiencies and malnutrition. Decreased food intake, impaired absorption and increased nutrient requirements contribute to these nutritional deficiencies and malnutrition in children (Gladstone *et al*., 2019; WHO, 2010a), thus, compromising the nutritional status of infants and children due to the HIV infection (Ricci *et al*., 2019).

HIV causes increased energy requirements, which results in wasting of lean body tissue and fat mass. Lean body tissue and fat mass are important for strengthening the immune system and providing the body with energy. HEU infants may have a lower length and weight due to failure to thrive, as opportunistic infections or other symptoms may be present. The ART programme improves weight, growth and development of HEU infants, whilst improving their survival (Arikawa, Rollins, Jourdain, Humphrey, Kourtis, Hoffman *et al.*, 2018; Di Lenardo, Ward, Pillet, Mann, Bornman, Obida *et al*., 2020).

2.5. UNDERNUTRITION IN INFANTS AGED 6–12 MONTHS

Nutrition is the main factor affecting the growth of 6–12-month-old infants, with inappropriate feeding practices, such as decreased breastfeeding rates with age, early introduction of solid foods, and inadequate nutrients in the infants' complementary foods. These factors can lead to nutrient deficiencies, such as iron, resulting in undernutrition in the form of stunting, underweight and wasting. Figure 2.2. highlights the immediate, underlying and basic causes of malnutrition (Mandela, 2020; UNICEF, 2021; World Health Organization (WHO), 2010), and how these impact on the growth of children under 5 years of age.

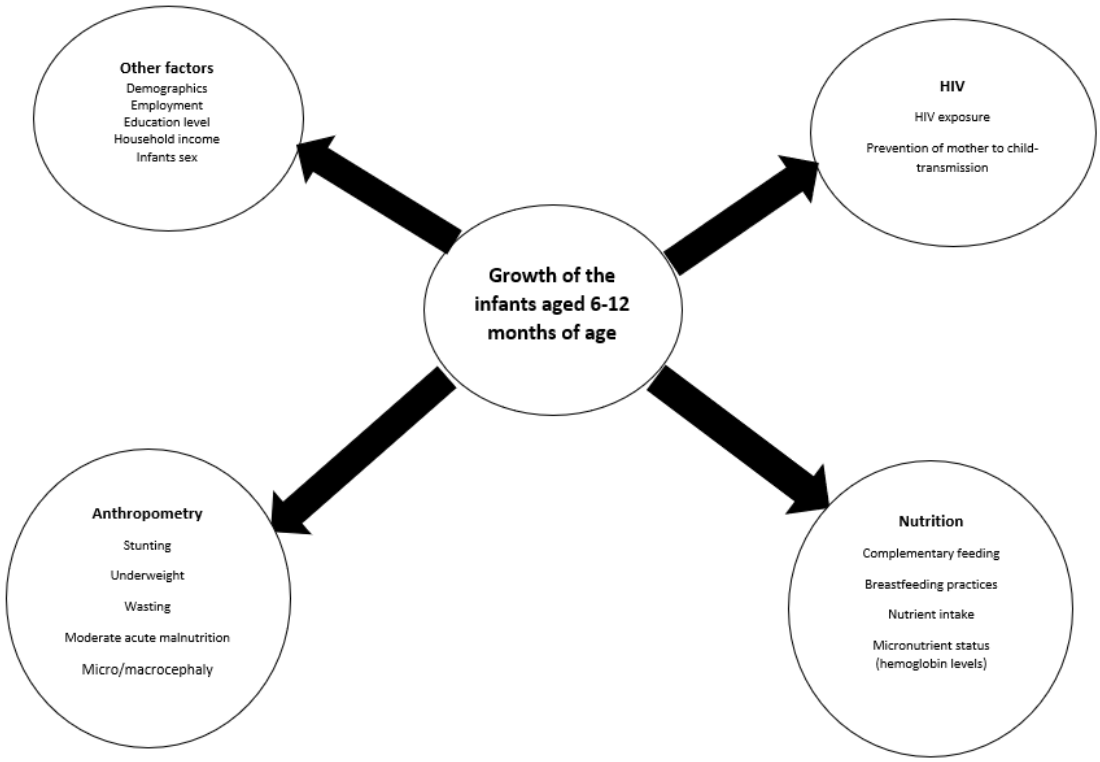


Figure 2.5: Factors affecting growth of infants (adapted from WHO, 2016)

Anthropometric measurements are suggestive of the protein and energy status of infants and they are used in the assessment of growth and nutritional health (Mamabolo, Alberts, Mbenyane, Steyn, Nthangeni, Delemarre-Van De Waal & Levitt, 2004). The assessment and monitoring of infants' growth in health facilities is conducted through plotting the following measurements on the South African Road to Health booklet: weight, length, head circumference (HC), and mid-upper-arm circumference (MUAC) (Patel & Rouster, 2020; WHO, 2009). Studies show that HEU infants had substantially higher rates of morbidity and mortality than infants who were unexposed to HIV (Evans *et al.*, 2016a; Evans, Humphrey,

Ntozini & Prendergast, 2016b; Evans *et al*., 2020; Locks, Manji, Kupka, Liu, & Kisenge, McDonald, 2017; Prendergast, Chasekwa, Evans, Mutasa, Mbuya, Stoltzfus *et al*., 2019; Tshiambara, Hoffman, Legodi, Botha, Mulol, Pisa *et al*., , 2023) leading to high rates of undernutrition in the form of underweight, stunting and wasting (Locks *et al*., 2017; Tshiambara *et al*., 2023).

2.5.1. Stunting (length-for-age Z-scores)

Globally, 149 million children under the age of 5 years were stunted, with South Africa accounting for 33.6% of the total (UNICEF, 2019). Although there has been a global drop in the number of stunted children below the age of 5 years, Africa remains the most affected region, with a reduction of only 28.9 to 22.4 million in 2000 and 2018 respectively (UNICEF, 2019). In Africa, in 2019, two out of every five children suffered from stunting (World Health Organization, 2020).

An infant is classified as stunted if their length-for-age is below the -2 Z-score according to the WHO child growth standard median (WHO, 2021). Stunting is an indicator of the infant's lack of well-being and the after-effects of chronic undernutrition associated with poor socio-economic conditions, maternal ill-health and improper nutrition, and inappropriate infant and young child feeding (IYCF) (WHO, 2020). Stunted infants may not achieve their full age-length, and their cognitive development is compromised. This may result in learning challenges, poor community participation, earning less than peers when reaching adulthood, and increased risk for developing NCDs (WHO, 2020). Greater risk of stunting and vulnerability to infections was also found in HEU infants in a randomised control trail conducted in Zimbabwe (Prendergast *et al*., 2019) and a review concluded that HIV-exposure may lead to poor growth and weight gain in infants, and can be observed as early as three months of age (Isanaka *et al*., 2009).

Stunting rates were reported to be higher in HEU than in HUU Indian infants (Ram, Gupte, Nayak, Kinikar, Khandave, Shankar *et al*., 2012) due to the increased risk of infection and poor appetite (Feucht, Van Bruwaene, Becker & Kruger, 2016). Higher rates of stunting was found in Kenyan HEU (20%) than HUU (10%) infants at 9 months (Neary, Langat, Singa, Kinuthia, Itindi, Nyaboe, Ng'anga, Katana, John-Stewart & Mcgrath, 2021), and a cross-sectional study investigating the relationship between maternal autonomy and undernutrition in children less than 2 years old found higher rates of stunting (31.4%) (Agu, Emechebe, Yusuf,

Falope & Kirby, 2019), with lack of breastfeeding, low income and body mass index (BMI) as predictors of stunting in Nigerian infants (Agu *et al*., 2019; McDonald, Kupka, Manji, Okuma, Bosch, Aboud *et al*., 2012). Locks (2017) reported that HEU children (24%) in Tanzania were stunted by 18 weeks follow up compared to six weeks baseline data (7%).

2.5.2. Underweight (weight-for-age Z-scores)

WHO (2020) defined underweight as a low weight-for-age, falling below the -2 length-for-age Z-scores on the WHO child growth standard median. A child who is underweight may also be stunted or wasted. Underweight is a form of undernutrition and may lead to micronutrient deficiency including anaemia (WHO, 2021). In Tanzania, high percentages of underweight was found in HEU than HUU infants (24% vs. 17%) by seven months of age (Locks, Manji, Kupka, Liu, Kisenge, McDonald &, 2017). A Nigerian cross-sectional study found 29.8% of their study population (children under 2 years) to be underweight (Agu *et al*., 2019). Limited studies are done focusing on HEU and HUU infants, however, one study reported that underweight is higher in the HEU infants than in the HUU infants (Ram et al., 2012).

2.5.3. Wasting (weight-for-length Z-score)

Infants are classified as being wasted when they fall below the -2 weight-for-length Z-score on the WHO child growth standard median (WHO, 2021). Wasting indicates recent and severe weight-loss due to insufficient food intake and infectious diseases such as diarrhoea (WHO, 2020). Infants or children suffering from wasting have weakened immunity, are susceptible to long-term developmental delays, and the risk of death is also high, particularly when wasting is severe (falling below -3 weight-for-length Z-scores). In 2020, 45.0 million children under-5 were wasted (WHO, 2020). Tanzanian HEU children were more wasted (27%) than HUU children (17%) by 18 months (Locks *et al*., 2017). This was also reported in in Zimbabwe, HEU children were significantly more wasted than HUU by 18 months (Evans *et al*., 2020).

2.5.4. Head circumference and acute malnutrition

HC may be used to detect any abnormalities or failure-to-thrive in children. The vulnerable group, such as HEU children, may have a smaller head or lower HC than infants and children who are not exposed to HIV. Limited studies exist on the HC of HEU as compared to HUU. Lower mean head circumference-for-age Z-score (HCZ) in HEU was found in Kenya (Neary *et al*., 2021) and Zimbabwe (Evans *et al*., 2020a) as comapred to HUU infants. Lower mean

HCZ in HEU infants maybe be due to developmental delay and small head size at birth in these infants (Evans *et al*., 2016a; Williams *et al.*, 2020).

Mid-upper-arm circumference (MUAC) is a simple, cost effective, quick and non-invasive measurement assessing nutritional status in children under-5. Subcutaneous fat and muscle are also found in the arm and the lower MUAC results may reflect reduction in muscle mass or fat, if not both. MUAC is a good indicator of infants' and children's nutritional status, it is simple, and cheap, and it is routinely done at health facilities as part of growth monitoring and evaluation on the infant's three-monthly visits (Dukhi, Sartorius & Taylor, 2017). MUAC may be more appropriate than weight-for-length Z-scores (WLZ) in identifying severe malnutrition in children, as it does not require date of birth and gestational age of birth. Limited studies is available on the MUAC of HEU 6-12 months old infants as compared to HUU infants (Evans *et a*l., 2020). The MUAC classifications in children under 5-years of age are shown in Figure 2.6.

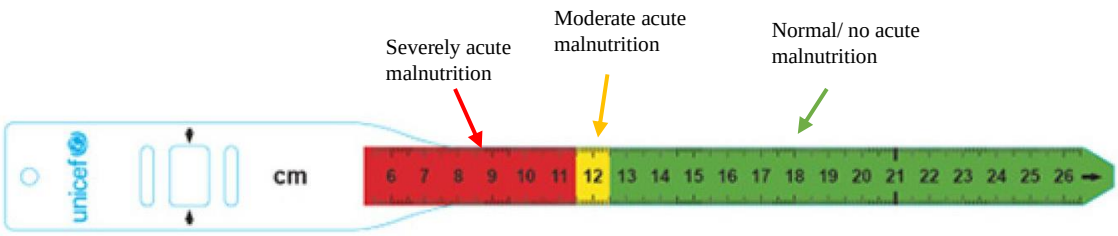


Figure 2.6 Malnutrition classification using MUAC in children under-5 years (Evans et al., 2020; WHO, 2021).

2.5.5. The importance of good nutrition in the prevention of undernutrition

Good nutrition plays a critical role in the prevention of undernutrition, as adequate nutrition is crucial for the growth and development of infants. Adequate intakes of macro- and micronutrients are important for optimal growth and development of the infants (WHO, 2021). Good nutrition is important for the prevention of undernutrition that may lead to unhealthy weight loss, leading to malnourishment and a weakened immune system. Therefore, the body becomes vulnerable to infections and diseases such pneumonia and diarrhoea. Variety of food in the infant's diet is essential in providing the nutrients needed for optimal growth and development, and supporting and maintaining a strong immune system (Mahan & Raymond, 2016). Adequate intake of micronutrients prevents common micronutrient deficiencies in children under 5-years old such as iron, zinc, iodine, and vitamin A, which can lead to poor

health, anaemia, impaired vision and poor cognitive development (Mahan & Raymond, 2016; WHO, 2021).

2.6. OTHER FACTORS CONTRIBUTING TO GROWTH OF INFANTS

2.6.1. Socio-Economic Status

Many MLWH continue to fear that breastfeeding will result in vertical HIV transmission (Matji *et al*., 2009; West *et al.*, 2019), despite the fact that this risk is considerably reduced in the setting of ART (Coutsoudis *et al*., 2001; Pillay *et al*., 2001; WHO, 2016). MLWH exhibited favourable attitudes, knowledge, and practices about the benefits of exclusive breastfeeding, but many mothers were not sure of exclusive breastfeeding, and, if offered formula, they would stop breastfeeding (Ashipala, Shikukumwa & Joel, 2021).

Infants' sex contributes to malnutrition as males have higher lean body mass associated with length than females, but females have higher fat percentage than males, even though these differences are observed later in the puberty stage (Mahan & Raymond, 2016). A systematic review showed that boys have higher odds or being stunted and underweight than girls (Thurstans, Opondo, Seal, Wells, Khara, Dolan *et al.*, 2020). Sambu (2020) reported that 30% of the South African males and 25% of females were stunted, and that the prevalence of stunting was higher in the children residing in the rural areas than urban areas (29% vs 26%).

High level of education (tertiary) is negatively associated with breastfeeding status of the mothers (Gebretsadik, Tadesse, Ambese & Mulugeta, 2023; Harvey, Newell & Padmadas, 2022). In Ethiopia, when investigating the predictors of meat consumption, a study found that mothers with no formal education are less likely to feed their children rich sources of iron food, such as meat (Yisak, Ambaw, Walle, Alebachew & Ewunetei 2020). Maternal education influences the nutritional status of the infants and their feeding choices (Ajami, Abdollahi, Salehi, Oldewage-Theron & Jamshidi-Naeini, 2018) and it was found in the Cambodian infants in Asia that maternal education indirectly influenced infants' length (Harvey *et al*., 2022).

Independent association between wealth of the mothers and stunting of the infants was found in Iran (Ajami et al., 2018). Children who were breastfed beyond 12 months in impoverished homes where at a higher risk of stunting in Thailand, as prolonged breastfeeding may be

adopted to compensate for a lack of or limited access to complementary foods (Cetthakrikul, Topothai, Suphanchaimat, Tisayaticom, Limwattananon & Tangcharoensathien, 2018). A qualitative study in Rwanda (Umugwaneza, Havemann-Nel, Vorster & Wentzel-Viljoen, 2021) revealed that poverty is the biggest contributor to poor complementary feeding, which increases the prevalence of undernutrition.

2.6.2. Body mass index of mothers and infants' growth

Maternal nutritional status contributes to the infant's nutritional status in the first 1000 days (WHO, 2021). BMI is a non-invasive, quantitative technique used to measure, record, and analyse the dimensions of a human body, such as height and weight (Zamboni, Mazzali, Fantin, Rossi & Di Francesco, 2008). BMI is not sufficient to analyse body composition thoroughly, although it has been used in research. Fat, muscle, water and other important indicators of underlying medical conditions are not considered in the BMI, but considered in the body composition. Table 2.5. shows the WHO classifications of the BMI, which also has additional cut-off points. BMI is measured by dividing the weight and height squared of the patients and measured in kilograms per meter squared.

MLWH and on ART are at a lessor risk of underweight and ART increases weight gain (Guaraldi et al., 2023; Stires et al., 2021). In Kenya and Ethopia, MLWH more likely to have lower weight and underweight (Gemede, Kaba, & Dufera, 2021; Widen *et al*., 2019). MLWH weight gain was positively associated with increased infants LAZ in HEU infants at 12 months in Rwanda (Lane et al., 2021).

Table 2.5. BMI classification of adults from the ages of 19-years and older

Classification	**BMI (kg/m^2)**	
	Principal cut-off points	**Additional cut-off points**
Underweight	<18.50	<18.50
Severe thinness		<16.00
Moderate thinness		16.00–16.99
Mild thinness		17.00–18.49
Normal (healthy weight)	18.50–24.99	18.50–24.99
Overweight	≥25.00	≥25.00
Pre-obese: lower	25.00–29.99	25.00–27.49
Pre-obese: upper		27.50–29.99

Obese	≥30.00	≥30.00
Obese class I	30.00–34.99	30.00–32.49
		32.50–34.99
Obese class II	35.00–39.99	35.00–37.49
		37.50–39.99
Obese class III	≥40.00	≥40.00

2.6.3. Maternal maid-upper arm circumference (MUAC) and infants growth

Mid-upper arm circumference (MUAC) is a good measure of muscle mass and, therefore, protein intake (as an indicator of acute malnutrition). MUAC does not change very much during pregnancy and is a useful measure of the body's muscle mass during pregnancy, quick and easy to measure as compared to BMI even in the context of HIV (Fakier, Petro, & Fawcus, 2017; Ramlal *et al*., 2012; Ververs *et al*., 2013; WHO, 2016). Lower MUAC while pregnant correlates with lower infants LAZ, resulting in a 1.6 times higher risk of infant stunting during the first months of life (Kpewou *et al*., 2020). Table 2.6. shows the MUAC classification in the adult population, adapted from the South African Department of Health. The table shows that a MUAC of less than 18.5 cm is severe wasting and a MUAC of more than 33 cm is classified as obese in women.

Table 2.6. Adults MUAC classification

Classification	**MUAC measurement**
Severe wasting	<18.5 cm
Moderate wasting	<21 cm
Undernutrition/chronic wasting illness	<23 cm
Normal MUAC	≥23 cm

Adapted from South African National Department of Health (Department of Health, 2014)

Growth and development of infants may be influenced by the feeding practices of infants with appropriate infant feeding including exclusive breastfeeding and complementary feeding (WHO, 2021). Poor growth of infants has been associated with poor breastfeeding practices, especially in resource-limited settings where water and sanitation are still a major challenge (WHO, 20202). HEU infants are evidence of this as there is often a lack of knowledge in

mothers regarding infant feeding in the context of HIV (Locks, 2017; Parker, 2013; WHO, 2020).

2.7. SUMMARY

Undernutrition is prevalent in LMICs, affecting infant growth and development. Factors affecting infant growth include feeding practices, haemoglobin levels, dietary intake, HIV exposure, and mother's breastmilk composition. HIV is the main determinant, and HVTP programmes reduce infection rates, but increase maternal HIV exposure risk. Breastmilk composition is influenced by a mother's age, dietary intake, and educational level, with HIV being a modifiable risk factor for poor nutritional outcomes, necessitating monitoring of HEU infants. Infants aged 6–12 months are at a higher risk of poor growth and development due to the transition from exclusive breastfeeding to solid foods. This is due to the lack of safe, diverse, and nutrient-dense foods; decreased breastfeeding rates; fear of vertical transmission; and reduced breastmilk concentration. Meeting the adequate intake of trace elements including iron and zinc are particularly problematic during the second half of infancy due to increased needs, low concentration in breastmilk, and inadequate dietary intake of these nutrients.

2.8. CONCLUSION

This chapter has described the available literature on the topic, the next three chapters, which are written according to the journals' instructions answer the objectives of the study in three (one article and 2 manuscripts) research papers.

2.9. REFERENCES

Abate, M.W., Nigat, A.B., Demelash, A.T., Emiru, T.D., Tibebu, N.S., Tiruneh, C.M., et al. 2022. Prevalence of timely complementary feeding initiation and associated factors among mothers having children aged 6–24 months in rural north-central ethiopia: Community based cross-sectional study. *PloS One*. 17(5)**:**e0267008.

Adeniyi, O.V., Ajayi, A.I., Issah, M., Owolabi, E.O., Goon, D.T., Avramovic, G. and Lambert, J. 2019. Beyond health care providers' recommendations: Understanding influences on infant feeding choices of women with HIV in the Eastern Cape, South Africa. *International Breastfeeding Journal*. 14(1).

Agu, N., Emechebe, N., Yusuf, K., Falope, O. and Kirby, R.S. 2019. Predictors of early childhood undernutrition in Nigeria: The role of maternal autonomy. *Public health nutrition*. 22(12)**:**2279-89.

Ajami, M., Abdollahi, M., Salehi, F., Oldewage-Theron, W. and Jamshidi-Naeini, Y. 2018. The association between household socioeconomic status, breastfeeding, and infants' anthropometric indices. *International journal of preventive medicine*. 9.

Allali, S., Brousse, V., Sacri, A.-S., Chalumeau, M. and De Montalembert, M. 2017. Anemia in children: Prevalence, causes, diagnostic work-up, and long-term consequences. *Expert review of hematology*. 10(11)**:**1023-8.

Andreas, N.J., Kampmann, B. and Le-Doare, K.M. 2015. Human breast milk: A review on its composition and bioactivity. *Early human development*. 91(11)**:**629-35.

Arikawa, S., Rollins, N., Jourdain, G., Humphrey, J., Kourtis, A.P., Hoffman, I., Essex, M., Farley, T., Coovadia, H.M., Gray, G. and Kuhn, L., 2018. Contribution of maternal antiretroviral therapy and breastfeeding to 24-month survival in human immunodeficiency virus-exposed uninfected children: an individual pooled analysis of African and Asian studies. *Clinical Infectious Diseases*, *66*(11), pp.1668-1677.

Ashipala, D.O., Shikukumwa, G. and Joel, M.H. 2021. Knowledge, attitudes and practices of HIV-positive mothers regarding the benefits of exclusive breastfeeding at a regional hospital in the north east of Namibia. *African Health Sciences*. 21(3)**:**1074-82.

Ballard, O. and Morrow, A.L. 2013. Human milk composition: Nutrients and bioactive factors. *Pediatric Clinics of North America.* 60(1)**:**49-74.

Blanton, L.V., Barratt, M.J., Charbonneau, M.R., Ahmed, T. and Gordon, J.I. 2016. Childhood undernutrition, the gut microbiota, and microbiota-directed therapeutics. *Science*. 352(6293)**:**1533-.

Boix-Amorós, A., Collado, M.C., Van't Land, B., Calvert, A., Le Doare, K., Garssen, J., Hanna, H., Khaleva, E., Peroni, D.G., Geddes, D.T. and Kozyrskyj, A.L., 2019. Reviewing the evidence on breast milk composition and immunological outcomes. *Nutrition reviews*, *77*(8), pp.541-556.

Boquien, C.-Y. 2018. Human milk: An ideal food for nutrition of preterm newborn. *Frontiers in pediatrics*. 6295.

Breckenridge, T.A., Black-Hughes, C., Rautenbach, J. and Mckinley, M. 2019. HIV/AIDS orphans in South Africa: NGO interventions supporting transitions to alternative care. *International Social Work.* 62(2)**:**502-17.

Burton, R., Giddy, J. and Stinson, K. 2015. Prevention of mother-to-child transmission in South Africa: An ever-changing landscape. *Obstetric medicine*. 8(1)**:**5-12.

Butts, C.A., Hedderley, D.I., Herath, T.D., Paturi, G., Glyn-Jones, S., Wiens, F., et al. 2018. Human milk composition and dietary intakes of breastfeeding women of different ethnicity from the Manawatu-Wanganui region of New Zealand. *Nutrients*. 10(9).

Bzikowska-Jura, A., Czerwonogrodzka-Senczyna, A., Oledzka, G., Szostak-Wegierek, D., Weker, H. and Wesolowska, A. 2018. Maternal nutrition and body composition during breastfeeding: Association with human milk composition. *Nutrients*. 10(10).

Cape, W., Faber, M. and Benadé, S. 2007. Breastfeeding, complementary feeding and nutritional status of 6–12-month-old infants in rural Kwazulu-Natal. *South African Journal of Clinical Nutrition*. 20(1)**:**16-24.

Cetthakrikul, N., Topothai, C., Suphanchaimat, R., Tisayaticom, K., Limwattananon, S. and Tangcharoensathien, V. 2018. Childhood stunting in Thailand: When prolonged breastfeeding interacts with household poverty. *BMC pediatrics*. 181-9.

Chalashika, P., Essex, C., Mellor, D., Swift, J.A. and Langley-Evans, S. 2017. Birthweight, HIV exposure and infant feeding as predictors of malnutrition in Botswanan infants. *Journal of Human Nutrition and Dietetic*s. 30(6)**:**779-90.

Chaponda, A., Goon, D.T. and Hoque, M.E. 2017. Infant feeding practices among HIV-positive mothers at Tembisa Hospital, South Africa. *African Journal of Primary Health Care & Family Medicine*. 9(1)**:**1-6.

Chezem, J., Friesen, C. and Boettcher, J. 2003. Breastfeeding knowledge, breastfeeding confidence, and infant feeding plans: Effects on actual feeding practices. *Journal of Obstetric, Gynecologic & Neonatal Nursing*. 32(1)**:**40-7.

Chisti, M.J., Salam, M.A., Smith, J.H., Ahmed, T., Ashraf, H., Bardhan, P.K. and Pietroni, M.A. 2011. Impact of lack of breast feeding during neonatal age on the development of clinical signs of pneumonia and hypoxemia in young infants with diarrhea. *PloS one*. 6(10)**:**e25817.

Coovadia, H.M., Rollins, N.C., Bland, R.M., Little, K., Coutsoudis, A., Bennish, M.L. and Newell, M.-L. 2007. Mother-to-child transmission of HIV-1 infection during exclusive breastfeeding in the first 6 months of life: An intervention cohort study. *The Lancet*. 369(9567)**:**1107-16.

Cortina, M., Jack, H.E., Pearson, R., Kahn, K., Tollman, S., Hlungwani, T., et al. 2019. Relationship between children's cognitions and later educational progress in rural South Africa: A longitudinal study. *Journal of Epidemiology and Community Health*.-2018-211361.

Coutsoudis, A., Goga, A., Rollins, N. and Coovadia, H.M. 2002. Free formula milk for infants of HIV-infected women: Blessing or curse?. *Health Policy Plan*. 17(2)**:**154-60.

Coutsoudis, A., Pillay, K., Kuhn, L., Spooner, E., Tsai, W.-Y., Coovadia, H.M. and Group, S.a.V.a.S. 2001. Method of feeding and transmission of HIV-1 from mothers to children by 15 months of age: Prospective cohort study from Durban, South Africa. *AIDS*. 15(3)**:**379-87.

Da Silva, L.L.S., Fawzi, W.W., Cardoso, M.A. and Group, E.W. 2018. Factors associated with anemia in young children in Brazil. *PloS One*. 13(9)**:**e0204504.

Deeney, M. and Harris-Fry, H. 2020. What influences child feeding in the northern triangle? A mixed-methods systematic review. *Maternal and child nutrition*. 10.1111/mcn.13018e13018.

Department of Health 2011. The Tshwane declaration of support for breastfeeding in South Africa. *South African Journal of Clinical Nutrition.*

Department of Health 2014. National consolidated guidelines for the prevention of mother-to-child transmission of HIV (PMTCT) and the management of HIV in children, adolescents and adults. Africa.

Desalu, O. Year. Published. Improving care and treatment for people living with HIV/AIDS. APHA 2017 Annual Meeting & Expo (Nov. 4-Nov. 8), 2017. *American Public Health Association.*

Dewey, K.G., 2001. Nutrition, growth, and complementary feeding of the brestfed infant. *Pediatric Clinics of North America*, *48*(1), pp.87-104.

Dewey, K.G. 2013. The challenge of meeting nutrient needs of infants and young children during the period of complementary feeding: An evolutionary perspective. *Journal of nutrition*. 143(12)**:**2050-4.

Dewey, K.G. and Brown, K.H. 2003. Update on technical issues concerning complementary feeding of young children in developing countries and implications for intervention programs. *Food and nutrition bulletin*. 24(1)**:**5-28.

Dewey, K.G. and Adu-Afarwuah, S. 2008. Systematic review of the efficacy and effectiveness of complementary feeding interventions in developing countries. *Maternal & child nutrition*. 424-85.

Di Lenardo, T.Z., Ward, B.J., Pillet, S., Mann, K., Bornman, R., Obida, M. and Chevrier, J. 2020. Exposure to lead and vaccine-specific igg titers in South African children participating in the Venda health examination of mothers, babies and their environment (Vhembe): A longitudinal study. *Environmental research*. 180108794.

Du Plessis, L., Peer, N., English, R. and Honikman, S. 2016. Breastfeeding in South Africa: Are we making progress?. *South African Health Review*. 2016(1)**:**109-23.

Dukhi, N., Sartorius, B. and Taylor, M. 2017. Mid-upper arm circumference (MUAC) performance versus weight for height in South African children (0–59 months) with acute malnutrition. *South African Journal of Clinical Nutrition*. 30(2).

Ejigu, Y., Magnus, J.H., Sundby, J. and Magnus, M.C. 2020. Differences in growth of HIV-exposed uninfected infants in Ethiopia according to timing of in-utero antiretroviral therapy exposure. *The Pediatric Infectious Disease Journal*. 39(8)**:**730.

Evans, C., Jones, C.E. and Prendergast, A.J. 2016a. HIV-exposed, uninfected infants: New global challenges in the era of paediatric HIV elimination. *Lancet infectious diseases*. 16(6)**:**e92-e107.

Evans, C., Humphrey, J.H., Ntozini, R. and Prendergast, A.J. 2016b. HIV-exposed uninfected infants in Zimbabwe: Insights into health outcomes in the pre-antiretroviral therapy era. *Front Immunol*. 7190.

Evans, C., Chasekwa, B., Ntozini, R., Majo, F.D., Mutasa, K., Tavengwa, N., et al. 2020. Mortality, HIV transmission and growth in children exposed to HIV in rural Zimbabwe. *Clinical Infectious Diseases*.

Faber, M. 2005. Complementary foods consumed by 6–12-month-old rural infants in South Africa are inadequate in micronutrients. Public health nutrition. 8(4)**:**373-81.

Faber, M. 2007. Dietary intake and anthropometric status differ for anaemic and non-anaemic rural South African infants aged 6–12 months. *Journal of Health, Population, and Nutrition*. 25(3)**:**285.

Faber, M., Laubscher, R. and Berti, C. 2017. Poor dietary diversity and low nutrient density of the complementary diet for 6- to 24-month-old children in urban and rural KwaZulu-Natal, South Africa. *Maternal & Child Nutrition*. 12(3)**:**528-45.

Faber, M., Rothman, M., Laubscher, R. and Smuts, C.M. 2020. Dietary patterns of 6–24-month-old children are associated with nutrient content and quality of the diet. *Maternal & Child Nutrition*. 16(2)**:**e12901.

Fadnes, L.T., Engebretsen, I.M., Wamani, H., Semiyaga, N.B., Tylleskär, T. and Tumwine, J.K. 2009. Infant feeding among hiv-positive mothers and the general population mothers: Comparison of two cross-sectional surveys in eastern Uganda. *BMC public health*. 9(1)**:**124.

Fakier, A., Petro, G. and Fawcus, S., 2017. Mid-upper arm circumference: A surrogate for body mass index in pregnant women. *South African Medical Journal*, *107*(7), pp.606-610.

Feucht, U.D., Van Bruwaene, L., Becker, P.J. and Kruger, M. 2016. Growth in HIV-infected children on long-term antiretroviral therapy. Tropical Medicine and International Health. 21(5)**:**619-29.

Filteau, S. 2009. The HIV-exposed, uninfected African child. *Tropical Medicine & International Health*. 14(3)**:**276-87.

Fouché, C., Van Niekerk, E. and Du Plessis, L.M. 2016. Differences in breast milk composition of HIV-infected and HIV-uninfected mothers of premature infants: Effects of antiretroviral therapy. *Breastfeeding Medicine*. 11(9)**:**455-60.

Friel, J., Qasem, W. and Cai, C. 2018. Iron and the breastfed infant. Antioxidants. 7(4)**:**54.

Frisbie, S.H., Mitchell, E.J., Roudeau, S., Domart, F., Carmona, A. and Ortega, R. 2019. Manganese levels in infant formula and young child nutritional beverages in the United States and France: Comparison to breast milk and regulations. *PloS One*. 14(11)**:**e0223636.

Gebretsadik, G.G., Tadesse, Z., Ambese, T.Y. and Mulugeta, A. 2023. Trends in and predictors of animal source food consumption among 6–23 months age children in Tigrai, northern Ethiopia: Evidence from three consecutive Ethiopian demographic and health surveys, 2005–2016. *BMC nutrition*. 9(1)**:**43.

Gemede, H.F., Kaba, F. and Dufera, M., 2021. Nutritional Knowledge, Practices, Nutritional Status and the Associated Factors Among HIV Positive Mothers On Antiretroviral Therapy: Evidence from Cross Sectional Survey in Abay Choman Health Centers, Western Ethiopia.

Gibson, R., Ferguson, E. and Lehrfeld, J. 1998. Complementary foods for infant feeding in developing countries: Their nutrient adequacy and improvement. *European journal of clinical nutrition*. 52(10)**:**764-70.

Gladstone, M.J., Chandna, J., Kandawasvika, G., Ntozini, R., Majo, F.D., Tavengwa, N.V., et al. 2019. Independent and combined effects of improved water, sanitation, and hygiene (wash) and improved complementary feeding on early neurodevelopment among children born to HIV-

negative mothers in rural Zimbabwe: Substudy of a cluster-randomized trial. *PLoS medicine*. 16(3):e1002766.

Goga, A.E., Doherty, T., Jackson, D.J., Sanders, D., Colvin, M., Chopra, M. and Kuhn, L. 2012. Infant feeding practices at routine PMTCT sites, South Africa: Results of a prospective observational study amongst HIV exposed and unexposed infants-birth to 9 months. *International Breastfeeding Journal*. 7(1):4.

Grote, V., Verduci, E., Scaglioni, S., Vecchi, F., Contarini, G., Giovannini, M., et al. 2016. Breast milk composition and infant nutrient intakes during the first 12 months of life. *European journal of clinical nutrition.* 70(2):250-6.

Guaraldi, G., Bonfanti, P., Di Biagio, A., Gori, A., Milić, J., Saltini, P., Segala, F.V., Squillace, N., Taramasso, L. and Cingolani, A., 2023. Evidence gaps on weight gain in people living with HIV: a scoping review to define a research agenda. *BMC Infectious Diseases*, *23*(1), p.230.

Hailemariam, T.W., Adeba, E. and Sufa, A. 2015. Predictors of early breastfeeding initiation among mothers of children under 24 months of age in rural part of west Ethiopia. *BMC Public Health*. 15(1):1076.

Hall, K., Sambu, W., Berry, L., Giese, S., Almeleh, C. and Rosa, S. 2017. South African early childhood review 2017. Cape Town: Children's Institute & Ilifa Labantwana.

Hampel, D., Dror, D.K. and Allen, L.H. 2018. Micronutrients in human milk: Analytical methods. *Advances in Nutrition*. 9(suppl_1):313S-31S.

Harvey, C.M., Newell, M.-L. and Padmadas, S. 2022. Maternal socioeconomic status and infant feeding practices underlying pathways to child stunting in Cambodia: Structural path analysis using cross-sectional population data. *BMJ Open*. 12(11):e055853.

Hernell, O. 2011. Human milk vs. Cow's milk and the evolution of infant formulas. *Milk and milk products in human nutrition*. 6717-28.

Horwood, C., Haskins, L., Vermaak, K., Phakathi, S., Subbaye, R. and Doherty, T. 2010. Prevention of mother to child transmission of HIV (PMTCT) programme in KwaZulu-Natal, South Africa: An evaluation of PMTCT implementation and integration into routine maternal, child and women's health services. *Tropical Medicine & International Health*. 15(9):992-9.

Huo, J., Sun, J., Fang, Z., Chang, S., Zhao, L., Fu, P., et al. 2015. Effect of home-based complementary food fortification on prevalence of anemia among infants and young children aged 6 to 23 months in poor rural regions of China. *Food and nutrition bulletin*. 36(4):405-14.

Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 2000. Using the estimated average requirement for nutrient assessment of groups. In *DRI Dietary Reference Intakes: applications in dietary assessment*. National Academies Press (US).

Irwin, J.J. and Kirchner, J.T. 2001. Anemia in children. *American family physician*. 64(8).

Isanaka, S., Duggan, C. and Fawzi, W.W. 2009. Patterns of postnatal growth in HIV-infected and HIV-exposed children. N*utrition reviews*. 67(6):343-59.

Jalbert, E., Williamson, K.M., Kroehl, M.E., Johnson, M.J., Cutland, C., Madhi, S.A., Nunes, M.C. and Weinberg, A., 2019. HIV-exposed uninfected infants have increased regulatory T cells that correlate with decreased T cell function. *Frontiers in Immunology*, 10, p.595.

Jones, S., Sherman, G. and Varga, C. 2005. Exploring socio-economic conditions and poor follow-up rates of HIV-exposed infants in Johannesburg, South Africa. *AIDS Care*. 17(4):466-70.

Kaldenbach, S. 2018. Infant growth in the KwaZulu-Natal province, South Africa: Results from the KwaZulu-Natal initiative for breastfeeding support (KIBS) survey ii at 13-15 weeks and 6 months of age. The University of Bergen.

Keikha, M., Bahreynian, M., Saleki, M. and Kelishadi, R. 2017. Macro-and micronutrients of human milk composition: Are they related to maternal diet? A comprehensive systematic review. *Breastfeeding Medicine*. 12(9):517-27.

Khaghani, S., Ezzatpanah, H., Mazhari, N., Givianrad, M.H., Mirmiranpour, H. and Sadrabadi, F.S. 2010. Zinc and copper concentrations in human milk and infant formulas. *Iranian Journal of Pediatrics*. 20(1):53.

Khanal, V., Scott, J., Lee, A., Karkee, R. and Binns, C. 2015. Factors associated with early initiation of breastfeeding in western Nepal. *International journal of environmental research and public health*. 12(8):9562-74.

Kimani-Murage, E.W., Norris, S.A., Pettifor, J.M., Tollman, S.M., Klipstein-Grobusch, K., Gómez-Olivé, X.F., et al. 2011. Nutritional status and HIV in rural South African children. *BMC pediatrics*. 11(1)**:**23.

Kindra, G., Coutsoudis, A., Esposito, F. and Esterhuizen, T. 2012. Breastfeeding in HIV exposed infants significantly improves child health: A prospective study. *Maternal and child health journal*. 16(3)**:**632-40.

Kpewou, D.E., Poirot, E., Berger, J., Som, S.V., Laillou, A., Belayneh, S.N. and Wieringa, F.T., 2020. Maternal mid-upper arm circumference during pregnancy and linear growth among Cambodian infants during the first months of life. *Maternal & child nutrition*, *16*, p.e12951.

Lane, C., Widen, E., Collins, S. and Young, S. 2020. HIV-exposed, uninfected infants in Uganda experience poorer growth and body composition trajectories than HIV-unexposed infants. *Journal of Acquired Immune Deficiency Syndromes*. 4(Supplement_2)**:**1023-.

Lane, C., Bobrow, E.A., Ndatimana, D., Ndayisaba, G.F. and Adair, L.S. 2019a. Decreased consumption of common weaning foods is associated with poor linear growth among HIV-exposed infants participating in the Kigali antiretroviral and breastfeeding assessment for the elimination of HIV (KABEHO) study. *American Journal of Human Biology*. 31(6)**:**e23308.

Lane, C.E., Bobrow, E.A., Ndatimana, D., Ndayisaba, G.F. and Adair, L.S. 2019b. Determinants of growth in HIV-exposed and HIV-uninfected infants in the KABEHO study. *Matern and Child Nutrition*. 10.1111/mcn.12776e12776.

Lane, C., Adair, L., Bobrow, E., Ndayisaba, G.F., Asiimwe, A. and Mugwaneza, P., 2021. Longitudinal interrelationship between HIV viral suppression, maternal weight change, breastfeeding, and length in HIV-exposed and uninfected infants participating in the Kabeho study in Kigali, Rwanda. *Annals of epidemiology*, *53*, pp.1-6.

Lang'at, P.C., Ogada, I., Steenbeek, A., Macdonald, N.E., Ochola, S., Bor, W. and Odinga, G. 2018. Infant feeding practices among HIV-exposed infants less than 6 months of age in Bomet County, Kenya: An in-depth qualitative study of feeding choices. *Archives of Disease in Childhood*. 103(5)**:**470-3.

Le Roux, S.M., Abrams, E.J., Donald, K.A., Brittain, K., Phillips, T.K., Nguyen, K.K., Zerbe, A., Kroon, M. and Myer, L., 2019. Growth trajectories of breastfed HIV-exposed uninfected and HIV-unexposed children under conditions of universal maternal antiretroviral therapy: a prospective study. *The Lancet Child & Adolescent Health*, 3(4), pp.234-244.

Li, Q., Liang, F., Liang, W., Shi, W. and Han, Y. 2019. Prevalence of anemia and its associated risk factors among 6-months-old infants in Beijing. *Frontiers in pediatrics*. 7286.

Locks, L.M., Manji, K.P., Kupka, R., Liu, E., Kisenge, R., Mcdonald, C.M., et al. 2017. High burden of morbidity and mortality but not growth failure in infants exposed to but uninfected with human immunodeficiency virus in Tanzania. *The Journal of pediatrics*. 180191-9. e2.

Lönnerdal, B. 2017. Development of iron homeostasis in infants and young children. *The American journal of clinical nutrition*. 106(suppl_6)**:**1575S-80S.

Maccow, G. 2013. Assessing children's cognitive development and learning.

Mahan, L.K. and Raymond, J.L. 2016. Krause's food & the nutrition care process-e-book, Elsevier Health Sciences.

Mamabolo, R.L., Alberts, M., Mbenyane, G.X., Steyn, N.P., Nthangeni, N.G., Delemarre-Van De Waal, H.A. and Levitt, N.S. 2004. Feeding practices and growth of infants from birth to 12 months in the central region of the Limpopo Province of South Africa. *Nutrition*. 20(3)**:**327-33.

Mandela, N. 2020. The slow violence of malnutrition. South African Child Gauge 2020.

Martin, S., Chinnock, P., Perales, J., Lee, H.Y., Lee, C.Y., Wu, Y.T. and Mavrodaris, A. 2017. Self-management interventions for people living with HIV/AIDS. *The Cochrane Database of Systematic Reviews*. 2017(8).

Matji, J.N., Wittenberg, D.F., Makin, J.D., Jeffery, B., Macintyre, U.E. and Forsyth, B.W. 2009. Factors affecting HIV-infected mothers' ability to adhere to antenatally intended infant feeding choice in Tshwane. *South African Journal of child health*. 3(1)**:**20-3.

Matsungo, T.M., Kruger, H.S., Faber, M., Rothman, M. and Smuts, C.M. 2017. The prevalence and factors associated with stunting among infants aged 6 months in a peri-urban South African community. *Public Health Nutrition*. 20(17)**:**3209-18.

Mcdonald, C.M., Kupka, R., Manji, K.P., Okuma, J., Bosch, R.J., Aboud, S., et al. 2012. Predictors of stunting, wasting and underweight among Tanzanian children born to HIV-infected women. *European journal of clinical nutrition.* 66(11)**:**1265-76.

Meyers, L.D. and Suitor, C.W. 2007. Dietary reference intakes research synthesis: Workshop summary, National Academies Press.

Morden, E., Technau, K.G., Giddy, J., Maxwell, N., Keiser, O. and Davies, M.A., 2016. Growth of HIV-exposed uninfected infants in the first 6 months of life in South Africa: the IeDEA-SA collaboration. *PLoS One*, 11(4), p.e0151762.

Motadi, S.A., Mbhenyane, X.G., Mbhatsani, H.V., Mabapa, N.S. and Mamabolo, R.L. 2015. Prevalence of iron and zinc deficiencies among preschool children ages 3 to 5 y in Vhembe District, Limpopo Province, South Africa. *Nutrition*. 31(3)**:**452-8.

Muchina, E. and Waithaka, P. 2010. Relationship between breastfeeding practices and nutritional status of children aged 0-24 months in Nairobi, Kenya. *African Journal of Food, Agriculture, Nutrition and Development*. 10(4).

Mugware, A., Motadi, S.A. and Mushaphi, L.F. 2022. Feeding practices and micronutrient status of children aged 0–36 months in Thulamela Municipality, Limpopo Province. *Health SA Gesondheid*. 279.

Mulol, H. and Coutsoudis, A. 2016. Breastmilk output in a disadvantaged community with high HIV prevalence as determined by the deuterium oxide dose-to-mother technique. *Breastfeeding Medicine*. 11(2)**:**64-9.

Musakwa, N., Feeley, A., Magwete, M., Patz, S., Mcnamara, L., Sanne, I., et al. 2020. Dietary intake among paediatric HIV-positive patients initiating antiretroviral therapy in Johannesburg, South Africa. *Vulnerable Children and Youth Studies*. 15(2)**:**155-70.

Nakai, J. 2018. Food and agriculture organization of the United Nations and the Sustainable Development Goals. *Sustainable development*. 221-450.

Nambiema, A., Robert, A. and Yaya, I. 2019. Prevalence and risk factors of anemia in children aged from 6 to 59 months in Togo: Analysis from Togo demographic and health survey data, 2013–2014. *BMC Public Health*. 191-9.

Nannan, N., Groenewald, P., Pillay-Van Wyk, V., Nicol, E., Msemburi, W., Dorrington, R. and Bradshaw, D. 2019. Child mortality trends and causes of death in South Africa, 1997-2012, and the importance of a National Burden of Disease Study. *South African Medical Journal.* 109(7)**:**480-5.

National Department of Health, S.A. 2019. ART clinical guidelines for the management of HIV in adults, pregnancy, adolescents, children, infants and neonates. National Department of Health Pretoria.

Neary, J., Langat, A., Singa, B., Kinuthia, J., Itindi, J., Nyaboe, E., et al. 2021. Higher prevalence of stunting and poor growth outcomes in HIV-exposed uninfected than HIV-unexposed infants in Kenya. *AIDS*.

Ntenda, P.A., Nkoka, O., Bass, P. and Senghore, T. 2018. Maternal anemia is a potential risk factor for anemia in children aged 6–59 months in Southern Africa: A multilevel analysis. *BMC Public Health.* 18(1)**:**650.

Omoni, A.O., Ntozini, R., Evans, C., Prendergast, A.J., Moulton, L.H., Christian, P.S. and Humphrey, J.H. 2017. Child growth according to maternal and child hiv status in Zimbabwe. *The Pediatric infectious disease journal*. 36(9)**:**869.

Parker, M.E., Tembo, M., Adair, L., Chasela, C., Piwoz, E.G., Jamieson, D.J., et al. 2013. The health of HIV-exposed children after early weaning. *Maternal & child nutrition*. 9(2)**:**217-32.

Patel, J.K. and Rouster, A.S. 2020. Infant nutrition requirements and options.

Pedersen, S.H., Wilkinson, A.L., Andreasen, A., Kinung'hi, S.M., Urassa, M., Michael, D., et al. 2016. Longitudinal analysis of mature breastmilk and serum immune composition among mixed HIV-status mothers and their infants. *Clinical nutrition*. 35(4)**:**871-9.

Prendergast, A.J., Chasekwa, B., Evans, C., Mutasa, K., Mbuya, M.N.N., Stoltzfus, R.J., et al. 2019. Independent and combined effects of improved water, sanitation, and hygiene, and improved complementary feeding, on stunting and anaemia among HIV-exposed children in rural Zimbabwe: A cluster-randomised controlled trial. *The Lancet Child & Adolescent Health.* 3(2)**:**77-90.

Provan, D., Singer, C.R. and Baglin, T. 2009. Oxford handbook of clinical haematology, Oxford University Press, USA.

Qasem, W.A. and Friel, J.K. 2015. An overview of iron in term breast-fed infants. *Clinical Medicine Insights: Pediatrics*. 979-84.

Rahamon, S.K., Arinola, G.O. and Akiibinu, M.O. 2013. Total antioxidant potential and essential trace metals in the breast milk and plasma of nigerian human immunodeficiency virus-infected lactating mothers. *Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences*. 18(1)**:**27.

Rahamon, S.K., Onifade, A.A. and Arinola, O.G. 2018. Antioxidants in breast milk of lactating mothers with HIV. *HIV/AIDS*. Elsevier.

Ram, M., Gupte, N., Nayak, U., Kinikar, A.A., Khandave, M., Shankar, A.V., et al. 2012. Growth patterns among HIV-exposed infants receiving nevirapine prophylaxis in Pune, India. *BMC infectious diseases*. 12(1)**:**282.

Ramlal, R.T., Tembo, M., Soko, A., Chigwenembe, M., Ellington, S., Kayira, D., King, C.C., Chasela, C., Jamieson, D., Van Der Horst, C. and Bentley, M.E., 2012. Maternal Mid–Upper Arm Circumference Is Associated With Birth Weight Among HIV-Infected Malawians. *Nutrition in Clinical Practice*, *27*(3), pp.416-421.

Ricci, C., Asare, H., Carboo, J., Conradie, C., Dolman, R.C. and Lombard, M. 2019. Determinants of undernutrition prevalence in children aged 0–59 months in Sub-Saharan Africa between 2000 and 2015. A report from the World Bank database. Public health nutrition. 1-9.

Rios-Leyvraz, M. and Yao, Q. 2023. Calcium, zinc, and vitamin d in breast milk: A systematic review and meta-analysis. International Breastfeeding Journal. 18(1)**:**1-14.

Rollins, N.C., Ndirangu, J., Bland, R.M., Coutsoudis, A., Coovadia, H.M. and Newell, M.-L. 2013. Exclusive breastfeeding, diarrhoeal morbidity and all-cause mortality in infants of HIV-infected and HIV uninfected mothers: An intervention cohort study in KwaZulu-Natal, South Africa. *PloS one*. 8(12)**:**e81307.

Rollins, N.C., Bhandari, N., Hajeebhoy, N., Horton, S., Lutter, C.K., Martines, J.C., et al. 2016. Why invest, and what it will take to improve breastfeeding practices? *The Lancet*. 387(10017)**:**491-504.

Rosala-Hallas, A., Bartlett, J.W. and Filteau, S. 2017. Growth of HIV-exposed uninfected, compared with HIV-unexposed, Zambian children: A longitudinal analysis from infancy to school age. *BMC pediatrics*. 17(1)**:**80.

Saloojee, H., De Maayer, T., Garenne, M.L. and Kahn, K. 2007. What's new? Investigating risk factors for severe childhood malnutrition in a high HIV prevalence South African setting. Scandinavian *Journal of Public Health*. 35(69_suppl)**:**96-106.

Sambu, W. 2020. Child nutrition. South African Child Gauge 2020.

Sherr, L., Hensels, I., Tomlinson, M., Skeen, S. and Macedo, A. 2018. Cognitive and physical development in HIV-positive children in South Africa and Malawi: A community-based follow-up comparison study. *Child: care, health and development*. 44(1)**:**89-98.

Slogrove, A.L., Johnson, L.F. and Powis, K.M. 2018. Population-level mortality associated with HIV exposure in HIV-uninfected infants in Botswana and South Africa: A model-based evaluation. *Journal of tropical pediatrics*.

Slogrove, A.L., Goetghebuer, T., Cotton, M.F., Singer, J. and Bettinger, J.A. 2016. Pattern of infectious morbidity in HIV-exposed uninfected infants and children. *Frontiers in immunology*. 7164.

Smith, B.L., Zizzo, S., Amzel, A., Wiant, S., Pezzulo, M.C., Konopka, S., et al. 2018. Integration of neonatal and child health interventions with pediatric HIV interventions in global health. *The International Journal of Maternal and Child Health (MCH) and AIDS*. 7(1)**:**192-206.

Smuts, C.M., Dhansay, M.A., Faber, M., Van Stuijvenberg, M.E., Swanevelder, S., Gross, R. and Benade, A.S. 2005. Efficacy of multiple micronutrient supplementation for improving anemia, micronutrient status, and growth in south african infants. *The Journal of nutrition*. 135(3)**:**653S-9S.

Stats, S. 2022. Mid-year population estimates 2022. Stats SA: Pretoria, South Africa.

Stires, H., LaMori, J., Chow, W., Zalewski, Z., Vidulich, A., Avina, M., Sloan, C., Hughes IV, R. and Hardy, H., 2021. Weight gain and related comorbidities following antiretroviral initiation in the

2000s: a systematic literature review. *AIDS Research and Human Retroviruses, 37*(11), pp.834-841.

Study, E.C. 2005. Mother-to-child transmission of HIV infection in the era of highly active antiretroviral therapy. *Clinical Infectious Diseases*. 40(3)**:**458-65.

Stuebe, A. 2009. The risks of not breastfeeding for mothers and infants. *Reviews in Obstetrics and Gynecology*. 2(4)**:**222.

Sugandhi, N., Rodrigues, J., Kim, M., Ahmed, S., Amzel, A., Tolle, M., et al. 2013. Hiv exposed infants: Rethinking care for a lifelong condition. AIDS (London, England). 27(0 2)**:**S187.

Swanepoel, E. 2018. Dietary intake of infants followed from age 6 to 18 months from a low socio-economic peri-urban community. Masters: North-West University, Potchefstroom Campus.

Swanepoel, E., Havemann-Nel, L., Rothman, M., Laubscher, R., Matsungo, T.M., Smuts, C.M. and Faber, M. 2019. Contribution of commercial infant products and fortified staple foods to nutrient intake at ages 6, 12, and 18 months in a cohort of children from a low socio-economic community in South Africa. *Maternal and Child Nutrition*. 15(2)**:**e12674.

Teklemariam, Z., Mitiku, H. and Mesfin, F. 2015. Prevalence of anemia and nutritional status among hiv-positive children receiving antiretroviral therapy in Harar, Eastern Ethiopa. *HIV/AIDS* (Auckland, NZ). 7191.

Theron, M., Amissah, A., Kleynhans, I., Albertse, E. and Macintyre, U. 2007. Inadequate dietary intake is not the cause of stunting amongst young children living in an informal settlement in gauteng and rural Limpopo Province in South Africa: The nutrigro study. *Public health nutrition.* 10(4)**:**379-89.

Thurstans, S., Opondo, C., Seal, A., Wells, J., Khara, T., Dolan, C., et al. 2020. Boys are more likely to be undernourished than girls: A systematic review and meta-analysis of sex differences in undernutrition. *BMJ global health.* 5(12)**:**e004030.

Tshiambara, P., Hoffman, M., Legodi, H., Botha, T., Mulol, H., Pisa, P. and Feucht, U. 2023. Comparison of feeding practices and growth of urbanized african infants aged 6–12 months old by maternal HIV status in Gauteng Province, South Africa. *Nutrients.* 15(6)**:**1500.

Umugwaneza, M., Havemann-Nel, L., Vorster, H.H. and Wentzel-Viljoen, E. 2021. Factors influencing complementary feeding practices in rural and semi-urban Rwanda: A qualitative study. *Journal of Nutritional Science.* 10e45.

UNAIDS. *Countdown to zero: Global plan towards the elimination of new HIV infections among children by 2015 and keeping their mother alive*, Geneva, Switzerland, UNAIDS

UNAIDS. *Update miles to go—closing gaps, breaking barriers, righting injustices*. 2018. Geneva, Switzerland, UNAIDS

UNAIDS. *In danger: Unaids global AIDS update 2022: Joint United Nations Programme on HIV/AIDS* Geneva, Switzerland.

UNAIDS. *Aids statistics—2023 fact sheet.* 2023,Geneva, Switzerland, UNAIDS.

UNICEF. *Strategy for health 2016-2030.* 2016. Unicef. Available from: https://nkhokwe.kuhes.ac.mw/items/4df36c0c-d6c2-44e5-883d-d5bc8c13f5ae.

UNICEF. *Malnutrition rates remain alarming: Stunting is declining too slowly while wasting still impacts the lives of far too many young children*, 2018. Unicef. Available from: https://data.unicef.org/topic/nutrition/malnutrition/.

UNICEF. *State of the World's Children Description: 146-205.* 2019. Unicef.

Ververs, M.T., Antierens, A., Sackl, A., Staderini, N. and Captier, V., 2013. Which anthropometric indicators identify a pregnant woman as acutely malnourished and predict adverse birth outcomes in the humanitarian context?. *PLoS currents*, *5*.

Wedderburn, C.J., Evans, C., Yeung, S., Gibb, D.M., Donald, K.A. and Prendergast, A.J. 2019. Growth and neurodevelopment of hiv-exposed uninfected children: A conceptual framework. Current *HIV/AIDS Reports*. 16(6)**:**501-13.

West, N.S., Schwartz, S.R., Yende, N., Schwartz, S.J., Parmley, L., Gadarowski, M.B., et al. 2019. Infant feeding by South African mothers living with HIV: Implications for future training of health care workers and the need for consistent counseling. *International Breastfeeding Journal*. 14(1)**:**1-7.

Whitney, E.N. and Rolfes, S.R. 2018. Understanding nutrition, Cengage Learning.

Williams, P.L., Yildirim, C., Chadwick, E.G., Van Dyke, R.B., Smith, R., Correia, K.F., DiPerna, A., Seage III, G.R., Hazra, R. and Crowell, C.S., 2020. Association of maternal antiretroviral use with microcephaly in HIV-exposed uninfected children in the prospective surveillance monitoring for ART toxicities (SMARTT) cohort study. *The lancet. HIV*, *7*(1), p.e49.

Woldesenbet, S., Lombard, C., Manda, S., Kufa, T., Ayalew, K. and Cheyip, M. 2021. The 2019 national antenatal sentinel HIV survey, South Africa, National Department of Health. The 2019 National Antenatal Sentinel HIV Survey, South Africa, National Department of Health.

World Health Organization. *Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity.* WHO. 2011. Available from: https://iris.who.int/handle/10665/85839.

World Health Organization.World Health Organisation. *WHO: Iron deficiency anaemia: Assessment, prevention, and control*. 2020.

World Health Organization. *Guiding principles for complementary feeding of the breastfed child*. Available from: https://www.who.int/maternal_child_adolescent/documents/a85622/en/.

World Health Organization. *WHO child growth standards and the identification of severe acute malnutrition in infants and children: Joint statement by the World Health Organization and the United Nations Children's Fund.* World Health Organization. 2009.

World Health Organization. *WHO child growth standards: Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: Methods and development. World Health Organization.* 2019.

World Health Organization. A*ntiretroviral therapy of HIV infection in infants and children: Towards universal access: Recommendations for a public health approach-2010 revision.* World Health Organization. 2010.

World Health Organization/. Indicators for assessing infant and young child feeding practices: Part 2: Measurement. Available from: https://www.who.int/nutrition/publications/infantfeeding/9789241599290/en/.

World Health Organization. *A conceptual framework for action on the social determinants of health.*

World Health Organization. G*uideline: Updates on HIV and infant feeding: The duration of breastfeeding, and support from health services to improve feeding practices among mothers living with HIV*. Geneva,WHO, Switzerland, 2016.

World Health Organization. *Vitamin and Mineral Nutrition Information System (VMNIS): Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity*. Geneva, switzerland: WHO, 2011. Available from: https://apps.who.int/iris/handle/10665/85839.

World Health Organization/World Health Organization. *Advocacy brief: Breastfeeding and HIV: Global breastfeeding collective*. World Health Organization. Available from: https://www.who.int/nutrition/publications/infantfeeding/breastfeeding-and-hiv-advocacybrief/en/.

World Health Organization. M*aintaining and improving quality of care within HIV clinical services*. World Health Organization. Geneva, Switzerland, 2019b.

World Health Organization. *UNICEF/WHO/the world bank group joint child malnutrition estimates: Levels and trends in child malnutrition: Key findings of the 2020 edition*. Geneva, WHO, Switzerland, 2021.

World Health Organization. *Advocacy brief: Nutrition for growth year of action: Nine smart breastfeeding pledges. 2021. WHO*

World Health Organization. *Global health estimates: Leading causes of death*. Geneva, Switzerland, 2021

Wu, J., Li, J., Li, Y., Loo, K.K., Yang, H., Wang, Q., et al. 2018. Neurodevelopmental outcomes in young children born to HIV-positive mothers in rural Yunnan, China. *Pediatrics International.* 60(7)**:**618-25.

Yeganeh, N., Watts, D.H., Xu, J., Kerin, T., Joao, E.C., Pilotto, J.H., et al. 2018. Infectious morbidity, mortality and nutrition in HIV-exposed, uninfected, formula fed infants: Results from the hptn 040/pactg 1043 trial. *The Pediatric infectious disease journal*. 37(12)**:**1271.

Yisak, H., Ambaw, B., Walle, Z., Alebachew, B. and Ewunetei, A. 2020. Minimum acceptable diet and associated factors among hiv-exposed children aged 6–24 months in Debre Tabor town, Ethiopia. *HIV/AIDS-Research and Palliative Care*. 639-45.

Zachara, B.A. and Pilecki, A. 2000. Selenium concentration in the milk of breast-feeding mothers and its geographic distribution. *Environmental Health Perspectives*. 108(11)**:**1043-6.

Zamboni, M., Mazzali, G., Fantin, F., Rossi, A. and Di Francesco, V. 2008. Sarcopenic obesity: A new category of obesity in the elderly. *Nutrition, Metabolism and Cardiovascular Diseases*. 18(5)**:**388-95.

CHAPTER 3: ARTICLE 1 (Manuscript 1)

Comparison of Feeding Practices and Growth of Urbanized African Infants Aged 6–12 Months Old by Maternal HIV Status in Gauteng Province, South Africa

Phumudzo Tshiambara [1,2,3,4,*], Marinel Hoffman [2,3,4], Heather Legodi [1], Tanita Botha [5], Helen Mulol [3,4,6], Pedro Pisa [1] and Ute Feucht [3,4,6]

[1] Department of Human Nutrition, Faculty of Health Sciences, University of Pretoria, Prinshof Campus, Pretoria 0084, South Africa
[2] Department of Consumer and Food Sciences, Faculty of Natural and Agricultural Sciences, University of Pretoria, Hatfield Campus, Pretoria 0028, South Africa
[3] Research Centre for Maternal, Fetal, Newborn and Child Health Care Strategies, University of Pretoria, Kalafong Provincial Tertiary Hospital, Pretoria 0001, South Africa
[4] Research Unit for Maternal and Infant Health Care Strategies, South African Medical Research Council, Pretoria 0001, South Africa
[5] Department of Statistics, Faculty of Natural and Agricultural Sciences, University of Pretoria, Hatfield Campus, Pretoria 0028, South Africa
[6] Department of Paediatrics, Faculty of Health Sciences, University of Pretoria, Prinshof Campus,
Pretoria 0084, South Africa
* Correspondence: phumudzo.mamphwe@up.ac.za or phumumamphwe@gmail.com; Tel.: +27-12-356-3205

Abstract

Background: Appropriate feeding practices are protective against malnutrition and poor growth.

Objective: We compared feeding practices and growth in HIV-exposed-uninfected (HEU) and HIV-unexposed-uninfected (HUU) between 6-12 months of age in urbanized African infants in South Africa.

Methods: A repeated cross-sectional analysis was used to determine differences in infant feeding practices and anthropometric measures by HIV exposure status at 6, 9, and 12 months in the Siyakhula study.

Results: The study included 181 infants (86 HEU; 95 HUU). Breastfeeding rates were lower in HEU vs. HUU infants at 9 (35.6% vs. 57.3%; $p = 0.013$) and 12 months (24.7% vs. 48.0%; $p = 0.005$). Introduction to early complementary foods was common (HEU = 16.2 ± 11.0 vs. HUU = 12.8 ± 9.3 weeks; $p = 0.118$). Lower weight-for-age Z-scores (WAZ) and head circumference-for-age Z-scores (HCZ) were found in HEU infants at birth. At 6 months, WAZ, length-for-age Z-scores (LAZ), HCZ, and mid-upper-arm circumference-for-age Z-scores (MUACAZ) were lower in HEU vs. HUU infants. At 9 months, lower WAZ, LAZ, and MUACAZ were found in HEU vs. HUU infants. At 12 months, lower WAZ, MUACAZ, and weight-for-length Z-scores (−0.2 ± 1.2 vs. 0.2 ± 1.2; $p = 0.020$) were observed.

Conclusion: HEU infants had lower rates of breastfeeding and poorer growth compared to HUU infants. Maternal HIV exposure affects the feeding practices and growth of infants.

Keywords: HIV exposure; infants; anthropometry; growth; feeding practices; breastfeeding; nutrition

3.1. INTRODUCTION

Globally 38.4 million people were living with HIV and 28.7 million were accessing antiretroviral therapy (ART) in the year 2022 (UNAIDS, 2022). In the high-prevalence country of South Africa, 8.2 million people were living with HIV, including 23.9% of all women of reproductive age in 2021 (Stats SA, 2021). The country introduced public health HIV programs, including the prevention of mother-to-child transmission (PMTCT) program in 2002 and the ART programme in 2004, to reduce mortality and prevent viral transmission (UNAIDS, 2011; World Health Organization (WHO), 2019a). The success of these programs has led to large numbers of infants being born with *in utero* exposure to maternal HIV infection, while remaining HIV-exposed-uninfected (HEU) (Evans, Chasekwa, Ntozini, Majo, Mutasa, Tavengwa *et al*., 2020; Sugandhi, Rodrigues, Kim,

Ahmed, Amzel, Tolle, Dzubien *et al*., 2013). In Sub-Saharan Africa (SSA) alone there are more than one million annual births of HEU children (UNAIDS, 2018).

Sub-optimal infant feeding practices negatively impact childhood growth, therefore the Infant and Young Child Feeding (IYCF) policy emphasizes breastfeeding as a cornerstone for health and survival, including in the context of HIV (WHO, 2019b). This is also in line with the WHO recommendations for breastfeeding for at least the first 24 months of life (WHO, 2016), preventing malnutrition, including stunting, underweight, and wasting (Becquet, Bland, Leroy, Rollins, Ekouevi, Coutsoudis *et al*., 2009; Chalashika, Essex, Mellor, Swift & Langley-Evans, 2017; Goga, Doherty, Jackson, Sanders, Colvin, Chopra *et al*., 2012). Many women living with HIV still fear that breastfeeding may lead to vertical HIV transmission (Matji, Wittenberg, Makin, Jeffery, MacIntyre & Forsyth, 2009; West, Schwartz, Yende, Schwartz, Parmley, Gadarowski *et al*., 2019), even though this risk is significantly lowered within the context of ART provision (Coutsoudis, Pillay, Kuhn, Spooner, Tsai, Coovadia *et al*., 2001; WHO, 2019a).

To ensure optimal growth, safe and nutritious food should be introduced to infants after the age of 6 months, in addition to continued breastfeeding (Dewey, 2003). However, early introduction of complementary feeding is a very common practice in South Africa (45-87.5%) (Faber, 2007; Faber, Laubscher & Berti, 2016; Matsungo, Kruger, Faber, Rothman & Smuts, 2017; Mugware, Motadi & Mushaphi, 2022; Rothman, Faber, Covic, Matsungo, Cockeran, Kvalsvig *et al*., Smuts, 2018; Sayed & Schönfeldt, 2020) which is an important public health concern (Stewart, Iannotti, Dewey, Michaelsen & Onyango, 2013), with stunting (30.5%), wasting (30.3%) and underweight (33.2%) reported already in 4-5-month-old infants (Masuke, Msuya, Mahande, Diarz, Stray-Pedersen, Jahanpour *et al*., Mgongo, 2021).

Infants who are HEU are known to be at greater risk of adverse birth outcomes, morbidity, and infections, affecting their growth and development (Department of Health, 2014; Slogrove, Johnson & Powis, 2018). These sub-optimal growth outcomes include being underweight, stunting, or even wasting (Chalashika *et al*., 2017; Wu, Li, Li, Loo, Yang,

Wang *et al.*, 2018). In addition, HEU infants are also smaller at birth in terms of the mean weight and length (Kidzeru, Hesseling, Passmore, Myer, Gamieldien, Tchakoute *et al*., 2014; Rollins, Ndirangu, Bland, Coutsoudis, Coovadia & Newell, 2013), which may contribute to their poor growth observed as early as three months of age, as documented in multiple African countries (Zambia: lower mean weight 2.9 kg vs 3.0 kg between 1-16 weeks, lower length in HEU infants at 6 months (64.3 cm ± 1.1) (Rosala-Hallas, Bartlett & Filteau, 2017); Zimbabwe: likelihood of stunting (25%), underweight (55%), and wasting (58%) high in HEU infants (Evans, Humphrey, Ntozini & Prendergast, 2016b); Ethiopia: risk of stunting of 51.9 per 100 person-years infants HIV exposed from conception (Ejigu, Magnus, Sundby, & Magnus, 2020); and South Africa:10% HEU stunted (Le Roux, Abrams, Donald, Brittain., Phillips., Nguyen., *et al*., 2019)). Limited research is available on HEU infants that focuses on the complementary feeding introduction phase, especially in terms of having an appropriate HIV-unexposed-uninfected (HUU) comparison group (Becquet *et al*., 2009; Fadnes, Engebretsen, Wamani, Semiyaga, Tylleskar & Tumwine, 2009; Iliyasu, Galadanci, Iliyasu, Babashani, Gajida, Nass *et al*., 2019; Kindra, Coutsoudis, Esposito & Esterhuizen, 2012; Rollins *et al*., 2013). Therefore, this study aimed to compare the feeding practices and growth of HEU and HUU infants between 6-12 months of age in Tshwane District, Gauteng Province, South Africa.

3.2 METHODS

3.2.1. Study design and setting

This study is a sub-study of the longitudinal Siyakhula cohort study (White, Feucht, du Toit, Rossouw & Connor, 2021), which aims to better understand how the in-utero and early postnatal environments, altered by maternal HIV infection and the treatment thereof, influence infants' growth trajectories and cognitive development, and alter their immune development and function, irrespective of the infants' own HIV status. For this study mother-infant dyads who attended the 6 (n=181), 9 (n=166), and 12 (n=155) month follow-up visits were included, with declining numbers due to loss to follow-up and relocation.

3.2.2. Data collection

All study-related information was collected at the central study site at Kalafong Provincial Tertiary Hospital, Gauteng Province, South Africa (White *et al.*, 2021). After obtaining informed consent, questionnaires were administered in the participants' preferred local languages by trained fieldworkers. Socio-demographic information was collected using a structured questionnaire and included maternal age, marital status, level of education, employment status, as well as the infants' age, sex, and HIV exposure status (with all women living with HIV self-reporting use of ART during and after pregnancy, with the first-line regimen at the time of study being a once-daily fixed-dose combination of tenofovir, emtricitabine, and efavirenz).

Infant growth was assessed by documenting weight (calibrated digital scale; Seca 354, Germany), length (mechanical infantometer; Seca 416, Germany), head circumference , and mid-upper-arm-circumference (MUAC) (non-stretchable tape measure; KDS measure, model F10-02DM 2m, Kyoto, Japan), wearing minimal clothing. These measurements were available for the time of birth (except MUAC) for baseline purposes, and then at 6, 9 and 12 months as part of study-related procedures. Z-score indices, including weight-for-age (WAZ), length-for-age (LAZ), weight-for-length (WLZ), HC-for-age (HCZ) & MUAC-for-age (MUACZ), were computed using the Intergrowth-21st and WHO Anthro child growth standards v3.2.2 according to sex, with correction for gestational age (Villar, Cheikh, Victora, Ohuma, Bertino, Altman *et al*., Lambert, Papageorghiou, Carvalho & Jaffer, 2014; WHO, 2010). Nutritional classifications of underweight, stunting, and wasting were defined as Z-scores below −2 standard deviations (SD) for WAZ, LAZ, and WLZ respectively, and for overweight WLZ above +2 SD of the median values of the reference data (WHO, 2006).

Infant feeding information, including breastfeeding and complementary feeding practices, was collected using maternal recall following the WHO global feeding practices indicators (WHO, 2010). Collected information included duration and type of feeding, age of introduction of complementary feedings, and type of food (WHO, 2010). The history of the usual food consumption was also collected using the unquantified food frequency questionnaire (FFQ) previously used in similar settings (Faber, 2007; Faber & Benade, 1999; Rothman *et al*., 2018; Smuts, Dhansay, Faber, van Stuijvenberg, Swanevelder *et al*., 2005), where mothers were asked about the usual infant food consumption during the past seven days.

3.2.3. Data processing and statistical analysis

The Research Electronic Data Capture v8.3.5 was used to capture data (Patridge & Bardyn, 2018). Z-scores were computed using the Intergrowth-21st and the WHO Anthro (Villar *et al*., 2014; World Health Organization, 2010), and values <-3 and >+3 were excluded from analysis due to implausibility from clinical settings. Descriptive statistics were used to present the socio-demographic information, anthropometric measurements, feeding practices and infant HIV exposure. All continuous data were presented as means and standard deviations with the categorical data represented as frequencies and percentages. Normality of the data was assessed using the Shapiro Wilk test. Comparisons between HEU and HUU groups were performed using the independent t-test (or its non-parametric equivalent Mann U Whitney test) for continuous variables or the Pearson Chi squared test for categorical variables. All statistical analyses were performed using R version 4.1.2 program (Team, 2013) and performed at a 5% level of significance.

3.2.4. Ethical consideration

The Faculty of Health Sciences Research Ethics Committee (Ref. no.: 294/2017) at the University of Pretoria approved the Siyakhula study. All relevant information was shared with the mothers prior to data collection. Mothers gave consent for themselves and their infants for each study visit, and the Declaration of Helsinki guidelines were followed. This sub-study was approved by the Faculty of Natural and Agricultural Sciences and the Faculty of Health Sciences Research Ethics Committee and the (Ref. no.: NAS063/2020) at the same university.

3.3. RESULTS

3.3.1. Description of the study population

A total of 181 mother-infant dyads (86 HEU; 95 HUU) were included in this study. The maternal socio-demographic characteristics are presented in Table 3.1. Mothers living with and without HIV were similar in terms of employment status, social grants, and access to electricity, while significant differences were found in terms of the mean maternal age (36.9 years ± 8.6 vs. 31.3 ± 6.3 years; $p < 0.001$) and education level ($p < 0.001$). There were no significant differences in the maternal socio-demographic characteristics when comparing the mothers in the overall Siyakhula study and this sub-study.

Table 3.1. Socio-demographic characteristics of the study mothers according to HIV status.

		Study Population	Mothers Living with HIV	Mothers not Living with HIV	*p*-Value
		(*n* = 181)	(*n* = 86)	(*n* = 95)	
Age (years) mean ± SD [1]		33.9 ± 7.9	36.9 ± 8.6	31.3 ± 6.3	*<0.001*
Education *n* (%) [2]	Formal education, but without school completion [3]	86 (48.6)	55 (66.3)	31 (33.0)	
	Completed secondary schooling	58 (32.8)	19 (22.9)	39 (41.5)	*<0.001*
	Tertiary education	33 (18.6)	9 (10.8)	24 (25.5)	
Employment *n* (%) [2]	Yes	84 (47.5)	41 (49.4)	43 (45.7)	0.738
Child support grant *n* (%) [2]	Yes	136 (76.8)	62 (74.7)	74 (78.7)	0.649
Marital status *n* (%) [2]	Single	134 (75.7)	60 (72.3)	74 (78.7)	0.412
	Married	43 (24.3)	23 (27.7)	20 (21.3)	
Access to water *n* (%) [2]	Communal tap	40 (22.6)	21 (25.3)	19 (20.2)	0.534
	Inside yard	88 (49.7)	42 (50.6)	46 (48.9)	
	Inside house	49 (27.7)	20 (24.1)	29 (30.9)	
Access to electricity *n* (%) [2]	Yes	165 (93.2)	76 (91.6)	89 (94.7)	0.601
Access to toilet *n* (%) [2]	None [4]	2 (1.1)	2 (2.4)	0 (0)	0.816
	Pit latrine	60 (33.9)	29 (34.9)	31 (33.0)	
	Flush toilet	115 (65.0)	52 (62.7)	63 (67.0)	

Values in *italic* font indicate significant *p*-values ($p < 0.05$); [1] non-normal distributed data; [2] excludes missing numbers; [3] formal education = includes any primary and secondary schooling; [4] none: not considered in the calculation. Mann–Whitney U test was used for continuous non-normally distributed data; Pearson's Chi-square test was used for categorical data to determine the differences in mothers living with HIV and mothers not living with HIV.

3.3.2. Birth characteristics of the infants

The birth characteristics of the HEU vs. HUU infants are presented in Table 3.2. More HEU vs. HUU infants were males, but the difference was not statistically significant. Significant differences were found in the mean birth weight (2.84 ± 0.49 kg vs. 3.06 ± 0.51 kg; p = 0.005); WAZ (−0.7 ± 0.9 vs. −0.2 ± 1.0; p = 0.003); head circumference (33.8 ± 1.8 cm vs. 34.5 ± 1.6 cm; p = 0.013) and HCZ (0.3 ± 1.3 vs. 0.7 ± 1.2; p = 0.038) of HEU vs. HUU infants. No significant differences were found between HEU vs. HUU infants in terms of the mean length (49.1 ± 4.1 cm vs. 49.9 ± 3.4 cm; p = 0.184) and LAZ (0.6 ± 1.4 vs. 0.7 ± 1.5; p = 0.804) at birth. In addition, no significant differences were found in the birth characteristics of the HEU vs. HUU infants when comparing the mothers in the overall Siyakhula study and this sub-study.

Table 3.2. Gestational age, sex, and anthropometric measurements and indices of HIV-exposed-uninfected and HIV-unexposed-uninfected infants at birth.

		Study Population	HEU Infants	HUU Infants	*p*-Value
		(*n* = 181)	(*n* = 86)	(*n* = 95)	
Gestational age (weeks) [1,2]		38.2 ± 1.7	38.2 ± 1.5	38.3 ± 1.8	0.293
Infant sex *n* (%)	Male	105 (58.0)	54 (62.8)	51 (53.7)	0.276
Birth body measurements [1]	Weight (kg) [3]	2.95 ± 0.51	2.84 ± 0.49	3.06 ± 0.51	*0.005*
	Length (cm) [2]	49.5 ± 3.8	49.1 ± 4.1	49.9 ± 3.4	0.184
	Head circumference (cm) [2]	34.1 ± 1.7	33.8 ± 1.8	34.5 ± 1.6	*0.013*
Birth Z-scores indices [1,3,4]	Weight-for-age	−0.5 ± 1.0	−0.7 ± 0.9	−0.2 ± 1.0	*0.003*
	Length-for-age	0.6 ± 1.5	0.6 ± 1.4	0.7 ± 1.5	0.804
	Head circumference-for-age	0.5 ± 1.3	0.3 ± 1.3	0.7 ± 1.2	*0.038*

Values in *italic* font indicate significant p-values ($p < 0.05$). Abbreviations: HEU: HIV-exposed-uninfected (born to mothers living with HIV); HUU: HIV-unexposed-uninfected (born to mothers not living with HIV); [1] data presented as mean ± SD; [2] non-normal distributed data; [3] normal distributed data; [4] the birth Z-scores indices sex-normalized were computed using INTERGROWTH-21st software, using gestation-adjusted age for preterm infants. Independent t-test was used for continuous normally distributed data, and the Mann–Whitney U test was used for continuous non-normally distributed data; Pearson's Chi-square test was used for categorical data to determine the differences in HEU and HUU infants.

3.3.3. Feeding practices

Breastfeeding and complementary feeding of HEU vs. HUU infants are further presented in Table 3.3. No significant differences were found in the early initiation of breastfeeding between HEU and HUU infants who were breastfed within one hour or after one hour after delivery ($p = 0.297$), based on maternal recall at the time of the study visit. The mean age of breastfeeding cessation was similar in both HEU vs. HUU infants (18.9 ± 15.8 vs. 18.1 ± 15.8 weeks; $p = 0.778$) before 6 months, and the mean age of formula milk introduction (18.4 ± 15.9 vs. 17.4 ± 15.4 weeks; $p = 0.770$). Early introduction of complementary foods before 6 months was common in both groups (16.2 ± 11.0 vs. 12.8 ± 9.3; $p = 0.118$).

Table 3.3. Feeding practices of HEU and HUU infants before 6 months based on maternal recall.

		HEU Infants	HUU Infants	*p*-Value
		***n* = 86**	***n* = 95**	
Initiation of breastfeeding *n* (%) [1,2]	<1 h after birth	40 (46.5)	55 (57.9)	0.297
	>1 h after birth	39 (45.3)	33 (34.7)	
	Never breastfed	7 (8.2)	7 (7.4)	
Baby received liquids/foods other than breastmilk/formula milk before age 6 months *n*Yes (%) [1,2]		60 (69.8)	76 (80.9)	0.120
Breastfeeding cessation age (weeks) [1,2,3]		18.9 ± 15.8	18.1 ± 15.8	0.778
Formula milk introduction mean age (weeks) [1,2,3]		18.4 ± 15.9	17.4 ± 15.4	0.770
Type of formula milk *n* (%) [1,2]	Commercial cow's milk-based formula	55 (93.2)	49 (84.5)	n/a
	Others [4]	4 (6.8)	9 (15.5)	
Main reason for introducing formula milk *n* (%) [1,2]	Return to work	17 (31.5)	29 (43.3)	0.281
	Insufficient milk/baby not growing	15 (27.8)	17 (25.4)	
	Convenience	6 (11.1)	10 (14.9)	
	Baby/mother unwell	16 (29.6)	11 (16.4)	
Complementary feeding introduction (weeks) [1,2,3]		16.2 ± 11.0	12.8 ± 9.3	0.118

First liquid introduced *n* (%) [1,2]	Water	71 (91.0)	85 (92.4)	0.966
	Others [5]	7 (9.0)	7 (7.6)	
First solid food introduced *n* (%) [1,2]	Mabelle/maize meal soft porridge	72 (83.7)	72 (75.8)	0.256
	Others [6]	14 (16.3)	23 (24.2)	

Abbreviations: HEU: HIV-exposed-uninfected; HUU: HIV-unexposed-uninfected; n/a: not applicable (no comparisons were performed due to one of the groups having less than five count leading to volatile results. [1] result based on maternal recall and *n* numbers vary as mothers with missing information were excluded; [2] data presented as mean ± SD; [3] non-normal distributed data; [4] others include lactose-free cow's milk and soy-based formula; [5] others include tea and juice; [6] others include baby cereal and instant porridge; Mann–Whitney U test used for continuous non-normally distributed data; Pearson's Chi-square test used for categorical data to determine the differences in HEU and HUU infants. Significant *p*-values were defined as $p < 0.05$.

The breastfeeding practices amongst HEU and HUU infants at 6, 9, and 12 months are shown in Figure 3.1. Similar percentages of EBF at birth and breastfeeding at 6 months were observed in the HEU and HUU infants, but significant differences were found at 9 (35.6% vs. 57.3%; $p = 0.013$) and 12 months (24.7% vs. 48.0%; $p = 0.005$).

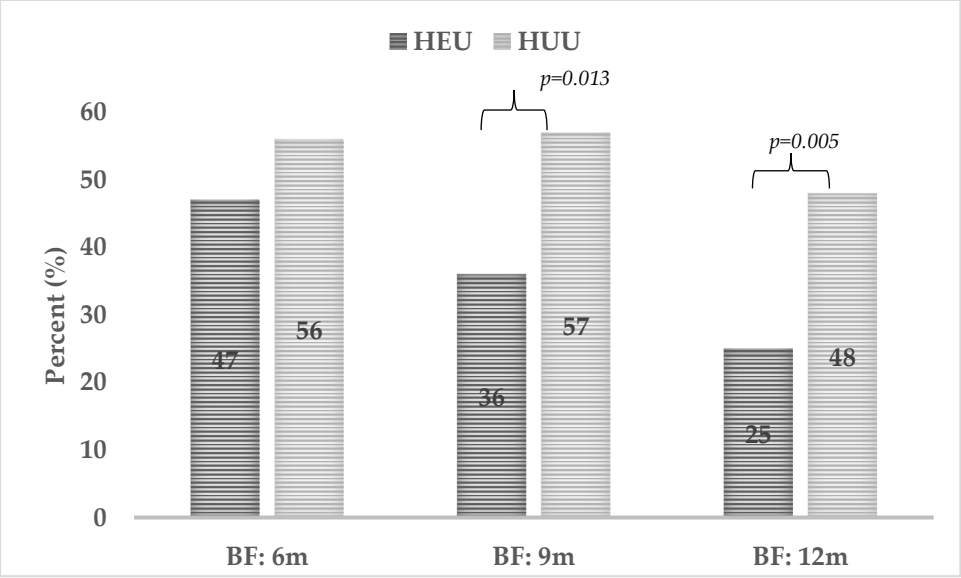


Figure 3.1. Infant breastfeeding practices by HIV exposure status in the first 12 months of life. Values in *italic* font indicate significant *p*-values ($p < 0.05$). Abbreviations: HEU: HIV-exposed-uninfected; HUU: HIV-unexposed-uninfected. The mixed feeding category was separately calculated, and no comparison tests were performed due to one group having <5 count, which led to volatile results. Pearson's Chi-square test was used for categorical data to determine the differences in HEU and HUU infants.

The foods frequently consumed by infants at 6 and 12 months are presented in Table 3.4. At 6 months, maizemeal soft porridge (11.4% vs. 10.1%), infant cereal (52.9% vs. 56.2%), and baby food in a jar (34.3% vs. 29.2%) were consumed at least four days per week by

HEU vs. HUU infants. At 12 months, carbonated/fizzy drinks (9.3% vs. 14.3%) and sweets/chocolates (22.7% vs. 11.7%) were consumed at least four days per week by HEU vs. HUU infants.

Table 3.4. Usual intake of food items by 6 and 12 months old HEU compared to HUU infants determined by quantified food frequency questionnaire (%).

Foods	Consumption at Age 6 Months (%) [1]						Consumption at Age 12 Months (%) [2]					
	Most Days [3]		Once a Week		Never		Most Days [3]		Once a Week		Never	
	HEU Infants	HUU Infants	HEU Infants	HUU Infants	HEU Infants	HUU Infants	HEU Infants	HUU Infants	HEU Infants	HUU Infants	HEU Infants	HUU Infants
Starches												
Maizemeal porridge—soft	11.4	10.1	7.1	3.4	81.4	86.5	49.3	42.9	6.7	15.6	44.0	41.6
Infant cereal	52.9	56.2	2.9	6.7	44.3	37.1	38.7	46.8	9.3	6.5	52.0	46.8
Instant porridge	10.0	11.2	2.9	0	87.1	88.8	28.0	16.9	14.7	13.0	57.3	70.1
Bread	4.3	0	1.4	4.5	94.3	95.5	56.0	54.5	28.0	27.3	16.0	18.2
Rice	1.4	0	2.9	1.1	95.7	98.9	17.3	18.2	42.7	37.7	40.0	44.2
Potato	15.7	11.2	15.7	13.5	68.6	75.3	54.7	42.9	36.0	44.2	9.3	13.0
Dairy products												
Fresh, fermented or powder milk	0	0	0	2.2	100	97.8	13.3	19.5	22.7	15.6	64.0	64.9
Yoghurt/dairy snack for baby	7.1	1.1	4.3	7.9	88.6	91.0	33.3	31.2	26.7	39.0	40.0	29.9
Animal foods/meat products												
Red meat	0	0	2.9	0	97.1	100	13.3	10.4	28.0	32.5	58.7	57.1
Liver	0	0	2.9	3.4	97.1	96.6	10.7	11.7	33.3	37.7	56.0	50.6
Chicken	0	5.6	4.3	3.4	95.7	91.0	36.0	40.3	32.0	27.3	32.0	32.5

Fish	1.4	0	2.9	2.2	95.7	97.8	12.0	11.7	34.7	32.5	53.3	55.8
Eggs	4.3	3.4	2.9	6.7	92.9	89.9	38.7	27.3	33.3	44.2	28.0	28.6
Vegetables												
Any vegetables [4]	9.0	8.7	14.5	10.1	76.8	80.9	48.0	51.9	40.0	31.2	12	16.9
• Orange [5]	87.5	88.2	n/a	n/a	n/a	n/a	70.7	68.8	n/a	n/a	n/a	n/a
• Dark-green leafy [6]	6.3	11.8	n/a	n/a	n/a	n/a	17.3	12.9	n/a	n/a	n/a	n/a
• Red/yellow [7]	6.2	0	n/a	n/a	n/a	n/a	0	0	n/a	n/a	n/a	n/a
Fruits												
Any fruits [8]	11.4	6.8	11.4	9.1	77.1	84.1	64.0	59.17	24.0	31.2	12.0	9.1
• Orange [9]	28.6	18.8	n/a	n/a	n/a	n/a	58.7	63.8	n/a	n/a	n/a	n/a
• Green [10]	28.6	12.5	n/a	n/a	n/a	n/a	29.3	36.2	n/a	n/a	n/a	n/a
• Red/yellow [11]	42.8	68.7	n/a	n/a	n/a	n/a	0	0	n/a	n/a	n/a	n/a
Food items added to porridge												
Salt	5.7	4.5	2.9	3.4	91.4	92.1	62.7	54.5	9.3	14.3	28.0	31.2
Oil	7.1	5.6	5.7	5.6	87.1	88.8	50.7	37.7	20.0	22.1	29.3	40.3
Margarine	8.6	7.9	10.0	6.7	81.4	85.4	42.7	29.9	26.7	28.6	30.7	41.6
Peanut butter	4.3	3.4	2.9	6.7	92.9	89.9	29.3	35.1	20.0	16.9	50.7	48.1
Miscellaneous												
Sweets/chocolates	1.4	0	7.1	5.6	91.4	94.4	22.7	11.7	29.3	29.9	48.0	58.4
Kids tea	4.3	2.2	4.3	3.4	91.4	94.4	46.7	35.1	18.7	15.6	34.7	49.4
Black/English tea	0	0	0	0	100	100	10.7	6.5	4.0	7.8	85.3	85.7

Chips	1.4	2.2	12.9	4.5	85.7	93.3	50.7	46.8	34.7	32.5	14.7	20.8
Carbonated/fizzy drinks	1.4	0	2.9	4.5	95.7	95.5	9.3	14.3	32.0	18.2	58.7	67.5
Fruit juice	4.3	5.7	7.2	8.0	88.5	86.3	21.3	36.4	34.7	28.6	44.0	35.1
Baby food in a jar/pureed	34.3	29.2	11.4	14.6	54.3	56.2	45.3	48.1	33.3	22.1	21.3	29.9
Juice concentrate	1.4	1.1	2.9	3.4	95.7	95.5	16.0	6.5	18.7	24.7	65.3	68.8

Abbreviations: HEU: HIV-exposed-uninfected; HUU: HIV-unexposed-uninfected; no comparisons were performed due to one of the groups having less than five counts leading to volatile results. [1] 6-months: HEU = 70, HUU = 89; [2] 12 months: HEU = 75, HUU = 77; [3] %: the categories 'every day' and 'most days' are grouped together; n/a: not applicable; [4] excludes infants who did not consume any vegetables; [5] orange colored vegetables (carrots, butternut, pumpkin, sweet potato); [6] dark-green-leafy colored vegetables (spinach, butternut leaves, cabbage); [7] red/yellow colored vegetables (tomatoes, beetroot, corn); [8] excludes infants who did not consume any fruits; [9] orange colored fruits (mangoes, peaches, oranges, mandarins); [10] green colored fruits (apples, grapes, avocado, pear) and [11] red/yellow colored fruits (banana, pineapple, watermelon, strawberry).

3.3.4. Growth of HEU vs. HUU infants

The anthropometric measurements, Z-score indices, and nutritional classification of the infants at 6, 9, and 12 months of age by HIV exposure status are presented in Table 3.5. HEU infants had a significantly lower mean weight compared to HUU infants at 6 months (7.3 ± 0.9 kg vs. 7.8 ± 1.0 kg; $p = 0.001$), and at 9 months (8.3 ± 1.0 vs. 8.8 ± 1.1 kg; $p = 0.002$), also at 12 months the mean weight was lower although this did not reach statistical significance (9.1 ± 1.2 kg vs. 9.4 ± 1.3 kg; $p = 0.106$). The mean WAZ was significantly lower in HEU as compared to HUU infants at 6 (−0.6 ± 1.1 vs. 0.1 ± 1.2; $p < 0.001$), 9 (−0.4 ± 1.1 vs. 0.1 ± 1.1; $p = 0.003$) and 12 months (−0.3 ± 1.1 vs. 0.1 ± 1.2; $p = 0.022$). No significant difference was found in the underweight classification of infants at 6, 9, and 12 months.

Table 3.5. Anthropometric measurements, Z-score indices, and nutritional classifications of the infants between 6–12 months of life by HIV exposure status.

	Age 6 Months			**Age 9 Months**			**Age 12 Months**		
	HEU Infants	**HUU Infants**	***p*-Value**	**HEU Infants**	**HUU Infants**	***p*-Value**	**HEU Infants**	**HUU Infants**	***p*-Value**

	(*n* = 86)	(*n* = 95)		(*n* = 80)	(*n* = 86)		(*n* = 75)	(*n* = 80)	
Anthropometric measurements									
Weight (kg) [1]	7.3 ± 0.9 [2]	7.8 ± 1.0 [2]	*0.001*	8.3 ± 1.0 [3]	8.8 ± 1.1 [3]	*0.002*	9.1 ± 1.2 [2]	9.4 ± 1.3 [2]	0.106
Length (cm) [1]	65.3 ± 3.5 [2]	66.6 ± 2.8 [2]	*0.014*	70.1 ± 3.1 [3]	71.2 ± 2.8 [3]	*0.012*	74.4 ± 3.1 [3]	74.5 ± 2.7 [3]	0.704
Head circumference (cm) [1]	43.5 ± 1.6 [2]	43.9 ± 1.6 [2]	0.106	45.3 ± 1.4 [3]	45.4 ± 1.7 [3]	0.621	46.5 ± 1.6 [3]	46.6 ± 1.6 [3]	0.655
Mid-upper-arm-circumference (cm) [1]	14.6 ± 1.3 [2]	15.2 ± 1.1 [2]	*0.002*	15.2 ± 1.2 [2]	15.7 ± 1.5 [2]	*0.026*	15.6 ± 1.2 [2]	16.0 ± 1.3 [2]	0.075
Z-score indices									
Weight-for-age Z-score [1,2]	−0.6 ± 1.1 [3]	0.1 ± 1.2 [3]	*<0.001*	−0.4 ± 1.1 [3]	0.1 ± 1.1 [3]	*0.003*	−0.3 ± 1.1 [2]	0.1 ± 1.2 [2]	*0.022*
Length-for-age Z-score [1,2]	−0.8 ± 1.4 [3]	−0.1 ± 1.2 [3]	*<0.001*	−0.5 ± 1.4 [3]	0.0 ± 1.3 [3]	*0.023*	−0.4 ± 1.3 [3]	−0.2 ± 1.1 [3]	0.308
Weight-for-length Z-score [1,2]	−0.1 ± 1.2 [3]	0.2 ± 1.1 [3]	0.074	−0.1 ± 1.2 [3]	0.2 ± 1.1 [3]	0.098	−0.2 ± 1.2 [3]	0.2 ± 1.2 [3]	*0.020*
Head circumference-for-age Z-score [1,2]	0.5 ± 1.2 [3]	0.9 ± 1.2 [3]	*0.019*	0.6 ± 1.2 [3]	0.8 ± 1.0 [3]	0.331	0.6 ± 1.2 [3]	0.9 ± 1.1 [3]	0.069
Mid-upper-arm-circumference-for-age Z-score [1,2]	0.5 ± 1.1 [3]	1.0 ± 0.9 [3]	*<0.001*	0.7 ± 1.0 [3]	1.1 ± 1.1 [3]	*0.013*	0.8 ± 1.1 [3]	1.3 ± 1.1 [3]	*0.025*
Nutritional classifications									
Underweight *n* (%) [5]	7 (8.9)	3 (3.4)	n/a	7 (8.8)	2 (2.4)	n/a	4 (5.5)	4 (5.1)	n/a

Stunted *n* (%) [6]	12 (15.0)	4 (4.6)	n/a	10 (12.5)	6 (7.1)	0.297	9 (12.3)	3 (3.8)	n/a
Wasted *n* (%) [7]	3 (3.7)	2 (2.3)	n/a	5 (6.2)	3 (3.6)	n/a	4 (5.5)	3 (3.9)	n/a
Overweight *n* (%) [8]	4 (4.9)	6 (6.8)	n/a	4 (5.0)	3 (3.6)	n/a	4 (5.5)	6 (7.8)	n/a
Acute malnutrition *n* (%) [9]	1 (1.3)	0 (0)	n/a	1 (1.2)	0 (0)	n/a	1 (1.4)	0 (0)	n/a
Macrocephalus *n* (%) [10]	8 (10.0)	17 (19.3)	0.140	10 (12.5)	10 (12.2)	>0.999	10 (13.7)	13 (16.9)	0.753

Values in *italics* font indicate significant *p*-values. Abbreviations: HEU: HIV-exposed-uninfected; HUU: HIV-unexposed-uninfected; n/a: not applicable (no comparisons were performed due to one of the groups having less than five counts leading to volatile results. [1] data presented as mean ± SD; [2] non-normal distributed data; [3] normal distributed data; [4] sex-normalized Z-scores indices at age 6–12 months were computed using World Health Organization Anthro software of 2010, using gestation-adjusted age for preterm infants; [5] underweight from weight-for-age Z-scores < −2; [6] stunted from length-for-age Z-scores < −2; [7] wasted from weight-for-length Z-scores (WLZ) < −2; [8] overweight from WLZ > +2; [9] acute-malnutrition from mid-upper-arm-circumference Z-scores < −2; [10] macrocephalus from head circumference-for-age Z-scores > +2. Independent t-test was used for continuous normally distributed data, and the Mann–Whitney U test was used for continuous non-normally distributed data; Pearson's Chi-square test was used for categorical determine the differences in HEU and HUU infants ($p < 0.05$).

The mean length was significantly lower in HEU vs. HUU infants at 6 months (65.3 ± 3.5 cm vs. 66.6 ± 2.8 cm; $p = 0.014$) and 9 months (70.1 ± 3.1 cm vs. 71.2 ± 2.8 cm; $p = 0.012$). The mean LAZ was also significantly lower in HEU vs. HUU infants at 6 months (−0.8 ± 1.4 vs. −0.1 ± 1.2; $p < 0.001$) and 9 months (−0.5 ± 1.4 vs. 0.0 ± 1.3; $p = 0.023$). Furthermore, HEU infants were at a higher risk of being stunted as compared to HUU infants at age 6 months (15.0% vs. 4.6%), although we could not perform significance tests due to low counts in the HUU group. The mean WLZ was significantly lower in HEU infants as compared to HUU infants at 12 months (−0.2 ± 1.2 vs. 0.2 ± 1.2; $p = 0.020$).

3.4. DISCUSSION

Our study showed significant differences in terms of breastfeeding practices and growth between HEU and HUU infants in the second half of the first year of life, with inappropriate infant feeding practices identified. Appropriate feeding practices, especially continued breastfeeding, is important from 6 months when a transition occurs from EBF to continued breastfeeding with complementary feeding, making this a critical time for growth monitoring and promotion. Furthermore, breastmilk is the best source of nutrition for infants (WHO, 2019a), with inappropriate feeding practices potentially resulting in malnutrition, leading to well-documented increased morbidity and mortality risk (UNICEF, 2015).

Early cessation of breastfeeding was found in our study, especially in the HEU infants. Lack of knowledge and mothers' education level are possible contributory reasons for too early cessation of breastfeeding, as reported in another South African study (Jackson, Swanevelder, Doherty, Lombard, Bhardwaj & Goga, 2019). Our study found the early introduction of complementary foods before 6 months in both groups, with HUU infants given complementary foods at an earlier age than HEU infants, although this difference was not significant. This was similar to another South African study which found the introduction of solids in HEU infants as early as 6 weeks of age (Budree, Goddard, Brittain, Cader, Myer & Zar, 2017), while an Ethiopian study found that 58% of HEU infants were not introduced to complementary feeding at the recommended age of 6 months (Haile, Belachew, Berhanu, Setegn & Biadgilign, 2015).

Almost half (47%) of HEU infants were breastfed at 6 months signifying the progress of the ART program in terms of breastfeeding promotion (Budree *et al*., 2017). Breastfeeding however decreased over time in our study, with mothers stopping breastfeeding their infants at a mean age of 18 weeks, with reasons including the need to return to work (31.4% vs 43.3%) and insufficient milk or not growing (27.7% vs 25.4%) in HEU vs HUU infants. Poor breastfeeding rates in our

study may result from cultural practices, lack of knowledge, and previous provision of formula milk through PMTCT programs (Rossouw, Cornell, Cotton & Esser, 2016).

Lower breastfeeding rates were found in HEU vs HUU infants at 9 months, lower than in Kenya (Neary, Langat, Singa, Kinuthia, Itindi, Nyaboe, Ng'anga *et al*., 2021) but higher than the South African study in which no HEU infants were breastfed in the year 2009 (Rossouw *et al*., 2016). Lack of knowledge and HIV stigma need to be addressed to increase the breastfeeding rates in the ART context (Lang'at, Ogada, Steenbeek, MacDonald, Ochola, Bor & Odinga, 2018). The IYCF policy needs to be used to promote, protect and support breastfeeding even in the context of HIV (WHO, 2021).

The unquantified food frequency questionnaire is simple and quick to administer and can be used to determine the foods frequently consumed (Cape, Faber & Benadé, 2007; Faber, 2007), which may affect the growth of infants. Our results show that HEU (34.3%) and HUU (29.2%) infants were mostly consuming baby food in a jar/ pureed at 6 months of age which also increased at 12 months to 45.3% vs 48.1%, which is similar to other South African data (Faber, 2007; Faber *et al*., 2016). Baby food purée in a jar has been reported to contain insufficient nutrients such as iron and zinc, which are important for good growth and development (Mir-Marqués, González-Masó, Cervera & de la Guardia, 2015). HEU infants consumed more miscellaneous junk food products such as chips (50.7% vs 46.8%) and juice concentrate diluted with water (16.0% vs 6.5%) at least 4 days per week at 12 months, similar to other South African studies (Budree *et al*., 2017; Faber, 2007). The IYCF policy is thus very important in emphasizing the correct and timely introduction of complementary foods which may improve the growth of HEU infants (WHO, 2016).

Our study found poor growth in HEU infants for the first 12 months of their life, similar findings have been reported in HEU infants who experience slower growth than HUU infants over the first 12 months of life resulting from a high risk of opportunistic infections such as pneumonia and lower respiratory tract infections (Kidzeru *et al*., 2014; Neary *et al*., 2021; Slogrove, 2021; Sugandhi *et al.*, 2013). The mechanism of higher morbidity in these infants has been suggested to be associated with multifactorial reasons such as maternal socioeconomic factors, abnormal immunological factors, and maternal nutrition, which have been identified as possible causes of the increased morbidity in HEU infants (Evans, Jones & Prendergast, 2016a; Ruck & Smolen, 2022). Furthermore the maternal use of ART affects the growth of HEU infants (Evans *et al.*, 2016b).

In our study, a lower mean LAZ was found in HEU infants at 6 months, which is similar to another South African study (Pillay, Moodley, Emel, Nkwanyana & Naidoo, 2021), and a lower mean HCZ was also reported in Zimbabwe at 6 months (Evans, Chasekwa, Ntozini, Humphrey & Prendergast, 2016c). A lower mean LAZ was also observed at 9 months in HEU infants as compared to HUU

infants, similar to other studies in Uganda (Lane, Widen, Collins & Young, 2020), Rwanda (Lane, Bobrow, Ndatimana, Ndayisaba & Adair, 2019), and Botswana (Chalashika *et al*., 2017). HEU infants were at a higher risk of being stunted (12.5% vs 7.1%) at 9 months as compared to HUU infants. A higher risk of stunting (20% vs 10%; $p<0.01$) was also found in Kenya at 9 months (Neary *et al*., 2021). Stunting has irreversible consequences yet it is important to identify it at an early age (WHO, 2016; 2020).

Our study differs from a study conducted in Botswana which found significant differences in HEU vs HUU infants in terms of the underweight and stunting status at 6-24 months (Chalashika *et al*., 2017). However, the study only included 37.2% of HEU infants. We found significantly lower mean WLZ in HEU infants as compared to HUU infants at 12 months. Similar findings were reported in another South African study at the age of 12 months (Le Roux *et al*., 2019). A higher risk of being overweight in infancy might result in higher rates of obesity later in life, which increases the risk of non-communicable diseases (WHO, 2020). This study found that maternal HIV exposure affects the feeding practices and growth of infants. Breastfeeding practices decreased with age.

The strength of our study lies in detailed infant feeding practices and anthropometric measurements collected by trained field workers to ensure quality control and validity. Further strengths include the repeated anthropometric measurements in infants at 6, 9, and 12 months, a control group (HUU infants), and a similar sample size in the HEU and HUU groups. Limitations of this study include uneven numbers across the time points due to loss of follow-up (some mothers moved away from the study site due to the SA national lockdown during the COVID-19 pandemic, amongst other reasons) and limited sample size. A further limitation is a potential recall bias as the unquantified FFQ asks for food consumed in the past seven days, and no associations were investigated due to the small number of counts in the different consumption frequencies in both the HEU and HUU groups. In addition, our results may only be partially generalizable, taking into account the sociodemographic and geographical context of this study. In addition, the results need to be interpreted with care as adjustment for confounders, such as maternal age, employment status, and education level, which could not be performed due to the low sample size.

3.5. CONCLUSIONS

In this study, we compared the feeding practices and growth of infants aged 6 and 12 months exposed and unexposed to maternal HIV infection. HEU infants had lower rates of breastfeeding at 9 and 12 months, and the breastfeeding rates decreased with age. HEU infants had lower LAZ, WAZ, and MUACZ at 6, 9, and 12 months and higher stunting rates compared to the HUU infants. Current findings will inform nutrition policy interventions aimed at educating mothers and

caregivers about appropriate complementary feeding in order to promote optimal growth, even in the context of HIV. For future studies, a bigger sample size will be more beneficial, as well as using structured questionnaires to determine nutrient intake from 6 months of age in HEU children.

Author Contributions: Conceptualization, P.T., M.H. and U.F.; methodology, P.T., M.H. and U.F.; formal analysis, T.B. and P.T.; data curation, P.T. and H.M.; writing—original draft preparation, P.T.; review and editing P.T., M.H., H.L., H.M., P.P. and UF.; visualization, P.T.; supervision, M.H., H.L. and U.F.; project administration, P.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the University of Pretoria Department of Research and Innovation UCDP, the National Research Foundation (NRF): new Generation of Academics Program grant ID: NGAP_RDG190128411410, and the Collaborative Initiative for Paediatrics HIV Education and Research (CIPHER) of the International AIDS Society grant ID: 2017/560_FEU.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of the University of Pretoria (protocol code NAS063/2020 on 04 June 2020). **Informed Consent Statement:** Informed consent was obtained from all participants involved in the study

Data Availability Statement: Data are available on request from the corresponding author, due to the University of Pretoria policy on data publication.

Acknowledgments: The authors would like to thank all the staff and research assistants (Maryjane Ntima, Sheilla Sono, Lidisa Mothiba, Sicebile Sibiya, and Kedibone Matshai) for their assistance in the data collection of the Siyakhula study. Special thanks to the mothers and infants who participated in the study.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design, analysis, or writing of this article or in the decision to publish the results. Any opinions, findings, and conclusions are, therefore, of the authors.

3.6. REFERENCES

Becquet, R., Bland, R., Leroy, V., Rollins, N.C., Ekouevi, D.K., Coutsoudis, A., et al. 2009. Duration, pattern of breastfeeding and postnatal transmission of hiv: Pooled analysis of individual data from west and south african cohorts. *PloS One.* 4(10)**:**e7397.

Budree, S., Goddard, E., Brittain, K., Cader, S., Myer, L. and Zar, H.J. 2017. Infant feeding practices in a south african birth cohort—a longitudinal study. *Matern Child Nutr.* 13(3)**:**e12371.

Cape, W., Faber, M. and Benadé, S. 2007. Breastfeeding, complementary feeding and nutritional status of 6–12-month-old infants in rural Kwazulu-Natal. *South African Journal of Clinical Nutrition*. 20(1)**:**16-24.

Chalashika, P., Essex, C., Mellor, D., Swift, J.A. and Langley-Evans, S. 2017. Birthweight, hiv exposure and infant feeding as predictors of malnutrition in botswanan infants. *J Hum Nutr Diet*. 30(6)**:**779-90.

Coutsoudis, A., Pillay, K., Kuhn, L., Spooner, E., Tsai, W.-Y., Coovadia, H.M. and Group, S.a.V.a.S. 2001. Method of feeding and transmission of hiv-1 from mothers to children by 15 months of age: Prospective cohort study from durban, south africa. *AIDS*. 15(3)**:**379-87.

Department of Health 2014. National consolidated guidelines for the prevention of mother-to-child transmission of HIV (PMTCT) and the management of HIV in children, adolescents and adults. Africa.

Dewey, K. 2003. Guiding principles for complementary feeding of the breastfed child. Guidelines for an integrated approach to the nutritional care of HIV-infected children (6 months-14 years). Geneva: World Health Organization; 2009. Appendix v, guiding principles for complementary feeding of the breastfed child. Geneva: World Health Organization.

Ejigu, Y., Magnus, J.H., Sundby, J. and Magnus, M.C. 2020. Differences in growth of HIV-exposed uninfected infants in Ethiopia according to timing of in-utero antiretroviral therapy exposure. *Pediatr. Infect. Dis.* J. 39(8)**:**730.

Evans, C., Jones, C.E. and Prendergast, A.J. 2016a. Hiv-exposed, uninfected infants: New global challenges in the era of paediatric hiv elimination. *Lancet Infect Dis*. 16(6)**:**e92-e107.

Evans, C., Humphrey, J.H., Ntozini, R. and Prendergast, A.J. 2016b. Hiv-exposed uninfected infants in Zimbabwe: Insights into health outcomes in the pre-antiretroviral therapy era. Front Immunol. 7190.

Evans, C., Chasekwa, B., Ntozini, R., Humphrey, J.H. and Prendergast, A.J. 2016c. Head circumferences of children born to HIV-infected and HIV-uninfected mothers in Zimbabwe during the preantiretroviral therapy era. *AIDS*. 30(15)**:**2323.

Evans, C., Chasekwa, B., Ntozini, R., Majo, F.D., Mutasa, K., Tavengwa, N., et al. 2020. Mortality, HIV transmission and growth in children exposed to HIV in rural Zimbabwe. Clin Infect Dis. 72586-94.

Faber, M. 2007. Complementary foods consumed by 6 – 12-month-old rural infants in South Africa are inadequate in micronutrients. Public Health Nutr. 8(4)**:**373-81.

Faber, M. and Benade, A. 1999. Nutritional status and dietary practices of 4–24-month-old children from a rural South African community. Public Health Nutr. 2(2)**:**179-85.

Faber, M., Laubscher, R. and Berti, C. 2016. Poor dietary diversity and low nutrient density of the complementary diet for 6- to 24-month-old children in urban and rural Kwazulu-natal, South Africa. *Matern Child Nutr*. 12(3)**:**528-45.

Fadnes, L.T., Engebretsen, I.M., Wamani, H., Semiyaga, N.B., Tylleskar, T. and Tumwine, J.K. 2009. Infant feeding among HIV-positive mothers and the general population mothers: Comparison of two cross-sectional surveys in eastern Uganda. *BMC Public Health.* 9124.

Goga, A.E., Doherty, T., Jackson, D.J., Sanders, D., Colvin, M., Chopra, M. and Kuhn, L. 2012. Infant feeding practices at routine pmtct sites, South Africa: Results of a prospective observational study amongst hiv exposed and unexposed infants-birth to 9 months. *International Breastfeeding Journal.* 7(1)**:**4.

Haile, D., Belachew, T., Berhanu, G., Setegn, T. and Biadgilign, S. 2015. Complementary feeding practices and associated factors among HIV positive mothers in southern Ethiopia. *Journal of Health, Population and Nutrition*. 34(1)**:**1-9.

Iliyasu, Z., Galadanci, H.S., Iliyasu, M.L., Babashani, M., Gajida, A.U., Nass, N.S. and Aliyu, M.H. 2019. Determinants of infant feeding practices among HIV-infected mothers in urban kano, Nigeria. *Journal of Human Lactation*. 35(3)**:**592-600.

Jackson, D., Swanevelder, S., Doherty, T., Lombard, C., Bhardwaj, S. and Goga, A. 2019. Changes in rates of early exclusive breast feeding in South Africa from 2010 to 2013: Data from three national surveys before and during implementation of a change in national breastfeeding policy. *BMJ Open*. 9(11)**:**e028095.

Kidzeru, E.B., Hesseling, A.C., Passmore, J.-a.S., Myer, L., Gamieldien, H., Tchakoute, C.T., et al. 2014. In-utero exposure to maternal HIV infection alters t-cell immune responses to vaccination in HIV-uninfected infants. *AIDS* (London, England). 28(10)**:**1421.

Kindra, G., Coutsoudis, A., Esposito, F. and Esterhuizen, T. 2012. Breastfeeding in HIV exposed infants significantly improves child health: A prospective study. *Maternal and child health journal.* 16(3)**:**632-40.

Lane, C., Widen, E., Collins, S. and Young, S. 2020. Hiv-exposed, uninfected infants in Uganda experience poorer growth and body composition trajectories than hiv-unexposed infants. *J Acquir Immune Defic Syndr.* 4(Supplement_2)**:**1023-.

Lane, C.E., Bobrow, E.A., Ndatimana, D., Ndayisaba, G.F. and Adair, L.S. 2019. Determinants of growth in hiv-exposed and hiv-uninfected infants in the kabeho study. *Matern Child Nutr.* 10.1111/mcn.12776e12776.

Lang'at, P.C., Ogada, I., Steenbeek, A., Macdonald, N.E., Ochola, S., Bor, W. and Odinga, G. 2018. Infant feeding practices among hiv-exposed infants less than 6 months of age in bomet county, kenya: An in-depth qualitative study of feeding choices. *Archives of Disease in Childhood.* 103(5)**:**470-3.

Le Roux, S., M, Abrams, E.J., Donald, K.A., Brittain., K., Phillips., T.K., Nguyen., K.K., et al. 2019. Growth trajectories of breastfed HIV-exposed uninfected and HIV-unexposed children under conditions of universal maternal antiretroviral therapy: A prospective study. *The Lancet Child & Adolescent Health.* 3(4)**:**234-44.

Masuke, R., Msuya, S.E., Mahande, J.M., Diarz, E.J., Stray-Pedersen, B., Jahanpour, O. and Mgongo, M. 2021. Effect of inappropriate complementary feeding practices on the nutritional status of children aged 6-24 months in urban moshi, northern tanzania: Cohort study. *PloS One.* 16(5)**:**e0250562.

Matji, J.N., Wittenberg, D.F., Makin, J.D., Jeffery, B., Macintyre, U.E. and Forsyth, B.W. 2009. Factors affecting HIV-infected mothers' ability to adhere to antenatally intended infant feeding choice in Tshwane. *SAJCH.* 3(1)**:**20-3.

Matsungo, T.M., Kruger, H.S., Faber, M., Rothman, M. and Smuts, C.M. 2017. The prevalence and factors associated with stunting among infants aged 6 months in a peri-urban south african community. *Public Health Nutr.* 20(17)**:**3209-18.

Mir-Marqués, A., González-Masó, A., Cervera, M.L. and De La Guardia, M. 2015. Mineral profile of spanish commercial baby food. *Food Chemistry.* 172238-44.

Mugware, A., Motadi, S.A. and Mushaphi, L.F. 2022. Feeding practices and micronutrient status of children aged 0–36 months in Thulamela Municipality, Limpopo Province. *Health SA Gesondheid.* 279.

Neary, J., Langat, A., Singa, B., Kinuthia, J., Itindi, J., Nyaboe, E., et al. 2021. Higher prevalence of stunting and poor growth outcomes in hiv-exposed uninfected than HIV-unexposed infants in Kenya. AIDS.

Patridge, E.F. and Bardyn, T.P. 2018. Research electronic data capture (redcap). *JMLA*. 106(1)**:**142.

Pillay, L., Moodley, D., Emel, L.M., Nkwanyana, N.M. and Naidoo, K. 2021. Growth patterns and clinical outcomes in association with breastfeeding duration in hiv exposed and unexposed infants: A cohort study in kwazulu natal, South Africa. *BMC Pediatrics*. 21(1)**:**1-9.

Stats SA Release, S. 2021. Mid-year population estimates 2021. Statistics South Africa. Available online: http://www. statssa. gov. za/publications P.

Rollins, N.C., Ndirangu, J., Bland, R.M., Coutsoudis, A., Coovadia, H.M. and Newell, M.L. 2013. Exclusive breastfeeding, diarrhoeal morbidity and all-cause mortality in infants of HIV-infected and hiv uninfected mothers: An intervention cohort study in Kwazulu Natal, South Africa. *PloS One*. 8(12)**:**e81307.

Rosala-Hallas, A., Bartlett, J.W. and Filteau, S. 2017. Growth of hiv-exposed uninfected, compared with hiv-unexposed, zambian children: A longitudinal analysis from infancy to school age. BMC pediatrics. 17(1)**:**80.

Rossouw, M.E., Cornell, M., Cotton, M.F. and Esser, M.M. 2016. Feeding practices and nutritional status of hiv-exposed and hiv-unexposed infants in the Western Cape. *Southern African journal of HIV medicine.* 17(1).

Rothman, M., Faber, M., Covic, N., Matsungo, T.M., Cockeran, M., Kvalsvig, J.D. and Smuts, C.M. 2018. Infant development at the age of 6 months in relation to feeding practices, iron status, and growth in a peri-urban community of South Africa. *Nutrients*. 10(1)**:**73.

Ruck, C.E. and Smolen, K.K. 2022. Effect of maternal hiv infection on infant development and outcomes. *Frontiers in Virology*. 31.

Sayed, N. and Schönfeldt, H.C. 2020. A review of complementary feeding practices in South Africa. *South African Journal of Clinical Nutrition.* 33(2)**:**36-43.

Slogrove, A.L. 2021. It is a question of equity: Time to talk about children who are HIV-exposed and “hiv-free”. Journal of the International AIDS Society. 24(11)**:**e25850.

Slogrove, A.L., Johnson, L.F. and Powis, K.M. 2018. Population-level mortality associated with hiv exposure in HIV-uninfected infants in Botswana and south africa: A model-based evaluation. *Journal of tropical pediatrics*.

Smuts, C.M., Dhansay, M.A., Faber, M., Van Stuijvenberg, M.E., Swanevelder, S., Gross, R. and Benade, A.S. 2005. Efficacy of multiple micronutrient supplementation for improving anemia,

micronutrient status, and growth in south african infants. *The Journal of nutrition*. 135(3)**:**653S-9S.

Stewart, C.P., Iannotti, L., Dewey, K.G., Michaelsen, K.F. and Onyango, A.W. 2013. Contextualising complementary feeding in a broader framework for stunting prevention. *Maternal & child nutrition.* 927-45.

Sugandhi, N., Rodrigues, J., Kim, M., Ahmed, S., Amzel, A., Tolle, M., et al. 2013. Hiv exposed infants: Rethinking care for a lifelong condition. *AIDS* (London, England). 27(0 2)**:**S187.

Team, R.C. 2013. R: A language and environment for statistical computing. R foundation for statistical computing, vienna, austria. http://www. R-project. org/.

UNAIDS. Countdown to zero: Global plan towards the elimination of new hiv infections among children by 2015 and keeping their mother alive. UNAIDS.

UNAIDS. Update miles to go—closing gaps, breaking barriers, righting injustices [internet], geneva, switzerland, unaids: July 2018.

UNAIDS. *In danger: Unaids global aids update 2022. Joint United Nations Programme on HIV/AIDS* Geneva, Switzerland.

Unicef. *Adopting optimal feeding practices is fundamental to a child's survival, growth and development, but too few children benefi*t. New York, 2015. UNICEF.

Villar, J., Cheikh Ismail, L., Victora, C.G., Ohuma, E.O., Bertino, E., Altman, D.G., et al. 2014. International fetal and newborn growth consortium for the 21st century (intergrowth-21st). International standards for newborn weight, length, and head circumference by gestational age and sex: The newborn cross-sectional study of the intergrowth-21st project. *Lancet.* 384(9946)**:**857-68.

West, N.S., Schwartz, S.R., Yende, N., Schwartz, S.J., Parmley, L., Gadarowski, M.B., et al. 2019. Infant feeding by south african mothers living with hiv: Implications for future training of health care workers and the need for consistent counseling. *Int Breastfeed J.* 1411.

White, M., Feucht, U.D., Du Toit, L.D.V., Rossouw, T. and Connor, K.L. 2021. Understanding the impact of maternal hiv infection on the health and well-being of mothers and infants in south africa: Siyakhula collaborative workshop report. *J. Multidiscip. Healthc.* 14:1987-99.

World Health Organization. *Who child growth standards: Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: Methods and development*. World Health Organization.

World Health Organization. *Indicators for assessing infant and young child feeding practices: Part 2: Measurement.* [updated 2019May 2019]. Available from: https://www.who.int/nutrition/publications/infantfeeding/9789241599290/en/.

World Health Organization. *Advocacy brief: Breastfeeding and HIV: Global breastfeeding collective.* World Health Organization [updated 2019; cited 2019 August]. Available from: https://www.who.int/nutrition/publications/infantfeeding/breastfeeding-and-hiv-advocacybrief/en/.

World Health Organization. *Global breastfeeding scorecard, 2019: Increasing commitment to breastfeeding through funding and improved policies and programmes.* World Health Organization.

World Health Organization/. Unicef/who/the world bank group joint child malnutrition estimates: Levels and trends in child malnutrition: Key findings of the 2020 edition.

World Health Organization. 2021. *Indicators for assessing infant and young child feeding practices: Definitions and measurement methods.*

Wu, J., Li, J., Li, Y., Loo, K.K., Yang, H., Wang, Q., et al. 2018. Neurodevelopmental outcomes in young children born to hiv-positive mothers in rural yunnan, china. *Pediatrics International.* 60(7)**:**618-25.

CHAPTER 4: ARTICLE 2 (Manuscript 2)

Dietary intake and growth of infants in the complementary feeding phase by maternal HIV status in an urban setting in Gauteng Province, South Africa

Phumudzo Tshiambara [a,b,c,d*], Marinel Hoffman [b,c,d], Heather Legodi [a], Yusentha Balakrishna [e], and Ute Feucht [c,d,f]

[a] Department of Human Nutrition, Faculty of Health Sciences, University of Pretoria, South Africa

[b] Department of Consumer and Food Sciences, Faculty of Natural and Agricultural Sciences, University of Pretoria, South Africa

[c] Research Centre for Maternal, Fetal, Newborn and Child Health Care Strategies, University of Pretoria, South Africa

[d] Research Unit for Maternal and Infant Health Care Strategies, South African Medical Research Council, South Africa

[e] Biostatistics Research Unit, South African Medical Research Council, Durban, South Africa

[f] Department of Paediatrics, Faculty of Health Sciences, University of Pretoria, South Africa

* Correspondence: phumudzo.mamphwe@up.ac.za or phumumamphwe@gmail.com

Abstract

Background: Inadequate dietary intake is an immediate cause of undernutrition. HIV is the main determinant of undernutrition.

Objective: To compare the dietary intake, micronutrient composition of breastmilk, and growth of HIV-exposed-uninfected (HEU) and HIV-unexposed-uninfected (HUU) infants aged 6 and 12 months in an urban setting.

Methods: This repeated cross-sectional study included socio-demographic data, dietary intake, breastmilk samples, and anthropometric measurements in HEU and HUU infants at 6 and 12 months of age participating in the Siyakhula Study.

Results: This study compared 86 HEU and 95 HUU infants at 6 months and found that HEU infants had lower weight-for-age Z-scores (WAZ) (-0.6±1.1 vs. 0.1±1.2; p<0.001), length-for-age Z-scores (LAZ) (-0.8±1.4 vs. -0.1±1.2; p<0.001), and mid-upper-arm circumference Z-scores (MUACAZ) (0.5±1.1 vs. 1.0±0.9; p<0.001) than HUU infants. At 12 months, HEU infants had lower WAZ, MUACAZ, and weight-for-length Z-scores compared to HUU infants. However, stunting rates remained higher in HEU than HUU infants who had similar LAZ at 12 months. Breastfeeding rates were lower in HEU than HUU infants at 6 (49% vs. 64%; p=0.005) and 12 months (24% vs. 46%; p=0.002), but HEU infants consumed more flesh foods than HUU infants (24% vs. 11%; p=0.046) at 12 months. Only 11% and 6% of HEU and HUU infants achieved minimal dietary diversity scores at 12 months. Micronutrient composition of breastmilk fed to HEU and HUU infants were similar at 6 and 12 months. Dietary intake of fat was similar in breastfed HEU and HUU infants, but iron and vitamin B12 were lower in non-breastfed HUU infants than in HEU infants at 12 months.

Conclusion: HEU infants have lower Z-scores and breastfeeding rates, but better food consumption and nutrient intakes than HUU infants. Continuous nutrition education and counselling are crucial for appropriate feeding practices.

Keywords: HIV exposure, infants, growth, dietary intake, complementary feeding, breastmilk, urban setting

4.1. INTRODUCTION

Globally, undernutrition is a pressing public health concern and is responsible for 45% of deaths in children under five years of age, mostly occurring in low-and middle-income countries (Prendergast and Evans, 2023). Malnutrition increases susceptibility to infections; compromising growth and development. Undernutrition can be prevented through appropriate feeding practices and adequate dietary intake (World Health Organization (WHO), 2016). In 2021, 38.4 million people worldwide were living with HIV, with 28.7 million receiving antiretroviral therapy (ART) (UNAIDS, 2022). In South Africa, 8.2 million people were living with HIV in 2021, with 24% being women of reproductive age (Stats, 2022). Many mothers living with HIV (MLWH) still fear vertical HIV transmission through breastfeeding despite the significant risk reduction offered by ART (WHO, 2019a). Breastfeeding has many known benefits, even in the context of maternal HIV infection (WHO, 2019b). Even so, South Africa has lower breastfeeding rates (32%) than the rest of Sub-Saharan Africa (45%) and globally (44%) (UNICEF, 2021b). Since the introduction of the prevention-of-mother-to-child

transmission (PMTCT) program and ART in 2002 and 2004, HIV-related mortality and vertical transmission rates have decreased (UNAIDS, 2011). Subsequently, more than one million children are born each year in Sub-Saharan Africa exposed to HIV in-utero, while remaining uninfected; these infants are HIV-exposed-uninfected (HEU) (Sugandhi *et al*., 2013).

Even in the context of HIV and ART, exclusive breastfeeding is recommended for the first 6 months of with the introduction of appropriate complementary foods thereafter and continued breastfeeding for 24 months or longer (WHO, 2019a). Ideally, the complementary feeding period is characterized by the introduction of a variety of appropriate, safe, and nutrient-dense foods from 6 months of age, whilst breastfeeding. Complementary feeding is crucial for optimal growth and development as infants transition from the exclusive breastfeeding period (Dewey, 2013). In Africa, inappropriate complementary feeding is common, with early introduction of solid foods and lack of dietary diversity in infant meals (Kulwa *et al*., 2015, Faber, 2007). In Ethiopia (2017), only 26% of HEU infants were given appropriate complementary foods (Esubalew et al., 2018), while South African studies reported early introduction of complementary foods in 45-88% of infants (Sayed and Schönfeldt, 2020, Mugware *et al*., 2022). Inappropriate complementary feeding increases the risk of poor growth, especially from 6 to 12 months, when toddlers have increased nutritional needs. At this stage, the quality, rather than quantity, of complementary foods is of paramount importance, particularly if breastfeeding is discontinued (Dewey, 2013). In South Africa, infants in urban settings face unique challenges to growth as their meals consist mostly of maize or commercial infant cereals (Faber *et al*., 2016).

Limited information is available on dietary intake in relation to growth and the trace element composition of human breastmilk fed to HEU compared to HUU infant aged 6-12 months and comparing the dietary intake and growth during the complementary feeding phase is essential, particularly in high HIV prevalence urban settings (Rahamon *et a*l., 2013, Pedersen *et al*., 2016, Parker *et al*., 2013). Therefore, this study aims to compare the dietary intake, breastmilk composition and growth of HEU and HUU infants aged 6 and 12 months.

Methods and materials

Study design and participants

This repeated cross-sectional study is part of the Siyakhula study, previously described elsewhere (Tshiambara *et al*., 2023). Mothers were screened for eligibility by trained research assistants in the participant's local language. Baseline mother-infant data were collected at birth

from October 2018 at the Kalafong Provincial Tertiary Hospital, Gauteng Province. Dietary intake and anthropometric measurements were collected in 181 infants (HEU=86 and HUU=95) at 6 months and 155 infants (HEU=75 and HUU=80) at 12 months. Twenty-six HEU and HUU infants were lost to follow-up, because they relocated or could not complete measurements due to illnesses, and one infant became HIV-infected by 12 months.

Data collection

Socio-demographic information

Sociodemographic information, including maternal age, education level, employment status, alcohol use and smoking, were collected after obtaining informed consent. Additionally, HIV information was collected, with all MLWH self-reporting initiation of ART either before or during pregnancy.

Anthropometry

Weight (calibrated digital scale; Seca 354, Seca, Hamburg, Germany), length (mechanical infantometer; Seca 416, Seca, Hamburg, Germany), head circumference, and mid-upper-arm-circumference (non-stretchable tape measure; KDS measure, model F10-02DM 2m, Kyoto, Japan) were measured while the infants were wearing minimal clothing. These measurements were provided at birth (except for mid-upper-arm-circumference [MUAC]) as a baseline previously reported (Tshiambara *et al*., 2023), and, subsequently at 6 and 12 months. Weight-for-age Z scores (WAZ), length-for-age Z-scores (LAZ), weight-for-length Zscores (WLZ), head circumference-for-age Zscores (HCAZ), and MUAC-for-age Zscores (MUACAZ) were computed using the Intergrowth-21st and World Health Organisation (WHO) Anthro child growth standards v3.2.2. (Villar et al., 2014, World Health Organization, 2006) Underweight (WAZ <2 standard deviations [SD]), stunting (LAZ <2 SD), wasting (WLZ <2 SD), and overweight (WLZ >2 SD) were determined for HEU and HUU infants, with reference to median values (WHO, 2006).

Dietary intake

Mothers were privately interviewed at follow-up visits by trained research assistants using their preferred local language. A 24-hour recall is easy to administer, and provides detailed information on foods consumed, time, type and amount, and the preparation method used and has been previously used in dietary studies of infants aged 6 months (Walker *et al*., 2018, Faber *et al*., 2020). A standardized dietary kit, containing samples of food and food containers,

household utensils, and photographs, was used to measure the previous day's food intake. Mothers indicated the amount eaten by the infants using dry oats and a measuring cup to quantify the amount of food consumed by infants. Breastmilk substitutes (formula milk) and commercial infant cereals were listed in dry and liquid amounts. In this study, the dietary intake of infants included food, beverages, and breastmilk substitutes, excluding breastmilk in line with other studies (Nyofane, 2020, Lane *et al*., 2019). Moreover, the dietary intake of breastfed and non-breastfed HEU and HUU infants was also determined.

Breast milk composition

The macronutrient composition of human breastmilk has been well studied and described elsewhere (Grote et al., 2016, Kim and Yi, 2020, Mabaya et al., 2022, Kemp et al., 2023). For the purpose of this study only micronutrient trace elements breastmilk composition was included. This is because of the critical role they play in infants' growth and development but is understudied, also because of the differences in the analytical methods of macronutrients and micronutrients. Micronutrient breastmilk composition was determined at 6 and 12 months postnatally using inductively coupled plasma mass spectroscopy (Hampel et al., 2018a). Breastmilk was analysed for trace elements including iron, zinc, manganese, copper, and selenium. Breastmilk fed to HEU and HUU infants was hand expressed (10 mL) in a glass labelled bottle with the participant number and study visit (6 or 12 months). The breastmilk samples were stored in a sealed cooler box after hand expression and in a -80˚C freezer until analysis. Before analysis, the breastmilk samples were acidified with 2% final acid concentrations using ultrapure nitric acid. The dissolved part of the breastmilk was analysed. The ion detector converts the ions into an electrical signal, and the results were interpreted using the MassHunter programme (Sun et al., 2015).

Data processing and analysis

Data were captured using Research Electronic Data Capture v 8.3.5 (Patridge and Bardyn, 2018). The South African Medical Research Council (SAMRC) Food Quantities Manual was used to convert reported food consumption quantities into weights for the 24-h recall data. The SAMRC food finder software was used to quantify infants' macro- and micronutrient intake (SAFOODS, 2018). Nutrient densities (nutrient amount per 100 kcal) of the complementary diet were estimated and a dietary diversity score (DDS) was calculated using 24-h recall data to determine the proportion of infants consuming a diet including at least five of the eight food groups (breast milk; grains, white/pale starchy roots, tubers and plantains; beans, peas, lentils, nuts and seeds; dairy products; flesh foods; eggs; vitamin A-rich fruits and vegetables; and

other fruits and vegetables) (DDS ≤4 vs. ≥5 groups) (World Health Organization, 2021b). Descriptive statistics were used to present socio-demographic information, dietary intake, micronutrient breastmilk composition, anthropometric measurements, and HIV exposure. All continuous data were reported as means with SDs or as medians with interquartile ranges (IQR) when data was skewed. Categorical data was reported as frequencies and percentages. The Shapiro-Wilk test was used to determine the data's normality. To test for differences in continuous variables, the independent t-test (or its non-parametric counterpart, the Mann-Whitney U test) was used. To test for associations between categorical variables, the Pearson Chi-squared test was used. All statistical analyses were carried out at a 5% level of significance using the Stata 16 program.

Ethical considerations

The Siyakhula study was approved by the University of Pretoria's Faculty of Health Sciences Research Ethics Committee (Ref. no. 294/2017). All relevant information was shared with the mothers before data collection commenced. Mothers provided consent for themselves and their infants, and the Declaration of Helsinki criteria were followed. The Research Ethics Committees of the Faculty of Natural and Agricultural Sciences and the Faculty of Health Sciences at the same University approved this sub-study (Ref. no.: NAS063/2020).

4.3. RESULTS

Maternal characteristics, stratified by HIV status, are presented in Table 4.1. Significant differences were found between MLWH and mothers not living with HIV (MnLWH) in terms of age, education, gravidity and parity. Gestational age did not differ between infants born from MLWH and MnLWH (38.2±1.5 vs. 38.3±1.8 weeks; p=0.293). Proportionately, more HEU infants were born with low birth weight than HUU infants (22% vs. 13%; p<0.001) and the mean birth WAZ was lower in HEU than HUU infants (-0.7±0.9 vs. -0.2±1.0; p=0.003). Early introduction of solids food was found in this study and similar in HEU and HUU infants (16.2±11.0 vs. 12.8±9.3 weeks; p=0.118). Water and Mabelle/maize meal soft porridge were introduced first by 84% and 76% of MLWH and MnLWH, respectively.

Table 4.1. Maternal characteristics stratified by maternal HIV status

	MLWH	MnLWH	p-value

		n=86	n=95	
Age (years) [1] mean ± SD		36.9 ± 8.6	31.3 ± 6.3	**<0.001**
Age (years) n (%)	20–29	11 (12.8)	39 (41.1)	**<0.001**
	30–39	55 (64.0)	45 (47.4)	
	≥40	20 (23.2)	11 (11.5)	
Education [1] n (%)	Formal education, but without school completion [2]	55 (66.3)	31 (33.0)	**<0.001**
	Completed secondary schooling	19 (22.9)	39 (41.5)	
	Tertiary education	9 (10.8)	24 (25.5)	
Employment (any) [1] n (%)	Yes	41 (49.4)	43 (45.7)	0.738
Monthly income of the household (ZAR) [1] n (%)	Do not know [3]	20 (23.3)	18 (18.9)	0.282
	R 0–4000	29 (33.8)	27 (28.4)	
	R 4001–8000	21 (24.4)	29 (30.5)	
	More than R 8001	16 (18.5)	21 (22.2)	
Child support grant [1] n (%)	Yes	64 (74.4)	74 (77.9)	0.583
Marital status [1] n (%)	Single/ divorced / widow	60 (72.3)	74 (78.7)	0.412
	Married/ cohabiting	23 (27.7)	20 (21.3)	
Access to water [1] n (%)	Communal tap	21 (25.3)	19 (20.2)	0.534
	Inside yard	42 (50.6)	46 (48.9)	
	Inside house	20 (24.1)	29 (30.9)	
Access to electricity [1] n (%)	Yes	79 (91.9)	90 (94.7)	0.437
Access to toilet [1] n (%)	Flush toilet	54 (62.8)	64 (67.0)	0.519
	Pit latrine [4]	32 (37.2)	31 (33.0)	
Smoking [1,5] n (%)	Yes	3 (3.5)	3 (3.2)	n/a
Drinks alcohol (any) [1,5] n (%)	Yes	12 (14.0)	12 (12.6)	0.793
Mode of delivery [1] n (%)	Vaginal delivery [6]	49 (57.0)	65 (68.4)	0.196
	Caesarean section	37 (43.0)	30 (31.6)	
Obstetric history median (IQR)	Gravidity	3 (2-4)	3 (2-3)	**0.024**
	Parity	2 (1-3)	2 (1-2)	**0.031**
	Previous pregnancy losses [7]	0 (0-1)	0 (0-1)	0.647

Abbreviations: MLWH: mothers living with HIV; MnLWH: mothers not living with HIV; ZAR: South African rand; SD: standard deviation; IQR: interquartile range

[1] excludes missing numbers; [2] formal education = includes any primary and secondary schooling; [3] Do not know category excluded from analysis; [4] Pit toilet includes n=2 MLWH with no access to a toilet in the yard); [5] at

delivery and 6 months postpartum; [6] includes assisted delivery; [7] includes abortions, miscarriages and terminations of pregnancy

Statistical analysis: to determine the difference in continuous data between mothers living and not living with HIV Mann–Whitney U test (non-normally distributed) and for categorical data Pearson's Chi-square test was used to determine the differences in mothers living with HIV and mothers not living with HIV; bold p-value shows significant difference of <0.05.

Anthropometric measurements, Z-score indices, and nutritional classification of infants at 6 and 12 months of life by HIV-exposure status are presented in Table 4.2. At 6 months, HEU infants had significantly lower mean WAZ, LAZ, HCAZ and MUACAZ than HUU infants. Among HEU infants, stunting (15%), underweight (9%) and wasting (4%) was found at age 6 months. At 12 months, HEU infants, had lower mean WAZ, WLZ, and MUACAZ than HUU infants. Although no statistical tests performed, stunting rates were higher in HEU than HUU infants (12% vs. 4%) at 12 months.

Table 4.2. Anthropometric measurements, Z-score indices and nutritional classifications of infants at 6 and 12 months of life by HIV exposure status

	Age 6 months			**Age 12 months**		
	HEU infants (***n*** **= 86)**	**HUU infants** (***n*** **= 95)**	**p-value**	**HEU infants** (***n*** **= 75)**	**HUU infants** (***n*** **= 80)**	**p-value**
Anthropometric measurements						
Weight [1] (kg) mean ± SD	7.3 ± 0.9	7.8 ± 1.0	**0.001**	9.1 ± 1.2	9.4 ± 1.3	0.106
Length (cm) mean ± SD	65.3 ± 3.5 [1]	66.6 ± 2.8 [1]	**0.014**	74.4 ± 3.1	74.5 ± 2.7	0.704
Head circumference (cm) mean ± SD	43.5 ± 1.6 [1]	43.9 ± 1.6 [1]	0.106	46.5 ± 1.6	46.6 ± 1.6	0.655
Mid-upper-arm-circumference [1] (cm) mean ± SD	14.6 ± 1.3	15.2 ± 1.1	**0.002**	15.6 ± 1.2	16.0 ± 1.3	0.075
Z-score indices						
Weight-for-age Z-score [2] mean ± SD	−0.6 ± 1.1	0.1 ± 1.2	**<0.001**	−0.3 ± 1.1 [1]	0.1 ± 1.2 [1]	**0.022**
Length-for-age Z-score [1,2] mean ± SD	−0.8 ± 1.4	−0.1 ± 1.2	**<0.001**	−0.4 ± 1.3	−0.2 ± 1.1	0.308
Weight-for-length Z score [1,2] mean ± SD	−0.1 ± 1.2	0.2 ± 1.1	0.074	−0.2 ± 1.2	0.2 ± 1.2	**0.020**
Head circumference-for-age Z-score [1,2] mean ± SD	0.5 ± 1.2	0.9 ± 1.2	**0.019**	0.6 ± 1.2	0.9 ± 1.1	0.069
Mid-upper-arm-circumference-for-age Z-score [1,2] mean ± SD	0.5 ± 1.1	1.0 ± 0.9	**<0.001**	0.8 ± 1.1	1.3 ± 1.1	**0.025**

Nutritional classifications						
Underweight [3] n (%)	7 (8.9)	3 (3.4)	n/a	4 (5.5)	4 (5.1)	n/a
Stunted [4] n (%)	12 (15.0)	4 (4.6)	n/a	9 (12.3)	3 (3.8)	n/a
Wasted [5] n (%)	3 (3.7)	2 (2.3)	n/a	4 (5.5)	3 (3.9)	n/a
Overweight [6] n (%)	4 (4.9)	6 (6.8)	n/a	4 (5.5)	6 (7.8)	n/a

Abbreviations: HEU: HIV-exposed-uninfected; HUU: HIV-unexposed-uninfected; n/a: not applicable (sample size< 5). SD: standard deviation. [1] non normally distributed data; [2] sex-normalized Z-scores indices at age 6 and 12 months were computed using World Health Organization Anthro software of 2010; [3] underweight from weight-for-age Z-scores < −2; [4] stunted from length-for-age Z-scores < −2; [5] wasted from weight-for-length Z-scores (WLZ) < −2; [6] overweight from WLZ > +2
Statistical analysis: Independent t-test was used for continuous normally distributed data and Mann–Whitney U test was used for continuous that was not normally distributed; Pearson's Chi-square test was used for categorical data determine the differences in HEU and HUU infants, bold p-value shows significant difference of <0.05.

Food groups consumed by HEU and HUU infants at 6 and 12 months are presented in Table 3.3. A smaller proportion of HEU consumed any breastmilk at 6 (49% vs. 64%; p=0.005) and 12 (24% vs. 46%; p=0.002) months than what was consumed by HUU infants. At 12 months, flesh foods consumption (any consumption thereof) was low overall, but higher among HEU than HUU infants (24% vs. 11%; p=0.046). HEU infants consumed eggs (8%), vitamin A rich foods (15%), and other fruits and vegetables (36%) at 12 months. Dietary diversity was low in this study with <11% achieved minimal DDS at 12 months.

Table 4.3. Food group consumption (any intake) and dietary diversity score of HEU and HUU infants at 6 and 12 months.

Food groups	**Any intake at age 6 months (%)**			**Any intake at age 12 months (%)**		
	HEU infants (*n* =86)	HUU infants (*n* = 95)	p-value	HEU infants (*n* =75)	HUU infants (*n* = 80)	p-value
Breast milk	48.8	64.2	**0.005**	24.0	46.3	**0.002**
Grains, roots, tubers and plantains	97.7	91.6	n/a	97.3	93.8	n/a
Pulses (beans, peas, lentils), nuts and seeds	1.2	1.1	n/a	4.1	4.0	n/a
Dairy products (milk, infant formula, yoghurt, cheese)	100	100	n/a	97.3	88.8	0.120
Flesh foods (meat, fish, poultry, organ meats)	1.2	4.2	n/a	24.0	11.3	**0.046**
Eggs	1.2	0.0	n/a	8.0	2.5	n/a
Vitamin-A-rich fruits and vegetables [1]	9.3	3.2	n/a	14.7	8.8	0.129
Other fruits and vegetables [2]	4.7	22.1	n/a	36.0	35.0	0.97
Achieved minimal dietary diversity score [3]	2.3	0.0	n/a	10.7	6.3	0.397

Abbreviations: HEU: HIV-exposed-uninfected; HUU: HIV-unexposed-uninfected;

[1] includes carrots, butternut, pumpkin, sweet potato; spinach, butternut leaves, tomatoes, mangoes, peaches, strawberry; [2] includes cabbage, beetroot, corn, mandarins, apples, grapes, avocado, pear, banana, pineapple, watermelon; [3] percentage of infants who consumed ≥ 5 food items from the food groups

Statistical analysis: Pearson's Chi-square test was used to analyse categorical data; bold p-value shows significant difference of <0.05.

Trace elements composition of breastmilk fed to HEU and HUU infants at 6 and 12 months is presented in Table 4.4. No significant differences were found in breastmilk composition in MLWH and MnLWH (p>0.05) at 6 and 12 months.

Table 4.4. Trace elements (mg/L) in human breastmilk nutrient composition in MLWH and MnLWH at 6 and 12 months postpartum

Trace elements (Median (IQR))	**At 6 months postpartum**			**At 12 months postpartum**		
	MLWH n=16	**MnLWH** n=32	**p-value**	**MLWH** n=5	**MnLWH** n=16	**p-value**
Iron	1.1 (0.5-1.5)	1.5 (0.9-2.3)	0.066	1.7 (1.0-2.1)	1.9 (1.6-2.3)	0.386
Zinc	6.6 (4.6-8.4)	5.3 (3.4-7.3)	0.182	6.4 (5.8-6.9)	4.2 (3.0-6.0)	0.052
Manganese	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.983	0.0 (0.0-0.0)	0.0 (0.0-0.1)	0.836
Copper	0.9 (0.8-1.2)	1.2 (0.8-1.5)	0.444	0.9 (0.7-1.1)	1.0 (0.8-1.2)	0.710
Selenium	0.1 (0.1-0.1)	0.1 (0.1-0.1)	0.197	0.1 (0.1-0.1)	0.1 (0.1-0.1)	0.836

Abbreviations: MLWH: mothers living with HIV; MnLWH: mothers not living with HIV; IQR: interquartile range

Statistical analysis: Mann-Whitney U test was used to determine the differences in continuous data; p-value shows a significant difference of <0.05.

The dietary intake of breastfed and non-breastfed HEU and HUU infants is presented in Table 4.5. In breastfed HEU and HUU infants, significant differences were found in the dietary intakes of protein (16.6 [8.7-28.9] vs. 11.7 [6.0-14.9] gram per day (g/d); p=0.014), and vitamin B12 (0.6 [0.3-1.2] vs. 0.2 [0.1-0.4] microgram per day (mcg/d); p=0.010) at 12 months. Non-breastfed HEU infants had higher dietary intakes of protein, fat, carbohydrates, calcium, iron, zinc, and vitamin B12 than HUU infants (p<0.05) at 12 months.

4.5. DISCUSSION

The study compared dietary intake, and growth in HEU and HUU infants at 6 and 12 months of age as well as micronutrient breastmilk composition of their mothers. More MLWH (66% vs. 33% MnLWH) in this study had not completed secondary education which is in agreement with a systematic review that found a higher risk of HIV infection among women who had not completed secondary school than those who completed secondary school, emphasizing the important role of female education (Pettifor *et al*., 2008).

HEU infants had higher low birth weight and lower WAZ and LAZ at birth and 6 months compared to HUU infants, a trend consistent with other African countries (Pillay *et al*., 2021, Chalashika *et al*., 2017, Le Roux *et al*., 2019). Stunting (HEU: 15% vs. HUU: 5%), and underweight (HEU: 9% vs. HUU: 3%) were noted in HEU and HUU infants at age 6 months in agreement with studies conducted in Botswana and Uganda (Chalashika *et al*., 2017, Osterbauer *et al*., 2012). Although HEU infants had lower LAZ than HUU infants at birth, HEU infants were able to catch-up on growth, supporting literature related to catch up growth (Le Roux *et al*., 2019, Slogrove *et al*., 2017). Lower WLZ was found in HEU compared to HUU infants at 12 months in our study, with similar findings reported in Zimbabwe (Omoni *et al*., 2017). Growth monitoring and promotion including continuous nutrition education and counselling on appropriate complementary feeding is crucial for improving HEU infant's growth and preventing stunting (Ara *et al*., 2019, Lassi *et al*., 2013, Prendergast *et al*., 2019).

Concerningly, we found early cessation of breastfeeding and introduction of complementary foods in both MLWH and MnLWH. These results corroborate with other African studies

(Faber *et al*., 2020, Mugware *et al*., 2022, Tshiambara *et al*., 2023). Breastfeeding rates were lower in HEU than HUU infants at 6 (49% vs. 64%; p=0.005) and 12 (24% vs. 46%; p=0.002) months with similar findings reported in Rwandan HEU infants (Lane *et al*., 2019). Despite the recommendations and benefits of breastfeeding, stigma, culture, and lack of knowledge still influence the breastfeeding practices of MLWH (Rossouw *et al*., 2016, Wanjohi *et al*., 2016). Breastfeeding is a lifesaving intervention in preventing undernutrition and needs to be encouraged throughout the first 1000 days for optimal growth and development. Nutrition education and counseling to the mothers, caregivers, and family members are important for raising awareness about the importance of breastfeeding, breastmilk as a food source of nutrition.

Although the practice of early introduction to solid foods is common in Africa, it is concerning as it may lead to undernutrition (WHO, 2021b) More attention and education are needed for mothers, and caregivers on the correct, timely and age-appropriate complementary foods to be introduced at 6 months of age. A South African study reveals that mothers introduce solid foods due to sleep deprivation, crying, and beliefs that breastmilk alone is not sufficient for optimal growth and development (Mohlala *et al*., 2022). This highlights the need for educating African mothers, caregivers and family members on the composition of breastmilk. The previous distribution of MLWH formula in public health facilities due to high rates of vertical transmission and poor adherence to ART medication contributes to South Africa's low breastfeeding rates prior to 2011 (Lake *et al*., 2019, Kagee *et al*., 2011). This study suggests that despite low household income and child support grants recipient, warrant an assumption that socio-economic status is low, yet more mothers were not breastfeeding and possibly relying on breastmilk substitutes that are expensive (WHO, 2016). The WHO recommends continued breastfeeding for MLWH in adherence to ART therapy for up to 24 months and beyond.

Dietary diversity is crucial for assessing well-balanced diets in terms of micronutrient intake, health, and growth of infants (Steyn *et al*., 2006). Similar to other studies in the African context (Yisak *et al*., 2020, Mugware *et al*., 2022), the minimum dietary diversity score was low in our study (≤11%) in HEU and HUU infants at 12 months. These results are concerning as they indicate possible inadequate micronutrient intake in most infants. Flesh food consumption was low in our study and even lower in HUU than in HEU infants (11% vs. 24%) at 12 months. This was also the case in another South African study in which only 17% of infants consumed flesh products by 12 months (Faber, 2007). Flesh foods are a rich source of heme iron needed

for oxygen transportation, but they are expensive. (Mahan and Raymond, 2016) Raising awareness of healthy and affordable alternatives of iron-rich sources through nutrition education will be beneficial in our setting, where the prevalence of HIV and anaemia is high (World Health Organization, 2021a, Stats, 2022).

The breastmilk composition of macronutrients has been previously described (Kim and Yi, 2020) but limited information exists on the composition of trace elements in the human breastmilk, which plays a crucial role in supporting macronutrients absorption and for optimal growth and development of the infant (Mahan and Raymond, 2016, Pedersen *et al.*, 2016). MLWH and MnLWH had similar breastmilk composition of iron at 6 and 12 months, contradicting other studies with bigger sample size, and geographical area possibly contributing to the different results (Hampel *et al*., 2018b, Bzikowska-Jura *et al*., 2021, Nakamori et al., 2009). Breastmilk zinc composition was similar in MLWH and MnLWH, (6.4[5.8-6.9] vs. 4.2[3.0-6.0] mg per litre; p=0.052) at 12 months with other studies reporting lower composition (Bzikowska-Jura et al., 2021, Nakamori *et al.*, 2009). The differences may be accounted by the sample size, geographical area, time of breastmilk expression and the analytical methods used (Rios-Leyvraz and Yao, 2023).

Dewey (2001) suggested that iron and zinc levels are insufficient in infancy and more pressing from 4 months onwards, but MLWH and MnLWH showed higher amounts due to factors affecting breastmilk composition like quantification, lactation stage, body composition, and maternal dietary intake. This has implications on the policy, and more studies with bigger sample size are required in this urban setting. Manganese is important for absorption and utilization of other nutrients, (Mahan and Raymond, 2016) and MLWH and MnLWH had similar composition. MLWH should be encouraged to breastfeed their infants while adhering to ART as the composition is similar. This was further indicated by Mulol and Coutsoudis, (2016) reporting that MLWH have adequate breastmilk to feed HEU infants without compromising their muscle mass.

Inadequate dietary intake is an immediate determinant of undernutrition (UNICEF, 2021a). Dietary fat intake was low in HEU and HUU infants who were breastfed and non-breastfed at 12 months. Low fat intakes were reported in Malawian HEU and South African infants (Parker *et al*., 2013, Faber *et al*., 2016, Swanepoel *et al*., 2019). These results are concerning as dietary fat plays a crucial role in growth and development, energy provision, improving the absorption of fat-soluble vitamins, and brain development (Mahan and Raymond, 2016). Breastfed HEU

and HUU infants (92[66-140] vs. 85[58-107] g/d; p=0.249) had similar carbohydrate intake at 12 months. However, significant differences were found in HEU and HUU non-breastfed infants (95 [62-138] vs. 125 [86-169] g; p=0.013) at 12 months. Higher intakes of carbohydrates were reported in African studies (Swanepoel *et al*., 2019, Parker *et al*., 2013, Musakwa *et al*., 2020) with sample size, age group, and HIV exposure status yielding different results from our study. Significant differences were found in the dietary intakes of protein of HEU and HUU infants who were breastfed and non-breastfed ($p<0.05$) at 12 months with similar findings reported in Rwandan (Lane *et al*., 2019) and South African infants (Faber et al., 2016). Dietary protein intake is important for muscle strengthening and supporting the immune system (Mahan and Raymond, 2016). There is a need for education on easily accessible and affordable protein-rich sources such as dark green leafy vegetables, eggs, and peanut butter.

Vitamin A intake of breastfed HEU and HUU infants (236 [157-359] vs. 259 [157-433] mcg/d; 0.332) were similar but low at 12 months, with low intakes previously reported in Malawian breastfed HEU children and South African infants (Parker *et al*., 2013, Faber et al., 2016). Vitamin A deficiency is common in children under five years of age and South Africa has implemented a supplementation programme for the prevention of vitamin A deficiency in children between 6-59 months old (Saitowitz *et al*., 2001). Dietary intake of vitamin A-rich foods should be encouraged as they are easily affordable as well as encouraging MLWH and MnLWH to start vegetable gardens at home in order to increase the access and consumption of these foods. Significant differences were found in the vitamin B12 intake of HEU and HUU infants who were breastfed and non-breastfed at 12 months, with breastfed HUU infants having lower vitamin B12 intake than HEU infants. This is concerning but expected as vitamin B12 is found in flesh foods and is essential for brain and nervous system nourishment as well as deoxyribonucleic acid formation (Mahan and Raymond, 2016). Eggs are an affordable and alternative source of vitamin B12 to flesh foods, but the consumption was very low (HEU=8% vs. HUU=2.5%) in our study Faber *et al*. (2022) in the North West Province of South Africa, found an improved intake of vitamin B12 after the daily intake of eggs for three months compared to baseline results (at 6 months). Encouragement of egg consumption through nutrition education and counselling will be beneficial in our setting with low household income.

Folate intakes were similar in HEU and HUU infants ($p>0.05$) but low at 12 months. Folate is a water-soluble vitamin and important for brain and spinal cord development hence mothers

are supplemented with folic acid during pregnancy to prevent neural tube defects and undernutrition is associated with poor brain development (Mahan and Raymond, 2016). Pulses, like lentils, are a good source of folate, but their consumption is low (4%) in study participants, suggesting the need for planting in South Africa. Calcium is essential for the development of strong bones and teeth, and supporting the immune system, with a lack of calcium intake from 6 months during growth spurts known to have detrimental effects on bone development, resulting in rickets and stunting (Mahan and Raymond, 2016). Although no significant differences were found in breastfed HEU and HUU infants, dietary intake of calcium was low at 12 months. Similar findings were reported in Rwandan infants (Lane *et al*., 2019) and Malawian HEU children (Parker *et al*., 2013). Significant differences were found in the calcium intake of HEU compared to HUU infants who were non-breastfed, with HUU having a lower intake than HEU (312[115-455] vs. 433[278-696] mg/d; p=0.014). Breastmilk is ideal for optimal growth and development and recommends up to 24 months and beyond even in the context of ART but mothers who choose not to breastfeed should be counselled on the proper handling and administration of breastmilk substitute, that is time-consuming and expensive (WHO, 2016)

Dietary iron intake was lower in HUU than HEU (5.9[3.4-8.2] vs. 8.9[5.6-11.6] mg/d) in non-breastfed infants at 12 months. Dietary intake of iron is important for optimal growth and development and the low intake may lead to anaemia. Therefore, efforts such as nutrition education and counselling during growth monitoring and promotion should be made to MnLWH to include iron-rich foods such as flesh foods, eggs, fruits, and vegetables that are affordable in infants' diet as our results suggest a risk of anaemia due to the low intake in HUU infants at 12 months. Tshiambara *et al*. (2023) in the same study population, reported a high consumption of miscellaneous food products that may inhibit iron absorption. Therefore, discouraging consumption of these products will be beneficial in this setting, as well as encouraging families to initiate a home vegetable garden.

Limited literature exists on dietary intakes and growth of infants, including HEU and HUU infants, during the complementary feeding phase with available HEU data often lacking an HUU comparison group, together inclusion of older children, limiting comparisons to the currently presented data (Williams *et al*., 2016, Musakwa *et al*., 2020, Kulwa *et al*., 2015).

The strength of our study lies in detailed data on infant complementary feeding practices (food groups, dietary diversity and dietary intake) and repeat anthropometric measurements collected

by trained field workers to ensure quality control and validity. Further strengths include determining the composition of breastmilk with special focus on trace elements, a control group (HUU infants), and a similar sample size in the HEU and HUU groups. However, our study is not without limitations including a small sample size, use of a single 24-hour recall, excluding breastmilk intakes in the dietary intakes due to lack of quantification, as well as inability to make comparison with the reference intakes due to the type of our study. Future studies should include a larger sample size, additional study visits during the complementary feeding phase, multiple 24-hour recalls, food diaries, daily adequacy of the nutrients, food security, body composition of the mothers', anaemia status of mothers and infants, as well as associating mothers' dietary intake and infants growth to better understand HEU infants' long-term growth trajectories and associated factors.

4.6. CONCLUSION

The study compared the dietary intakes and growth of African HEU and HUU infants during the complementary feeding phase, as well as their mothers' micronutrient breastmilk composition. HEU infants had lower WAZ, and MUACAZ than HUU infants, but were able to catch up on LAZ as they grew older. Breastfeeding rates were lower in HEU than HUU infants, but they had better food consumption than HUU infants, while the DDS of the both HEU and HUU infants were poor. Micronutrient breastmilk composition was similar in MLWH and MnLWH. Dietary intake of fat and iron was lower in HUU compared to HEU infants. These findings can guide nutrition policies and interventions focusing on growth to strengthen the nutrition education and counselling components to promote appropriate feeding practices and ensuring optimal growth and development for all infants, regardless of HIV status.

Acknowledgments - The authors would like to thank all the staff and research assistants (Maryjane Ntima, Sheilla Sono, Lidisa Mothiba, Sicebile Sibiya, and Kedibone Matshai) for their assistance in the data collection of the Siyakhula study. Special thanks to the mothers and infants who participated in the study, Malory Links for FoodFinder software assistance, and Dr C Tosh (University of Pretoria) for editing the manuscript.

Disclosure statement - no potential conflict of interest was reported by the authors.

Funding - The authors disclosed receipt of the following financial support: the University of Pretoria Department of Research and Innovation's University Capacity Development

Programme (UCDP), the National Research Foundation (NRF) of South Africa (NGAP_RDG190128411410), and the Collaborative Initiative for Paediatrics HIV Education and Research (CIPHER) of the International AIDS Society grant (2017/560_FEU). Funders had no role in the design, analysis, and interpretation of this research.

Institutional Review Board Statement - The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of the University of Pretoria (protocol code NAS063/2020 on 04 June 2020).

Informed Consent Statement - Informed consent was obtained from all participants involved in the study.

Author Contributions - Conceptualization, P.T., M.H., H.L., and U.F.; methodology, P.T., M.H., and U.F.; formal analysis, Y.B., and P.T.; data curation, P.T.; writing—original draft preparation, P.T.; review and editing P.T.; H.L.; M.H.; Y.B.; and UF.; visualization, P.T.; project administration, P.T. The co-authors granted permission that this manuscript be submitted for the attainment of the PhD degree.

Consent for publication - All the authors consent to the publication of the present paper.

Data availability statement - The data supporting the findings will be made accessible upon request from the primary investigator of the Siyakhula Study (Ute Feucht) following an embargo period to allow for the conclusion of research and publishing of findings.

Table 4.5. Dietary intake of HEU and HUU infants according to breastfeeding practices at 6 and 12 months, to account for the unmeasured breastmilk intake in breastfed infants, presented as median (IQR).

	6 months						12 months					
	Breastfed infants			Non-breastfed infants			Breastfed infants			Non-breastfed infants		
	HEU (n=42)	**HUU (n=65)**	**p-value**	**HEU (n=42)**	**HUU (n=27)**	**p-value**	**HEU (n=18)**	**HUU (n=37)**	**p-value**	**HEU (n=54)**	**HUU (n=38)**	**p-value**
Energy [1] (kJ)	1433 (779-3276)	1540 (871-2417)	0.566	2620 (16356-3168)	3253 (2884-4532)	**0.015**	2477 (1587-3831)	1950 (1500-2474)	0.132	3220 (2389-4303)	2195 (1424-3273)	**0.015**
Protein [2] (g)	9.0 (4.4-16.2)	7.5 (4.6-12.8)	0.522	14.8 (11.2-23.1)	18.8 (14.6-27.6)	0.079	16.6 (8.7-28.9)	11.7 (6.0-14.9)	**0.014**	22.0 (15.8-29.6)	15.3 (10.2-23.5)	**0.008**
Fat [2] (g)	5.7 (2.3-12.2)	4.5 (1.8-14.8)	0.964	17.4 (12.7-25.5)	23.4 (18.2-35.2)	0.059	11.9 (8.2-26.0)	7.4 (4.3-14.0)	0.140	19.0 (13.1-26.7)	15.8 (8.3-23.4)	**0.047**
Carbohydrates [2] (g)	68 (35-148)	65 (32-102)	0.604	96 (54-120)	126 (97-182)	**0.028**	92 (66-140)	85 (58-107)	0.249	125 (86-169)	95 (62-138)	**0.013**
Calcium [2] (mg)	166 (52-436)	204 (78-417)	0.566	479 (333-667)	653 (467-1119)	**0.039**	194 (72-325)	131 (61-240)	0.258	433 (278-696)	312 (115-455)	**0.014**
Iron [3] (mg)	6.2 (2.2-14.9)	7.4 (3.2-12.0)	0.931	8.8 (5.8-13.0)	9.8 (6.8-18.6)	0.212	4.4 (2.4-7.7)	4.1 (3.1-5.4)	0.701	8.9 (5.6-11.6)	5.9 (3.4-8.2)	**0.026**
Zinc [3] (mg)	3.1 (1.3-5.8)	3.4 (1.5-6.9)	0.951	6.4 (4.1-8.5)	6.3 (5.3-9.7)	0.305	3.3 (1.9-7.6)	2.6 (1.9-4.3)	0.337	5.6 (4.1-7.1)	4.0 (2.1-5.9)	**0.038**
Copper [2] (mg)	0.1 (0.0-0.2)	0.1 (0.0-0.3)	0.297	0.3 (0.2-0.5)	0.4 (0.3-0.6)	0.260	0.4 (0.2-0.5)	0.3 (0.2-0.4)	0.249	0.5 (0.4-0.7)	0.3 (0.2-0.5)	**0.005**
Vitamin A [2] (mcg)	362 (152-1121)	486 (272-800)	0.847	609 (431-999)	1063 (592-1437)	0.077	259 (157-433)	236 (158-359)	0.332	516 (276-793)	404 (171-631)	0.087
Thiamine [2] (mg)	0.6 (0.2-1.2)	0.6 (0.3-1.4)	0.915	1.0 (0.8-1.5)	0.9 (0.7-1.9)	0.760	0.5 (0.3-1.0)	0.5 (0.3-0.8)	0.716	1.0 (0.6-1.3)	0.6 (0.4-1.0)	**0.022**
Riboflavin [2] (mg)	0.5 (0.2-1.2)	0.5 (0.2-1.1)	0.903	1.1 (0.9-1.7)	1.3 (0.9-1.9)	0.487	0.6 (0.3-0.7)	0.3 (0.2-0.8)	0.352	1.1 (0.6-1.6)	0.8 (0.5-1.3)	0.135
Niacin [2] (mg)	4.0 (1.5-8.9)	3.9 (2.0-7.3)	0.699	6.0 (4.2-9.0)	6.8 (4.8-9.5)	0.487	5.0 (2.9-10.9)	4.4 (3.3-6.4)	0.413	6.5 (5.1-10.8)	5.2 (3.3-8.9)	0.070
Vitamin B6 [2] (mg)	0.2 (0.1-0.6)	0.4 (0.1-0.5)	0.643	0.6 (0.4-0.9)	0.8 (0.6-0.8)	0.218	0.5 (0.4-1.4)	0.7 (0.4-0.9)	0.936	0.8 (0.6-1.1)	0.6 (0.3-0.9)	0.096
Folate [2] (mcg)	0.0 (0.0-0.0)	0.0 (0.0-4.0)	0.290	0.0 (0.0-0.0)	0.0 (0.0-10.0)	**0.010**	0.0 (0.0-4.6)	0.0 (0.0-4.9)	0.848	0.1 (0.0-5.1)	0.0 (0.0-7.3)	0.247

Vitamin B12 [2] (mcg)	0.2 (0.0-0.5)	0.4 (0.1-1.0)	0.056	1.0 (0.7-1.6)	1.2 (0.7-1.7)	0.375	0.6 (0.3-1.2)	0.2 (0.1-0.4)	**0.010**	1.0 (0.5-1.6)	0.6 (0.3-0.9)	**0.004**
Pantothenic acid [2] (mg)	0.5 (0.2-1.4)	1.2 (0.2-2.5)	0.098	3.7 (2.4-5.0)	3.9 (2.7-5.4)	0.487	1.3 (0.6-2.1)	1.2 (0.6-2.2)	0.864	3.4 (1.7-5.4)	2.1 (0.7-4.5)	**0.023**
Vitamin C [2] (mg)	61 (20-138)	68 (36-111)	0.927	84 (52-127)	135 (98-181)	**0.029**	45 (26-87)	25 (15-44)	0.051	67 (33-110)	49 (17-77)	0.087

Abbreviations: Dietary reference Intakes, HEU: HIV-exposed-uninfected (born to mothers living with HIV); HUU: HIV-unexposed-uninfected (born to mothers not living with HIV); IQR: Interquartile range; g: gram; mg; milligram; mcg: microgram; RE: retinol equivalence; KJ: kilojoule

[1]: Dietary reference Intakes at 6 months for boys=2709 and girls=2535 and at 12 months for boys=3245 and girls=2985; [2] Dietary reference intakes at 6-12 months; [3] estimated average requirements at 6-12 months; Statistical analysis: Mann-Whitney U test was used to determine the differences in continuous data, bold p-value shows significant difference of <0.05.

4.7. REFERENCES

Ara, G., Khanam, M., Papri, N., Nahar, B., Kabir, I., Sanin, K.I., et al. 2019. Peer counseling promotes appropriate infant feeding practices and improves infant growth and development in an urban slum in Bangladesh: A community-based cluster randomized controlled trial. Current Developments in Nutrition. 3(7):nzz072.

Bzikowska-Jura, A., Sobieraj, P., Michalska-Kacymirow, M. and Wesołowska, A. 2021. Investigation of iron and zinc concentrations in human milk in correlation to maternal factors: An observational pilot study in Poland. Nutrients. 13(2):303.

Chalashika, P., Essex, C., Mellor, D., Swift, J.A. and Langley-Evans, S. 2017. Birthweight, HIV exposure and infant feeding as predictors of malnutrition in Botswanan infants. *J Hum Nutr Diet*. 30(6):779-90.

Dewey, K.G. 2001. Nutrition, growth, and complementary feeding of the breastfed infant. Pediatric Clinics of North America. 48(1):87-104.

Dewey, K.G. 2013. The challenge of meeting nutrient needs of infants and young children during the period of complementary feeding: An evolutionary perspective. *The Journal of Nutrition*. 143(12):2050-4.

Esubalew, F., Atenafu, A. and Abebe, Z. 2018. Feeding practices according to the who-recommendations for HIV exposed children in northwest Ethiopia: A cross-sectional study. *Clinical nutrition ESPEN*. 28114-20.

Faber, M. 2007. Complementary foods consumed by 6 – 12-month-old rural infants in South Africa are inadequate in micronutrients. *Public Health Nutr*. 8(4):373-81.

Faber, M., Laubscher, R. and Berti, C. 2016. Poor dietary diversity and low nutrient density of the complementary diet for 6- to 24-month-old children in urban and rural Kwazulu-natal, South Africa. *Matern Child Nutr*. 12(3):528-45.

Faber, M., Rothman, M., Laubscher, R. and Smuts, C.M. 2020. Dietary patterns of 6–24-month-old children are associated with nutrient content and quality of the diet. *Maternal & Child Nutrition*. 16(2):e12901.

Faber, M., Malan, L., Kruger, H.S., Asare, H., Visser, M., Mukwevho, T., et al. 2022. Potential of egg as complementary food to improve nutrient intake and dietary diversity. *Nutrients.* 14(16):3396.

Grote, V., Verduci, E., Scaglioni, S., Vecchi, F., Contarini, G., Giovannini, M., et al. 2016. Breast milk composition and infant nutrient intakes during the first 12 months of life. *European journal of clinical nutrition*. 70(2):250-6.

Hampel, D., Dror, D.K. and Allen, L.H. 2018. Micronutrients in human milk: Analytical methods. *Advances in Nutrition*. 9(suppl_1):313S-31S.

Hampel, D., Shahab-Ferdows, S., Gertz, E., Flax, V.L., Adair, L.S., Bentley, M.E., et al. 2018. The effects of a lipid-based nutrient supplement and antiretroviral therapy in a randomized controlled trial on iron, copper, and zinc in milk from HIV-infected m alawian mothers and associations with maternal and infant biomarkers. *Maternal & child nutrition.* 14(2):e12503.

Kagee, A., Remien, R.H., Berkman, A., Hoffman, S., Campos, L. and Swartz, L. 2011. Structural barriers to art adherence in southern africa: Challenges and potential ways forward. *Global public health*. 6(1):83-97.

Kemp, J.E., Becker, P. and Wenhold, F.a.M. 2023. High protein content in breast milk from South African mothers of preterm infants. *Acta Paediatrica*. 112(10):2129-36.

Kim, S.Y. and Yi, D.Y. 2020. Components of human breast milk: From macronutrient to microbiome and microrna. *Clinical and experimental pediatrics*. 63(8):301.

Kulwa, K., Mamiro, P.S., Kimanya, M.E., Mziray, R. and Kolsteren, P.W. 2015. Feeding practices and nutrient content of complementary meals in rural central Tanzania: Implications for dietary adequacy and nutritional status. *BMC Pediatrics.* 15(1):1-11.

Lake, L., Kroon, M., Sanders, D., Goga, A., Witten, C., Swart, R., et al. 2019. Child health, infant formula funding and south African health professionals: Eliminating conflict of interest. *South African Medical Journal*. 109(12):902-6.

Lane, C., Bobrow, E.A., Ndatimana, D., Ndayisaba, G.F. and Adair, L.S. 2019. Decreased consumption of common weaning foods is associated with poor linear growth among HIV-exposed infants participating in the Kigali antiretroviral and breastfeeding assessment for the elimination of hiv (kabeho) study. *American Journal of Human Biology*. 31(6):e23308.

Lassi, Z.S., Das, J.K., Zahid, G., Imdad, A. and Bhutta, Z.A. 2013. Impact of education and provision of complementary feeding on growth and morbidity in children less than 2 years of age in developing countries: A systematic review. *BMC Public Health*. 13(3):1-10.

Le Roux, S., M, Abrams, E.J., Donald, K.A., Brittain., K., Phillips., T.K., Nguyen., K.K., et al. 2019. Growth trajectories of breastfed HIV-exposed uninfected and hiv-unexposed children under conditions of universal maternal antiretroviral therapy: A prospective study. *The Lancet Child & Adolescent Health*. 3(4):234-44.

Mabaya, L., Matarira, H.T., Tanyanyiwa, D.M., Musarurwa, C., Mukwembi, J., Mudluli, T.E. and Marera, T. 2022. Polyunsaturated fatty acid composition in breast milk plasma of HIV-infected and uninfected mothers in relation to infant clinical outcomes. Nutrition & Metabolic Insights. doi:1-9.

Mahan, L.K. and Raymond, J.L. 2016. Krause's food & the nutrition care process-e-book, Elsevier Health Sciences.

Mohlala, T.B., Mathunjwa, S.N., Legodi, H.M. and Mataboge, M.S. 2022. Exploring the infant feeding experiences of mothers living in selected tshwane informal settlements: A qualitative study. *South African Journal of Clinical Nutrition*. 1-8.

Mugware, A., Motadi, S.A. and Mushaphi, L.F. 2022. Feeding practices and micronutrient status of children aged 0–36 months in Thulamela municipality, Limpopo province. *Health SA Gesondheid*. 279.

Mulol, H. and Coutsoudis, A. 2016. Breastmilk output in a disadvantaged community with high HIV prevalence as determined by the deuterium oxide dose-to-mother technique. *Breastfeeding Medicine.* 11(2):64-9.

Musakwa, N., Feeley, A., Magwete, M., Patz, S., Mcnamara, L., Sanne, I., et al. 2020. Dietary intake among paediatric HIV-positive patients initiating antiretroviral therapy in Johannesburg, South Africa. *Vulnerable Children and Youth Studies*. 15(2):155-70.

Nakamori, M., Ninh, N.X., Isomura, H., Yoshiike, N., Hien, V.T.T., Nhug, B.T., et al. 2009. Nutritional status of lactating mothers and their breast milk concentration of iron, zinc and copper in rural Vietnam. *Journal of Nutritional Science and Vitaminology.* 55(4):338-45.

Nyofane, M. 2020. Cost of diet in relation to nutrient intake of infants residing in an HIV-exposed environment. University of Pretoria.

Omoni, A.O., Ntozini, R., Evans, C., Prendergast, A.J., Moulton, L.H., Christian, P.S. and Humphrey, J.H. 2017. Child growth according to maternal and child HIV status in Zimbabwe. *The Pediatric infectious disease journal*. 36(9):869.

Osterbauer, B., Kapisi, J., Bigira, V., Mwangwa, F., Kinara, S., Kamya, M.R. and Dorsey, G. 2012. Factors associated with malaria parasitaemia, malnutrition, and anaemia among HIV-exposed and unexposed Ugandan infants: A cross-sectional survey. *Malaria journal.* 11(1):1-6.

Parker, M.E., Tembo, M., Adair, L., Chasela, C., Piwoz, E.G., Jamieson, D.J., et al. 2013. The health of HIV-exposed children after early weaning. *Maternal & child nutrition.* 9(2):217-32.

Patridge, E.F. and Bardyn, T.P. 2018. Research electronic data capture (redcap). JMLA. 106(1):142.

Pedersen, S.H., Wilkinson, A.L., Andreasen, A., Kinung'hi, S.M., Urassa, M., Michael, D., et al. 2016. Longitudinal analysis of mature breastmilk and serum immune composition among mixed HIV-status mothers and their infants. *Clinical nutrition*. 35(4):871-9.

Pettifor, A.E., Levandowski, B.A., Macphail, C., Padian, N.S., Cohen, M.S. and Rees, H.V. 2008. Keep them in school: The importance of education as a protective factor against hiv infection among young south African women. International Journal of Epidemiology. 37(6):1266-73.

Pillay, L., Moodley, D., Emel, L.M., Nkwanyana, N.M. and Naidoo, K. 2021. Growth patterns and clinical outcomes in association with breastfeeding duration in hiv exposed and unexposed infants: A cohort study in KwaZulu Natal, South Africa. *BMC pediatrics*. 21(1):1-9.

Prendergast, A.J. and Evans, C. 2023. Children who are HIV-exposed and uninfected: Evidence for action. *AIDS* (London, England). 37(2):205-15.

Prendergast, A.J., Chasekwa, B., Evans, C., Mutasa, K., Mbuya, M.N.N., Stoltzfus, R.J., et al. 2019. Independent and combined effects of improved water, sanitation, and hygiene, and improved complementary feeding, on stunting and anaemia among HIV-exposed children in rural Zimbabwe: A cluster-randomised controlled trial. *The Lancet Child & Adolescent Health.* 3(2):77-90.

Rahamon, S.K., Arinola, G.O. and Akiibinu, M.O. 2013. Total antioxidant potential and essential trace metals in the breast milk and plasma of Nigerian human immunodeficiency virus-infected lactating mothers. *Journal of research in medical sciences*: the official journal of Isfahan University of Medical Sciences. 18(1):27.

Rios-Leyvraz, M. and Yao, Q. 2023. Calcium, zinc, and vitamin d in breast milk: A systematic review and meta-analysis. *International Breastfeeding Journal.* 18(1):1-14.

Rossouw, M.E., Cornell, M., Cotton, M.F. and Esser, M.M. 2016. Feeding practices and nutritional status of HIV-exposed and HIV-unexposed infants in the Western Cape. *Southern African journal of HIV medicine.* 17(1).

SAfoods 2018. SAMRC food quantities manual for South Africa. South African Medical Research Council Cape Town, South Africa.

Saitowitz, R., Hendricks, M., Fiedler, J., Le Roux, I., Hussey, G. and Makan, B. 2001. Proposed vitamin a supplementation programme for South Africa, coverage and cost. *South African Medical Journal.* 91(9):755-60.

Sayed, N. and Schönfeldt, H.C. 2020. A review of complementary feeding practices in South Africa. *South African Journal of Clinical Nutrition*. 33(2):36-43.

Slogrove, A.L., Esser, M.M., Cotton, M.F., Speert, D.P., Kollmann, T.R., Singer, J. and Bettinger, J.A. 2017. A prospective cohort study of common childhood infections in South African HIV-exposed uninfected and HIV-unexposed infants. *The Pediatric infectious disease journal*. 36(2):e38.

Stats, S. 2022. Mid-year population estimates 2022. Stats SA: Pretoria, South Africa.

Steyn, N.P., Nel, J.H., Nantel, G., Kennedy, G. and Labadarios, D. 2006. Food variety and dietary diversity scores in children: Are they good indicators of dietary adequacy? *Public health nutrition.* 9(5):644-50.

Sugandhi, N., Rodrigues, J., Kim, M., Ahmed, S., Amzel, A., Tolle, M., et al. 2013. Hiv-exposed infants: Rethinking care for a lifelong condition. *AIDS*. 27 Suppl 2S187-95.

Sun, J., Yang, Z., Lee, H. and Wang, L. 2015. Simultaneous speciation and determination of arsenic, chromium and cadmium in water samples by high performance liquid chromatography with inductively coupled plasma mass spectrometry. *Analytical Methods*. 7(6):2653-8.

Swanepoel, E., Havemann-Nel, L., Rothman, M., Laubscher, R., Matsungo, T.M., Smuts, C.M. and Faber, M. 2019. Contribution of commercial infant products and fortified staple foods to nutrient intake at ages 6, 12, and 18 months in a cohort of children from a low socio-economic community in South Africa. *Matern Child Nutr*. 15(2):e12674.

Tshiambara, P., Hoffman, M., Legodi, H., Botha, T., Mulol, H., Pisa, P. and Feucht, U. 2023. Comparison of feeding practices and growth of urbanized African infants aged 6–12 months old by maternal HIV status in Gauteng province, South Africa. Nutrients. 15(6):1500.

UNAIDS. Countdown to zero: Global plan towards the elimination of new HIV infections among children by 2015 and keeping their mother alive. UNAIDS.

UNAIDS. In danger: UNAIDS global aids update 2022. Joint United Nations Programme on HIV/AIDS Geneva, Switzerland.

UNICEF. Unicef global databases—infant and young child feeding. [01 October 2021].

Villar, J., Cheikh Ismail, L., Victora, C.G., Ohuma, E.O., Bertino, E., Altman, D.G., et al. 2014. International fetal and newborn growth consortium for the 21st century (intergrowth-21st). International standards for newborn weight, length, and head circumference by gestational age and sex: The newborn cross-sectional study of the intergrowth-21st project. LANCET. 384(9946):857-68.

Walker, J.L., Ardouin, S. and Burrows, T. 2018. The validity of dietary assessment methods to accurately measure energy intake in children and adolescents who are overweight or obese: A systematic review. *European journal of clinical nutrition*. 72(2):185-97.

Wanjohi, M., Griffiths, P., Wekesah, F., Muriuki, P., Muhia, N., Musoke, R.N., et al. 2016. Sociocultural factors influencing breastfeeding practices in two slums in Nairobi, Kenya. *International Breastfeeding Journal*. 121-8.

Williams, A.M., Chantry, C., Geubbels, E.L., Ramaiya, A.K., Shemdoe, A.I., Tancredi, D.J. and Young, S.L. 2016. Breastfeeding and complementary feeding practices among HIV-exposed infants in coastal Tanzania. *Journal of Human Lactation.* 32(1):112-22.

World Health Organization. Who child growth standards: Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: Methods and development. World Health Organization.

World Health Organization/. Guideline: Updates on HIV and infant feeding: The duration of breastfeeding, and support from health services to improve feeding practices among mothers living with HIV.

World Health Organization. Global breastfeeding scorecard, 2019: Increasing commitment to breastfeeding through funding and improved policies and programmes. World Health Organization.

World Health Organization. Advocacy brief: Breastfeeding and HIV: Global breastfeeding collective. World Health Organization [updated 2019; cited 2019 August]. Available from: https://www.who.int/nutrition/publications/infantfeeding/breastfeeding-and-hiv-advocacybrief/en/.

World Health Organization/. Edition global anaemia estimates in women of reproductive age, by pregnancy status, and in children aged 6–59 months.

World Health Organization/. Indicators for assessing infant and young child feeding practices: Definitions and measurement methods.

Yisak, H., Ambaw, B., Walle, Z., Alebachew, B. and Ewunetei, A. 2020. Minimum acceptable diet and associated factors among hiv-exposed children aged 6–24 months in debre tabor town, ethiopia. *HIV/AIDS-Research and Palliative Care*. 639-45.

CHAPTER 5: ARTICLE 3 (Manuscript 3)

Haemoglobin levels and growth in South African infants, aged 6–12 months, exposed to maternal HIV infection

Phumudzo Tshiambara[1,2,3,4], Heather Legodi[1], Marinel Hoffman[2,3,4], Helen Mulol[3,4], and Ute Feucht[3,4,5]

[1] Department of Human Nutrition, Faculty of Health Sciences, University of Pretoria, Prinshof Campus, Pretoria 0084, South Africa

[2] Department of Consumer and Food Sciences, Faculty of Natural and Agricultural Sciences, University of Pretoria, Hatfield Campus, Pretoria 0028, South Africa

[3] Research Centre for Maternal, Fetal, Newborn and Child Health Care Strategies, University of Pretoria, Kalafong Provincial Tertiary Hospital, Pretoria 0001, South Africa

[4] Research Unit for Maternal and Infant Health Care Strategies, South African Medical Research Council, Pretoria 0001, South Africa

[5] Department of Paediatrics, Faculty of Health Sciences, University of Pretoria, Prinshof Campus, Pretoria 0084, South Africa

Correspondence: phumudzo.mamphwe@up.ac.za or phumumamphwe@gmail.com; Tel.: +27-12-356-3205

Abstract

Background: Anaemia is a public health concern commonly affecting women of reproductive age and children under five years.

Objective: To determine the differences in haemoglobin levels and growth parameters between HIV-exposed-but-uninfected (HEU; n=86) and HIV-unexposed-uninfected (HUU; n=95) infants, with further determination of correlations between haemoglobin levels and growth.

Methods: Multiple cross-sectional analysis in the Siyakhula study with anthropometric measurements and haemoglobin levels data collected from 6-, 9- & 12-months old infants.

Results: Maternal haemoglobin levels and anaemia status significantly differed by HIV status at 6, 9, and 12 months postpartum ($p<0.05$), but similar mean haemoglobin levels and anaemia statuses were found in HEU and HUU infants at the same visits. Anaemia occurred in 26.5% and 33.3% of HEU infants (6 (and 9 months respectively). Weight-for-age Z-scores (WAZ), length-for-age Z-scores (LAZ), and mid-upper-arm circumference-for-age Z-scores (MUACZ) were lower in HEU compared to HUU infants at 6 months, while at 9 months WAZ, LAZ, and MUACZ were lower, with lower WAZ (−0.3 ± 1.1 vs. 0.1 ± 1.2; p=0.022), MUACZ (0.8 ± 1.1 vs. 1.3 ± 1.1; p=0.025), and weight-for-length Z-scores (WLZ) (-0.2 ± 1.2 vs. 0.2 ± 1.2; p=0.020) at 12 months. Regarding haemoglobin levels, positive correlations were seen in HEU but not HUU infants with WAZ (p=0.039), LAZ (p=0.007), and MUACZ (p=0.039) at 9 months, and with WAZ (p=0.018) and WLZ (p=0.041) at 12 months. At 12 months, negative correlations were found in HEU infants between haemoglobin levels and 6 and 12 months breastfeeding practices ($p<0.001$).

Conclusion: Exposure to maternal HIV infection affects growth in infants.

KEYWORDS

Anaemia, anthropometry, growth, haemoglobin, HIV exposure, infants, nutrition

5.1. INTRODUCTION

Anaemia is a global public health concern commonly affecting women of reproductive age (15–49 years) and young children (below five years) (Turawa *et al*., 2021). One in three women of reproductive age suffered from anaemia globally in the year 2020 (World Health Organization (WHO), 2020a), with a high prevalence of anaemia (<11 g/dL) of 61% -71% previously described in South African pregnant women living with HIV (Turawa et al., 2021). Anaemia can lead to suboptimal birth outcomes as iron demands increase during pregnancy, especially in the third trimester, also impacting the transfer of iron from the mother to the foetus (O'Brien *et al*., 2003; Tabrizi & Barjasteh, 2015). A haemoglobin level of <11 g/dL has previously been used in classifying anaemia in 6–12 months old infants (Allali *et al.*, 2017; Huo *et al*., 2015; Li *et al*., 2020; Provan, Singer, & Baglin, 2009; WHO, 2017; WHO, 2021a). Low haemoglobin levels restrict the transportation of oxygen in the blood, reduce physical and mental capacity (Smith et al., 2018; WHO, 2020a), increase the risk of infections (Santos *et a*l., 2011),

and lead to poor nutrition and growth in children (World Health Organization, 2021a). Anaemia has multiple causes, including infections such as HIV, diarrhoea, undernutrition, prematurity, and low birth weight (Abu-Ouf & Jan, 2015; Allali *et al*., 2017; da Silva et al., 2018; Sanou & Ngnie-Teta, 2012; Siekmans, Receveur, & Haddad, 2014; Tantracheewathorn & Lohajaroensub, 2005).

The transition from the exclusive breastfeeding period to the complementary feeding phase poses a higher risk of anaemia in infants from 6 months of age as the iron content in breastmilk is insufficient to meet the daily requirements for optimal growth and development (Mahan & Raymond, 2016; WHO, 2020a). From the age of 6 months, infants need 11 mg of iron a day (National Heart & Institute, 2011), but they struggle to meet this required intake in the context of poor-quality complementary feeding, especially in low-and middle-income countries (Faber, 2007). In 2019 high rates of anaemia were reported globally (40%), in Africa (60%), and in South Africa (44%) in children under the age of five years (WHO, 2021). Poor nutrition in the first 1000 days causes anaemia, and anaemic children are at a higher risk of undernutrition (WHO, 2020b). Anaemic infants are also at greater risk of growth failure, including underweight and stunting, as previously described in South Africa (Faber, 2007), Ethiopia (Appiah *et al*., 2020), and India (Stiller *et al*., 2020).

The prevalence of anaemia is higher in people living with HIV, with blood loss, reduced production of red cells and decreased red cell survival being potential causes (Volberding et al., 2004; WHO, 2020b). In 2021, 38 million people were living with HIV globally, with more than half (54%) being women and girls (UNAIDS, 2022a). In South Africa, 8.45 million people were living with HIV in the year 2021 (Stats, 2022). The country's high access to the antiretroviral (ART) programme has resulted in the large numbers of infants born who are exposed to maternal HIV infection but uninfected (HEU), due to the greatly decreased vertical transmission (UNAIDS, 2022a). Anaemia has been reported to be more prevalent in HEU than HUU infants (Teklemariam *et al*., 2015). In Ethiopia the odds of being anaemic if born to mothers living with HIV (MLWH) were 2.54 times higher than when born to mothers not living with HIV (MnLWH) (Feleke, 2016). In the same country, a high prevalence of anaemia and growth failure among HEU children was also found (Teklemariam et al, 2015), with particularly lower length (mean length-for-age Z-scores (LAZ)) in HEU infants when

compared to HUU infants in multiple studies (Ejigu *et al*., 2020; Le Roux *et al*., 2019; Rosala-Hallas, Bartlett & Filteau, 2017; Tshiambara *et al*., 2023).

Limited research is available on haemoglobin levels and growth in infants from 6 months of age who are exposed to maternal HIV infection in South Africa, a country with a high prevalence of HIV (Stats, 2022; UNAIDS, 2022a, 2022b). Most published studies are from other African countries and had deployed a single cross-sectional analysis (Dryden-Peterson et al., 2011; Berhane et al., 2004; Nabakwe et al., 2018), and others included children living with HIV, or lacked a comparison group of HUU infants (Nabakwe *et al*., 2018; Odhiambo *et al*., 2015; Teklemariam *et al*., 2015). Therefore, this study aims to compare and determine the correlation between haemoglobin levels and growth of urbanised 6- to 12-month-old South African HEU infants, compared to HUU infants.

Key messages

- Prevalence of anaemia was higher in mothers living with HIV compared to mothers not living with HIV at 6, 9, and 12 months postpartum, whilst HIV-exposed-uninfected (HEU) and HIV-unexposed-uninfected (HUU) infants had similar haemoglobin levels.
- HEU infants had lower WAZ, and MUACZ than HUU infants at 6 and 12 months, while LAZ differed significantly at 6 and 9 months.
- Positive correlations were found between haemoglobin levels and WAZ, LAZ and MUACZ at 9 months in HEU infants.
- Exposure to maternal HIV infection increases the risk of suboptimal growth, with lower mean anthropometric Z-scores in 6–12 months old infants.

5.2. METHODS AND MATERIALS

5.2.1. Study design, setting and participants

The multiple cross-sectional study design embedded in the Siyakhula Study, with the overall study aiming “to understand how the in-utero and early postnatal environments, altered by maternal HIV-positive status, influence infants’ growth trajectories, and cognitive development, and alter their immune development and function, irrespective

of the infants' HIV status". The study was conducted at the Research Centre for Maternal, Fetal, Newborn and Child Health Care Strategies at Kalafong Provincial Tertiary Hospital and the University of Pretoria in Gauteng Province, South Africa, as has been described elsewhere (Nyofane *et al*., 2022; Tshiambara *et al*., 2023; White *et al*., 2021). The sub-study participants included 181 mother-infant dyads with birth, 6-, 9-, and 12-months follow-up data, as summarized in Figure 1.

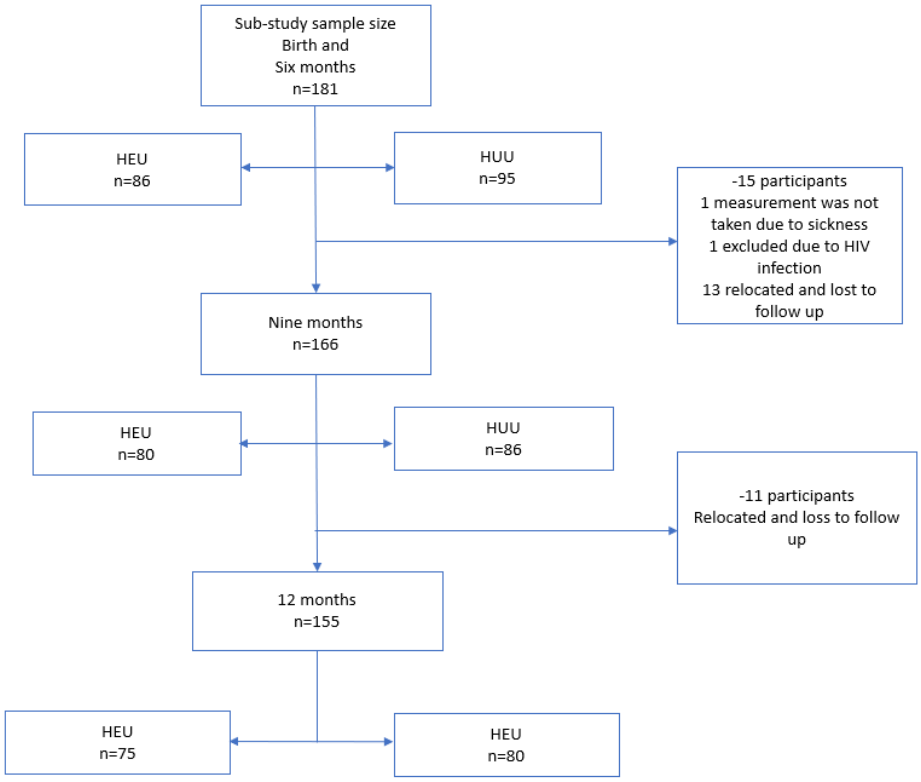


Figure 5.1. *Sub-study flow diagram of participants at 6, 9, and 12 months*

Abbreviations: HEU: HIV-exposed-uninfected; and HUU: HIV-unexposed-uninfected.

5.2.2. Data collection

5.2.2.1. Socio-demographic

Maternal demographic information was collected, including age, marital status, education, employment, access to water, toilet, and electricity. Additionally, socio-economic background, lifestyle and feeding practices, and current habits such as the use of tobacco and alcohol, and HIV status were collected, with MLWH self-reporting the use of ART throughout and after pregnancy, with the first-line regimen at the time of the study being a once-daily fixed-dose combination of tenofovir, emtricitabine, and efavirenz. Information was collected by trained fieldworkers in the participant's

preferred local language using a structured questionnaire (Tshiambara *et al*., 2023; White *et al*., 2021). A structured infant and mother follow-up questionnaire was subsequently used to gather and record the mother-infant dyad's information and measurements.

5.2.2.2. Haemoglobin levels

The HemoCue® device is a commonly used, point-of-care test to measure haemoglobin and diagnose anaemia. Capillary blood samples were collected by appropriately trained research staff at postpartum visits (6, 9, and 12 months) in mothers and their infants. A lancet was used to prick the finger, the first two or three drops of blood were wiped away before filling the microcuvette with a single millilitre of blood; the microcuvette was then inserted into the portable photometer (Hb 201^{+}; HemoCue®, Angelholm, Sweden) device, recommended by the World Health Organization (2001) for use in both clinical and survey settings in resource-limited locations for determining the prevalence of anaemia (Levy *et al*., 2017; Nkrumah *et al*., 2011). A haemoglobin level cut-off of <11.0 gram per decilitre (g/dL) was used to classify anaemia in 6–12 months infants and pregnant women, and <11.9 g/dL for non-pregnant women (Allali *et al*., 2017; Huo et al., 2015; Li *et al*., 2020; Provan, Singer, & Baglin, 2009; World Health Organization, 2017; WHO, 2021).

5.2.2.3. Anthropometric measures

Infant anthropometry was measured at birth, 6, 9, and 12 months to measure the infant's body size including the length, weight, head circumference (HC), and mid-upper-arm-circumference (MUAC) (the latter from 6 months onwards). A mechanical infantometer (Seca 416, Germany), calibrated digital scale (Seca 354, Germany), and non-stretchable tape measures (KDS measure, model F10-02DM 2m, Kyoto, Japan) were used to collect the length, weight, MUAC and HC of the infants.

The infants' Z-scores for weight, length, MUAC and HC were computed using the WHO Anthro child growth criteria v3.2.2 with gestational age correction and standardised for age and sex (World Health Organization, 2019). Stunting underweight, and wasting were nutritional classifications defined as Z-scores less than -2 SD for WAZ, LAZ, and WLZ, respectively, and overweight as a WLZ more than +2 SD of the reference data median values.

Maternal body size was assessed using weight, height and MUAC measurements and the body mass index was calculated. All anthropometric measurements were taken twice

and, as per the International Society for the Advancement of Kinanthropometry (ISAK) guidelines. If weight and lengths differed by >0.05 kg or 0.5 cm, a third measurement was taken, and the two closest values were used. The mean was then used for both measurements. (Stewart *et al*., 2012).

5.2.3 Data processing and statistical analysis

Descriptive data analysis was used to present continuous data such as maternal age, haemoglobin levels, anthropometric measures, and Z-scores indices for the study population. Normality of the data was determined by histograms, and the Shapiro-Wilk test. Age, weight, length, HC, and MUAC are examples of continuous variables with normally distributed data that were reported as mean and standard deviation. In the case of non-normally distributed data, the median and interquartile range (IQR) were reported. All categorical variables such as HEU status, employment status, prematurity and low birth weight were presented as frequencies and percentages.

The independent t-test (normally distributed) or the Mann-Whitney U test (non-normally distributed) was used to compare the data from HEU and HUU for continuous variables and the Pearson Chi-square test was used to compare categorical variables. The relationship between haemoglobin levels and growth parameters (infant Z-scores) and further potential founders was examined using the Pearson correlation coefficient. The Statistical Package for the Social Sciences was used for all statistical analyses (Version 28, IBM, Armonk New York) and the level of significance was set at $p<0.05$ (Haryono, Slamet, & Septian, 2023).

5.2.4. Ethical considerations

The Faculty of Health Sciences Research Ethics Committee (FHSREC) at the University of Pretoria, South Africa, gave its approval to the Siyakhula Study (Ref. no. 294/2017). The Faculty of Natural and Agricultural Sciences and the FHSREC at the same institution provided extra clearance for this sub-study (Ref. no. NAS063/2020). Prior to data collection, all pertinent information was disclosed to the mothers, who then gave their informed consent on behalf of both themselves and their infants.

5.3. RESULTS

The socio-demographic characteristics of the MLWH and MnLWH (n=181; 86 (47.5%) vs. 95 (52.5%), respectively) are presented in Table 5.1, with significant differences found in the maternal age and education levels (both p<0.001) between the two groups, while no significant differences were found in terms of employment status, monthly household income, and access to electricity, toilet, and water and mode of delivery (p>0.05). All the mothers living with HIV were on antiretroviral therapy, with the majority (98.8%) on tenofovir/emtricitabine/efavirenz. Most mothers-initiated ART before pregnancy (76.7%) and the mean latest CD4 count was 473 ± 273 cells/mm^3.

Table 5.1. Maternal characteristics stratified by maternal HIV status

		MLWH	MnLWH	p-value
		n=86	n=95	
Age (years) mean ± SD		36.9 ± 8.6	31.3 ± 6.3	<0.001*
Age (years) n (%) †	20-29	11 (12.8)	39 (41.1)	<0.001*
	30-39	55 (64.0)	45 (47.4)	
	≥40	20 (23.2)	11 (11.5)	
Education n (%) †	Formal education, but without school completion ‡	55 (66.3)	31 (33.0)	<0.001*
	Completed secondary schooling	19 (22.9)	39 (41.5)	
	Tertiary education	9 (10.8)	24 (25.5)	
Employment n (%) †	Yes	41 (49.4)	43 (45.7)	0.738
Monthly income of the household (ZAR) n (%) †	Don't know §	20 (23.3)	18 (18.9)	0.282
	R 0 - R 4000	29 (33.8)	27 (28.4)	
	R 4001-R 8000	21 (24.4)	29 (30.5)	
	More than R 8001	16 (18.5)	21 (22.2)	
Child support grant n (%) †	Yes	64 (74.4)	74 (77.9)	0.583
Marital status n (%) †	Single/ divorced / widow	60 (72.3)	74 (78.7)	0.412
	Married/ cohabiting	23 (27.7)	20 (21.3)	
Access to water n (%) †	Communal tap	21 (25.3)	19 (20.2)	0.534
	Inside yard	42 (50.6)	46 (48.9)	
	Inside house	20 (24.1)	29 (30.9)	
Access to electricity n (%) †	Yes	79 (91.9)	90 (94.7)	0.437
Access to toilet n (%) †	None ¶	2 (2.4)	0 (0)	n/a
	Pit latrine	29 (34.9)	31 (33.0)	0.816

	Flush toilet	52 (62.7)	63 (67.0)	
Smoking n (%) †, ¥	Yes	3 (3.5)	3 (3.2)	n/a
Drinks alcohol n (%) †, ¥	Yes	12 (14.0)	12 (12.6)	0.793
Mode of delivery n (%) †	Vaginal delivery ¦	49 (57.0)	65 (68.4)	0.196
	Caesarean section	37 (31.6)	30 (31.6)	
Obstetric history	Gravidity	3 [2, 4]	3 [2, 3]	0.024 *
median [IQR]	Parity	2 [1, 3]	2 [1, 2]	0.031 *
	Previous pregnancy losses ¢	0 [0, 1]	0 [0, 1]	0.647

Abbreviations: MLWH: mothers living with HIV; MnLWH: mothers not living with HIV; ZAR: South African rand; SD: standard deviation; IQR: interquartile range

† excludes missing numbers; ‡ formal education = includes any primary and secondary schooling; § Don't know category excluded from analysis; ¶ none: not considered in the calculation; ¥ at delivery and 6 months postpartum; ¦ includes assisted delivery; ¢ includes abortions, miscarriage and termination of pregnancy

Statistical analysis: to determine the difference in continuous data between MLWH and MnLWH the Mann–Whitney U test (non-normally distributed) was used; for categorical data Pearson's Chi-square test was used to determine the differences between MLWH and MnLWH; * p-value shows significant difference of <0.05.

The infants' characteristics at birth are presented in Table 5.2. Significant differences were found in HEU vs HUU infants in the low-birth-weight (birthweight below 2500g) category (22.1% vs. 12.6%; p<0.001). There was a significant difference between the infants' birth weight in HEU and HUU infants (2844 ± 490 g vs. 3058 ± 507 g; p=0.005) and in the birth weight-for-age Z-score (-0.7 ± 0.9 vs. -0.2 ± 1.0; p=0.003). No significant differences were found in the gestational age in weeks of HEU vs HUU infants (38.2 ± 1.5 vs. 38.3 ± 1.8; p=0.293). HEU infants had started with vertical transmission HIV prevention and 55.8% were initiated on a single drug nevirapine prophylaxis, with the remainder receiving a dual prophylaxis containing nevirapine and zidovudine (AZT).

Table 5.2. Characteristics of HIV-exposed-uninfected and HIV-unexposed-uninfected infants at birth

		HEU infants	**HUU infants**	**p-value**
		n=86	n=95	
Gestational age (weeks) mean ± SD †		38.2 ± 1.5	38.3 ± 1.8	0.293
Premature n (%)	Yes	11 (12.8)	12 (12.6)	0.974
	Very preterm (28-<32w)	0 (0)	1 (1.1)	n/a
	Moderate preterm (32-<34w)	0 (0)	1 (1.1)	
	Late preterm (34-<37w)	11 (12.8)	10 (12.6)	
Low birth weight n (%) ‡		19 (22.1)	12 (12.6)	<0.001*
Infant sex n (%)	Female	32 (37.2)	44 (46.3)	0.276

APGAR score median [IQR]	1 minute	8 [8, 9]	9 [9, 9]	0.145
	5 minutes	9 [9, 9]	9 [9, 10]	0.199
Body measurements mean ± SD	Weight (g) †	2844 ± 490	3058 ± 507	0.005*
	Length (cm)	49.1 ± 4.1	49.9 ± 3.4	0.184
	Head circumference (cm)	33.8 ± 1.8	34.5 ± 1.6	0.013*
Z-scores indices mean ± SD †,	Weight-for-age	−0.7 ± 0.9	−0.2 ± 1.0	0.003*
	Length-for-age	0.6 ± 1.4	0.7 ± 1.5	0.804
	Head circumference-for-age	0.3 ± 1.3	0.7 ± 1.2	0.038*

Abbreviations: HEU: HIV-exposed-uninfected (born to mothers living with HIV); HUU: HIV-unexposed-uninfected (born to mothers not living with HIV); n/a: not applicable (no comparisons were performed due to one of the groups having less than five counts leading to volatile results; g: gram; cm: centimetre; IQR: interquartile range; SD: standard deviation.

‡ Low birth weight is classified as infants born with a weight of less than 2500g; § the birth Z-score indices are sex-normalized and were computed using INTERGROWTH-21st software.

Statistical analysis: to determine the difference in continuous data between mothers living and not living with HIV, Independent-t-test (normally distributed) and Mann–Whitney U test († non-normally distributed) were used; for categorical data, Pearson's Chi-square test was used to determine the differences. * p-value shows significant difference of <0.05.

The infants' anthropometric measurements, Z-score indices, and nutritional categories at 6, 9, and 12 months, according to their HEU status, are shown in Supplementary Table 1. The mean weight of HEU infants was significantly lower than that of HUU infants at 6 months (7.3±0.9 kg vs. 7.8±1.0 kg; p=0.001), and their mean WAZ was also significantly lower (−0.6±1.1 vs. 0.1±1.2; p<0.001) than that of HUU infants. WAZ and LAZ were significantly lower in HEU than HUU infants at 9 months (p = 0.003 and 0.023, respectively). Stunting (15.0%), underweight (8.9%), and wasting (3.7%) were found in HEU infants at 6 months.

The maternal and infant haemoglobin levels and anaemia status at 6, 9, and 12 months postpartum, stratified by maternal HIV status, are presented in Table 5.3. Significant differences were found between MLWH and MnLWH in terms of the mean haemoglobin levels and anaemia status at 6, 9, and 12 months postpartum (p < 0.05). MLWH were more anaemic at 6 months (36.2% vs. 13.1%; p<0.001), 9 months (26.0% vs. 13.5%; p<0.001), and 12 months (28.6% vs. 17.9%; p<0.001) compared to MnLWH. No significant differences were found between HEU and HUU infants in terms of the mean haemoglobin levels and anaemia status at ages 6, 9, and 12 months (p>0.05).

Table 5.3. Haemoglobin levels and anaemia status of mothers and infants at 6, 9, and 12 months postpartum, stratified by maternal HIV status

	At 6 months postpartum			At 9 months postpartum			At 12 months postpartum		
	MLWH/ HEU	**MnLWH/ HUU**	p-value	**MLWH/ HEU**	**MnLWH/ HUU**	p-value	**MLWH/ HEU**	**MnLWH/ HUU**	p-value
	n=86	n=95		n=80	n=86		n=75	n=80	
Maternal haemoglobin levels (g/dL) mean ± SD	12.6 ± 1.6	13.2 ± 1.6	0.027*	12.6 ± 1.3	13.3 ± 1.2	<0.001 *	12.6 ± 1.5	13.1 ±1.3	0.030*
Maternal anaemia n (%) †	25 (36.2)	8 (13.1)	<0.001 *	19 (26.0)	10 (13.5)	<0.001 *	20 (28.6)	14 (17.9)	<0.001 *
Infant haemoglobin levels (g/dL) mean ± SD	11.9 ± 1.4	11.8 ± 1.2	0.996	11.7 ± 1.5 ‡	11.8 ± 1.4 ‡	0.440	11.9 ± 1.2 ‡	11.6 ± 1.2 ‡	0.089
Infant anaemia n (%)†	18 (26.5)	10 (18.2)	0.382	23 (33.3)	21 (28.8)	0.684	10 (16.9)	19 (29.7)	0.147

Abbreviations: HEU: HIV-exposed-uninfected, HUU: HIV-unexposed-uninfected; Hb: haemoglobin, MLWH: mothers living with HIV; MnLWH: mothers not living with HIV; g: gram; dL: decilitre. Mothers n without missing Hb levels at 6 months: MLWH=69 and MnLWH=61, at 9 months: MLWH=73 and MnLWH=74, at 12 months: MLWH=70 and MnLWH=65; infants n without missing Hb levels at 6 months: HEU=55 and HUU=68, at 9 months: HEU=69 and HUU=73, at 12 months: HEU=59 and HUU=64.

† World Health Organization anaemia cut-offs: non-pregnant women: <11.9 g/dL; infants from 6-59 months: <10.9 g/dL

Statistical analysis: to determine the difference in continuous data between MLWH and MnLWH, Independent-t-test (normally distributed) and Mann–Whitney U test (‡ non-normally distributed) were used; for categorical data, Pearson's Chi-square test was used to determine the differences in MLWH and MnLWH; * p-value shows significant difference of <0.05.

The maternal anthropometric measurements at 6, 9, and 12 months postpartum are presented in Table 5.4. The mean body mass index of MLWH was significantly lower than MnLWH at 6 months (25.6±4.5 vs. 27.7±4.7; p=0.011), 9 months (25.4±4.5 vs. 27.9±4.8; p=0.002), and at 12 months (26.0±4.5 vs. 28.2±4.9; p=0.007), with significantly lower percentages of overweight in MLWH than MnLWH (p<0.05) at 6,

9, and 12 months. The mean MUAC was significantly lower in MLWH than in MnLWH at 12 months ($p<0.05$).

Maternal haemoglobin levels, anaemia status, and anthropometric measurements at antenatal care visits and delivery, according to HIV status, are presented in Supplementary Table 2. MLWH had significantly higher mean weight than MnLWH (69.1±11.1 kg vs. 65.1±11.7 kg; p=0.009) at the second trimester antenatal care visits. No significance differences were found in maternal haemoglobin levels, anaemia status, height, body mass index (BMI), MUAC and normal MUAC category ($p>0.05$) at all antenatal/delivery visits. The breastfeeding practices of HEU vs HUU infants at 6, 9, and 12 months are shown in Supplementary Figure 1. HEU and HUU children had similar percentages of any breastfeeding at 6 months, but there were significant differences at 9 (35.6% vs. 57.3%; p=0.013) and 12 months (24.7% vs. 48.0%; p=0.005).

The correlation between infant haemoglobin levels, Z-scores indices and confounding factors in HEU and HUU infants at 6, 9, and 12 months are presented in Table 5.5. At 9 months, infants' mean haemoglobin levels had a positive but weak correlation with the mean WAZ (r=0.3, p=0.039), LAZ (r=0.3, p=0.007), and MUACZ (r=0.3, p=0.039) in HEU infants but not in HUU infants, while at 12 months, positive correlations were found with WAZ (r=0.3, p=0.018) and WLZ (r=0.3, p=0.017). In HEU infants, haemoglobin levels had a weak but negative correlation with breastfeeding practices at 6 (r=-0.3, p=0.026) and 12 months (r=-0.3, p=0.041), while at 9 months (r=-0.4, $p<0.001$) a moderate but negative correlation existed between infant haemoglobin levels and breastfeeding practices. Furthermore, haemoglobin levels in HUU infants at 6 months showed a significant positive correlation with maternal haemoglobin levels at 6 months (r=0.3; p=0.028). Infant haemoglobin levels at 6 months also showed a moderate and positive correlation with maternal MUAC at the second trimester (r=0.4; $p< 0.001$) and third trimester (r=0.3; p=0.025) antenatal care visits in HEU infants. No correlations were found between the infants' haemoglobin levels and maternal age, and education levels at 6, 9 or 12 months in both HEU and HUU infants.

Table 5.4. Maternal anthropometric measurements at 6, 9, and 12 months postpartum, stratified by maternal HIV status.

	6 months postpartum			9 months postpartum			12 months postpartum		
	MLWH	**MnLWH**	p-value	**MLWH**	**MnLWH**	p-value	**MLWH**	**MnLWH**	p-value
	n=86	n=95		n=80	n=86		n=75	n=80	
Weight mean ± SD	65.3 ± 12.8	68.8 ± 11.7	0.079	65.0 ± 12.7	69.2 ± 12.1	0.043*	65.6 ± 13.1	69.9 ± 12.5	0.056
Height mean ± SD	159.5 ± 6.0	157.4 ± 4.8	0.010*	159.6 ± 6.3 †	157.5 ± 5.7 †	0.332	158.8 ± 6.2	157.7 ± 5.1	0.506
BMI mean ± SD	25.6 ± 4.5 †	27.7 ± 4.7 †	0.011*	25.4 ± 4.5 †	27.9 ± 4.8 †	0.002*	26.0 ± 4.5	28.2 ± 4.9	0.007*
Underweight n (%) ‡	5 (6.9)	1 (1.4)	n/a	5 (7.0)	1 (1.4)	n/a	2 (2.9)	1 (1.5)	n/a
Normal weight n (%) §	29 (40.3)	20 (27.0)	0.425	29 (40.8)	21 (28.4)	0.099	29 (42.6)	18 (26.9)	0.225
Overweight obese n (%) ¥	38 (52.8)	53 (71.6)	0.019*	37 (52.0)	52 (70.3)	0.025*	37 (54.4)	48 (71.6)	0.038*
MUAC mean ± SD	29.9 ± 4.1	30.7 ± 3.8	0.220	29.6 ± 3.9	30.7 ± 3.7	0.073	29.9 ± 3.9	31.4 ± 3.4	0.021*
Normal MUAC n (%) ¦	67 (94.4)	73 (97.3)	0.367	68 (94.4)	69 (98.6)	0.182	65 (97.0)	66 (100)	0.157

Abbreviations: MLWH: mothers living with HIV; MnLWH: mothers not living with HIV; BMI: body mass index; MUAC: Mid-upper-arm circumference; SD: standard deviation.

Body mass Index classifications ‡ underweight category <18.5 kg/m2, § normal weight=18.5-24.9 kg/m2, ¥ overweight and obese ≥25.0 kg/m2; ¦ Mid-upper-arm-circumference normal >23cm; n/a: not applicable (no comparisons were performed due to one of the groups having less than five counts leading to volatile results

Statistical analysis: to determine the difference in continuous data between MLWH and MnLWH, Independent-t-test and Mann-Whitney U test († non-normally distributed) were used; and for categorical data, Pearson's Chi-square test was used to determine the differences in MLWH and MnLWH; * p-value shows significant difference of <0.05.

Table 5.5. Correlation between infant haemoglobin levels, Z-scores indices, and confounding factors in HEU and HUU infants at 6, 9, and 12 months.

		Infant haemoglobin (g/dL) level at 6 months				Infant haemoglobin (g/dL) level at 9 months				Infant haemoglobin (g/dL) level at 12 months			
		HEU		HUU		HEU		HUU		HEU		HUU	
		r	p	r	p	r	p	r	p	r	p	r	p
Infants factors													
Z-scores †	Weight-for-age	0.2	0.102	0.2	0.115	0.3	0.039*	0.1	0.268	0.3	0.018*	0.0	0.978
	Length-for-age	0.0	0.971	0.3	0.068	0.3	0.007*	0.1	0.279	0.1	0.522	0.0	0.754
	Weight-for-length	0.1	0.297	0.0	0.801	0.0	0.939	0.1	0.344	0.3	0.017*	0.0	0.357
	Head circumference-for-age	-0.1	0.275	0.1	0.733	0.2	0.222	-0.2	0.176	0.0	0.830	0.0	0.732
	Mid-upper-arm-circumference-for-age	0.2	0.076	0.3	0.047*	0.3	0.039*	0.0	0.934	0.2	0.142	0.0	0.442
Breastfed (any breastfeeding)	At 6 months	-0.3	0.026*	0.1	0.678								
	At 9 months					-0.4	<0.001*	-0.1	0.232				
	At 12 months									-0.3	0.036*	0.0	0.890
Maternal factors													
Age at delivery		0.0	0.946	-0.1	0.345	0.1	0.681	0.1	0.422	-0.1	0.556	0.0	0.697
Haemoglobin levels	First trimester	0.2	0.094	0.1	0.540	0.0	0.787	0.2	0.054	0.0	0.875	0.1	0.619
	Second trimester	0.0	0.833	0.0	0.896	0.1	0.682	0.0	0.826	-0.2	0.178	0.1	0.577
	Third trimester	0.0	0.978	-0.2	0.343	-0.2	0.141	0.0	0.923	-0.2	0.224	0.2	0.141
	Delivery	0.1	0.498	0.1	0.369	0.1	0.363	0.0	0.763	0.1	0.383	0.1	0.415
	6 months postpartum	0.1	0.271	0.3	0.028*								

	9 months postpartum					0.1	0.620	0.1	0.678				
	12 months postpartum									0.0	0.746	0.0	0.768
BMI	First trimester	0.1	0.642	0.1	0.448	0.1	0.477	0.1	0.656	0.1	0.557	0.1	0.348
	Second trimester	0.0	0.955	0.2	0.244	0.0	0.732	0.1	0.245	-0.1	0.406	0.2	0.143
	Third trimester	0.0	0.816	0.1	0.347	0.1	0.269	0.0	0.882	-0.2	0.116	0.1	0.670
	Delivery	0.1	0.642	0.2	0.185	0.1	0.409	0.0	0.934	0.0	0.905	-0.1	0.413
	6 months postpartum	0.0	0.840	0.2	0.153								
	9 months postpartum					-0.1	0.492	0.2	0.181				
	12 months postpartum									0.0	0.829	-0.3	0.032*
MUAC	First trimester	-0.1	0.449	0.1	0.465	-0.1	0.561	0.0	0.823	0.0	0.783	0.0	0.794
	Second trimester	0.4	< 0.001*	0.0	0.751	-0.2	0.114	0.0	0.934	0.0	0.988	-0.4	0.002*
	Third trimester	0.3	0.025*	0.0	0.944	0.0	0.886	0.0	0.909	0.1	0.413	-0.3	0.006
	Delivery	0.2	0.237	0.2	0.237	0.2	0.267	-0.2	0.280	0.1	0.361	-0.2	0.091
	6 months postpartum	0.0	0.752	0.1	0.543								
	9 months postpartum					0.0	0.825	0.2	0.197				
	12 months postpartum									0.0	0.887	-0.3	0.033
Education status at 6 months		-0.2	0.181	0.0	0.830	-0.1	0.330	0.0	0.713	-0.1	0.370	0.2	0.120
Monthly household income		-0.1	0.291	0.1	0.621	0.0	0.971	0.1	0.282	0.2	0.155	-0.1	0.568

Abbreviations: HEU: HIV-exposed-uninfected; HUU: HIV-unexposed-uninfected; BMI: body mass index; MUAC: Mid-upper-arm circumference; SD: standard deviation; r= Pearson correlation coefficient; p: p-value; r: † Z-scores indices at age 6–12 months were computed using World Health Organization Anthro software of 2010; blank space: no correlation performed at the visit; * p-value shows significant difference of <0.05.

5.4. DISCUSSION

Anaemia in infants has multifaceted causes, and determining correlations between infant haemoglobin levels and growth is of great public health importance, especially in HEU infants. This will help in identifying and tailoring interventions aimed at improving haemoglobin levels in infants, such as nutrition education focusing on appropriate infant and young child feeding policies.

Our study found significant differences between MLWH and MnLWH in terms of the haemoglobin levels and anaemia status at 6, 9, and 12 months, although no differences were found during the antenatal period or at the time of delivery. Similar findings were observed in the United States in six different sites, where higher rates of anaemia were found in women living with HIV than women not living with HIV (37% vs. 17%; $p<0.001$), suggesting an association between anaemia and HIV infection (Levine et al., 2001). MLWH have previously been described to be at a greater risk (1.17) of having children with anaemia than MnLWH (Musuka *et al*., 2021). We found no correlation between the haemoglobin levels of HEU infants and MLWH at 6 months, which may be due to the high coverage of ART in our mothers. However, there was a correlation between the haemoglobin levels of HUU infants and MnLWH at 6 months, which has also been previously reported (Berhane *et al*., 2004).

The combined prevalence of anaemia in HEU and HUU infants in this study was 22.7%, 30.1%, and 23.6% at 6, 9, and 12 months, which is lower than the global average (39.8%) and the African region (60.2%) in children under 59 months (WHO, 2022) and found in two other South African studies (36.4% and 40.2%) at 6 months (Rothman *et al*., 2018); (Smuts *et al*., 2005). No significant differences were found in our study in terms of the HEU vs HUU infants haemoglobin levels at 6, 9, and 12 months. Similar findings were observed in Mozambique at 9 and 12 months (Moraleda *et al*., 2014). Our results contradict a Ugandan study, which found lower haemoglobin levels in HEU children from 6 months (Tam *et al*., 2018). The differences may be due to the smaller sample size in their HEU group (n=25) vs HUU (n=291) where the majority of the study participants where HUU.

We found a similar prevalence of anaemia in our study at 6, 9, and 12 months between HEU and HUU infants. This finding was also reported in a Ugandan study, where no differences were found in anaemia between HEU and HUU infants (4–6 months) after

controlling for other factors such as maternal age, infant sex, exclusive breastfeeding, and wealth (Osterbauer *et al*., 2012). However, Dryden-Peterson *et al*. (2011) in Botswana found a 7.4% prevalence of anaemia in HEU infants at 6 months when assessing the impact of ART on severe anaemia and found that prenatal exposure to zidovudine (AZT) was associated with severe anaemia. The non-existent differences in our study may be explained by the fact that the majority of MLWH in our study were on tenofovir/emtricitabine/efavirenz and not AZT, which has been shown to increase the prevalence of anaemia in MLWH (Bennett, Dolin, & Blaser, 2019), as supported by multiple studies in Botswana (Dryden-Peterson *et al*., 2011) and Ethiopia (Berhane, Haile, & Tolessa, 2020). The use of ART increases the haemoglobin levels and decreases the prevalence of anaemia (Belperio & Rhew, 2004), even in the case of maternal anaemia, where the use of ART was protective against anaemia in MLWH (Berhane *et al*., 2004). A randomised control trial conducted in three African countries (Burkina Faso, Kenya, and South Africa) also found a significant reduction in the incidence of anaemia with the use of ART during and after pregnancy (Sartorius *et al*., 2013).

At 6 months, HEU infants had lower mean LAZ, WAZ, and MUACZ. In addition, stunting (15.0%), underweight (8.9%), and wasting (3.7%) was found in HEU infants at 6 months, similar to Ugandan study (Osterbauer *et al*., 2012). In Botswana, higher rates of underweight (15.6% vs. 6.9%; p=0.01) and stunting (15.6% vs. 7.3%; p=0.05) were found in HEU versus HUU infants from 6 months (Chalashika *et al*., 2017). Our study found no differences in terms of the mean WLZ in HEU vs HUU infants (p>0.05) at 6 and 9 months; similar findings were also observed in Botswana where no differences were found in the percentage of infants who were wasted in HEU and HUU infants (Chalashika *et al*., 2017). However, we found lower WLZ in HEU vs. HUU infants at 12 months, similar to a study conducted in China (Chen *et al*., 2019). This can be explained by the lower WAZ found in HEU compared to HUU infants, but in terms of LAZ, no significant differences were found at 12 months as the infant's weight in relation to their length is the basis of WLZ (WHO, 2009).

In the current study no correlation was found between haemoglobin levels and the Z-scores indices at 6 months in HEU infants, which aligns with the findings of a study conducted amongst 4–6-month-old Ugandan HEU infants (Osterbauer *et al*., 2012). A recent study based on this population found significant differences between HEU and

HUU infants at 9 months in terms of breastfeeding, with breastfeeding rates decreasing with age (Tshiambara *et al*., 2023). The decreased rates may be a result of cultural norms, lack of knowledge, fear, and prior use of formula milk provided by prevention of vertical transmission programmes (Rossouw *et al*., 2016). Our study found a correlation between the infants' haemoglobin levels and breastfeeding practices, similar to another study in Iran (Dalili *et al.*, 2015). Iron in breastmilk is highly bioavailable and easily absorbed in the body, however, the concentration is quite low from 6 months of age, posing a risk factor for anaemia (Mahan & Raymond, 2016). Anaemia can have serious functional effects, particularly for the infant's growth and development as complementary feeding starts at 6 months and the risk of inadequate intake of iron rich foods is high (Miniello *et al*., 2021), especially in the rural areas (Faber, 2007a). Poor quality of complementary foods in HEU infants may increase the risk of mother-to-child-transmission due to the decreased immunological responses (Haile *et al*., 2015; Obeagu *et al*., 2022).

A South African study comparing the feeding practices and growth of HEU versus HUU infants (Tshiambara *et al*., 2023) based on the same sub-study population, reported early introduction of complementary feeding in HEU and HUU infants and at 12 months, the mothers of infants reported that about 30% of HEU and HUU infants had never consumed meat products such as red meat, chicken, or fish in the previous seven days prior to the time of the interview. Meat products are rich sources of heme iron and the consumption of such increases the haemoglobin levels (Mahan & Raymond, 2016). In Ethiopia, HEU children aged 6–24 months were not introduced timeously to complementary foods (32%), with an inadequate minimum dietary diversity (42%), meal frequency (24%), and acceptable diet (65%), and 40% were not consuming iron rich foods (Yisak et al., 2020). In the same study, mothers with an inadequate minimal acceptable diet had poor knowledge on complementary feeding (81%) and working mother attributed to 73% of the inadequate minimal acceptable diet.

Employment is one of the factors contributing to inappropriate complementary feeding (Mphasha *et al*., 2023), with working mothers less likely to give appropriate complementary foods (Joshi et al., 2012), even in the context of HIV (Goon, Ajayi, & Adeniyi, 2020). The WHO infant and young child feeding (IYCF) policy recommends an intake of at least five (5) out of eight (8) food groups to be consumed a day (WHO, 2021). Hence the IYCF policy is important tool to be used and followed in order to

decrease the high prevalence of undernutrion in Africa and in the context of HIV (Obeagu *et al*., 2022; WHO, 2021a; Yisak *et al*., 2020).

We found a correlation between HEU infant haemoglobin levels and maternal MUAC at the second trimester antenatal care visit. MUAC is a good indicator for acute malnutrition, which is easy and quick to measure, and includes cut-offs during pregnancy as compared to BMI (Fakier *et al*., 2017; Ververs *et al*., 2013; World Health Organization, 2016), even in the context of HIV (Ramlal *et al*., 2012). A Cambodian study by Kpewou *et al*. (2020) reported that lower maternal MUAC was associated with lower infant LAZ and a low MUAC during pregnancy resulted in a 1.6 times higher risk of infant stunting during the first months of life.

In our study, no MLWH and MnLWH were underweight, which supports the return to health phenomenon and the use of ART increasing weight gain (Guaraldi *et al*., 2023; Stires et al., 2021). This result contradicts those in Kenya (Widen et al., 2019) and Ethiopia (Gemede, Kaba, & Dufera, 2021), which found MLWH more likely to have lower weight and be underweight. During pregnancy, MLWH and MnLWH had similar BMIs at the three antenatal care visits, but during postpartum visits, MLWH in our study had a lower mean BMI than MnLWH at 6, 9 and 12 months, even though it was within the normal range of the WHO classifications (WHO, 1995). Maternal nutritional status contributes to the infant's nutritional status in the first 1000 days. MLWH weight gain was positively associated with increased infants LAZ, and being breastfed was associated with increased LAZ at 12 months among HEU infants in Rwanda (Lane et al., 2021). A study in Uganda found that HEU infants were less likely to be breastfed, and for those who were breastfed, a shorter duration of breastfeeding was practiced by MLWH (Fadnes *et al*., 2009).

The strength of our study lies with the multiple cross-sectional analysis with known infant characteristics at baseline, as well as the availability of maternal anthropometric and haemoglobin measurements at antenatal and postnatal visits, with measurements performed by research staff ensuring quality control. The repeated haemoglobin and anthropometric measurements in the HEU infants, as well as the control group of HUU infants, with similar sample sizes in the two groups, are further strengths of this study. In addition, correlating infants haemoglobin levels and Z-scores indices with maternal haemoglobin and anthropometric measurements at antenatal and postpartum visits,

stratified by maternal HIV status, adds relevant information to the link between maternal and infant haemoglobin levels. The limitation of this study includes a relatively small sample size, lack of pre-pregnancy weight, and use of the Hemocue® machine for haemoglobin measurements, which may not be ideal, but has previously been used in other studies to classify anaemia in resource-limited settings. To better understand HEU's long-term growth trajectories, future studies should examine the growth and haemoglobin levels over a longer time period and further determine the dietary intake of iron in the complementary foods given to HEU and HUU infants.

5.5. CONCLUSION

In this study, we determined, compared and correlated the haemoglobin levels and growth of urbanised African infants from 6–12 months by maternal HIV status. MLWH were more likely to be anaemic than MnLWH, but HEU and HUU infants had similar percentages of anaemia. At 6, 9, and 12 months, HEU infants had lower LAZ, WAZ, and MUACZ and higher stunting rates than HUU infants. Infants' haemoglobin levels positively correlated with WAZ, LAZ and MUACZ at 9 months, but negatively correlated with breastfeeding practices at 6 and 12 months in HEU infants. Exposure to maternal HIV infection affects the growth of the infants, but not anaemia status.

DECLARATIONS

ETHICAL STATEMENT

The Siyakhula Study was approved by the Faculty of Health Sciences Research Ethics Committee (FHSREC) (Ref. no: 294/2017) of the University of Pretoria. For this study, approvals were granted by the Faculty of Natural and Agricultural Sciences and the FHSREC at the same institution (Ref. no: NAS063/2020). All relevant information was shared with the mothers, and the mothers provided informed consent on behalf of themselves and their infants prior to data collection.

ACKNOWLEDGMENTS

The authors would like to thank all of the Siyakhula Study's personnel and research assistants (Maryjane Ntima, Sheilla Sono, Lidisa Mothiba, Sicebile Sibiya, and Kedibone Matshai) for their assistance in data collecting. Thank you to all of the women and babies who took part in the study. This study was supported by the University of Pretoria Department of Research and

Innovation' University Capacity Development Programme (UCDP), the National Research Foundation (NRF): New Generation of Academics Programme grant ID: NGAP_RDG190128411410, and the International AIDS Society's Collaborative Initiative for Paediatrics HIV Education and Research (CIPHER) grant ID: 2017/560_FEU. Dr Cheryl Tosh (University of Pretoria) for editing the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

UF and PT conceptualised the study and formulated the research questions with supervision from UF, HL and MH. PT conducted the formal analysis, and interpreted the results with input from HM and UF. PT drafted and wrote the first draft of the manuscript and UF, HL, MH, and HM reviewed and edited the manuscript. PT completed the visualisation, and PT and HM undertook the project management of this work. All authors critically reviewed the manuscript and approved the final version. The co-authors granted permission that this manuscript be submitted for the attainment of the PhD degree.

DATA AVAILABILITY STATEMENT

The data supporting the findings will be made accessible upon request from the primary investigator of the Siyakhula Study (UF) following an embargo period to allow for the conclusion of research and publishing of findings.

Table 5.6. Anthropometric measurements, Z-score indices, and nutritional classifications of the infants between 6–12 months of life by HIV exposure status.

	Age 6 Months			Age 9 Months			Age 12 Months		
	HEU Infants (n = 86)	**HUU Infants** (n = 95)	**p-value**	**HEU Infants** (n = 80)	**HUU Infants** (n = 86)	**p-value**	**HEU Infants** (n = 75)	**HUU Infants** (n = 80)	**p-value**
Anthropometric measurements									
Weight (kg) mean ± SD	7.3 ± 0.9 †	7.8 ± 1.0 †	0.001*	8.3 ± 1.0 †	8.8 ± 1.1 †	0.002*	9.1 ± 1.2 †	9.4 ± 1.3 †	0.106
Length (cm) mean ± SD	65.3 ± 3.5 †	66.6 ± 2.8 †	0.014*	70.1 ± 3.1	71.2 ± 2.8	0.012*	74.4 ± 3.1	74.5 ± 2.7	0.704
Head circumference (cm) mean ± SD	43.5 ± 1.6 †	43.9 ± 1.6 †	0.106	45.3 ± 1.4	45.4 ± 1.7	0.621	46.5 ± 1.6	46.6 ± 1.6	0.655
Mid-upper-arm-circumference (cm) mean ± SD †	14.6 ± 1.3 †	15.2 ± 1.1 †	0.002*	15.2 ± 1.2	15.7 ± 1.5	0.026*	15.6 ± 1.2	16.0 ± 1.3	0.075
Z-score indices									
Weight-for-age Z-score mean ± SD ‡	−0.6 ± 1.1	0.1 ± 1.2	<0.001*	−0.4 ± 1.1	0.1 ± 1.1	0.003*	−0.3 ± 1.1 †	0.1 ± 1.2 †	0.022*
Length-for-age Z-score mean ± SD †, ‡	−0.8 ± 1.4	−0.1 ± 1.2	<0.001*	−0.5 ± 1.4	0.0 ± 1.3	0.023*	−0.4 ± 1.3	−0.2 ± 1.1	0.308
Weight-for-length Z-score mean ± SD †, ‡	−0.1 ± 1.2	0.2 ± 1.1	0.074	−0.1 ± 1.2	0.2 ± 1.1	0.098	−0.2 ± 1.2	0.2 ± 1.2	0.020*
Head circumference-for-age Z-score mean ± SD †, ‡	0.5 ± 1.2	0.9 ± 1.2	0.019*	0.6 ± 1.2	0.8 ± 1.0	0.331	0.6 ± 1.2	0.9 ± 1.1	0.069
Mid-upper-arm-circumference-for-age Z-score mean ± SD †, ‡	0.5 ± 1.1	1.0 ± 0.9	<0.001*	0.7 ± 1.0	1.1 ± 1.1	0.013*	0.8 ± 1.1	1.3 ± 1.1	0.025*
Nutritional classifications									
Underweight *n* (%) §	7 (8.9)	3 (3.4)	n/a	7 (8.8)	2 (2.4)	n/a	4 (5.5)	4 (5.1)	n/a
Stunted *n* (%) ¶	12 (15.0)	4 (4.6)	n/a	10 (12.5)	6 (7.1)	0.297	9 (12.3)	3 (3.8)	n/a
Wasted *n* (%) ¥	3 (3.7)	2 (2.3)	n/a	5 (6.2)	3 (3.6)	n/a	4 (5.5)	3 (3.9)	n/a
Overweight *n* (%) ¦	4 (4.9)	6 (6.8)	n/a	4 (5.0)	3 (3.6)	n/a	4 (5.5)	6 (7.8)	n/a
Acute malnutrition *n* (%) ¤	1 (1.3)	0 (0)	n/a	1 (1.2)	0 (0)	n/a	1 (1.4)	0 (0)	n/a
Macrocephalus *n* (%) #	8 (10.0)	17 (19.3)	0.140	10 (12.5)	10 (12.2)	>0.999	10 (13.7)	13 (16.9)	0.753

Abbreviations: HEU: HIV-exposed-uninfected; HUU: HIV-unexposed-uninfected; n/a: not applicable (no comparisons were performed due to one of the groups having less than five counts leading to volatile results. SD: standard deviation. ‡ sex-normalized Z-scores indices at age 6–12 months were computed using World Health Organization Anthro software of 2010; § underweight from weight-for-age Z-scores < −2; ¶ stunted from length-for-age Z-scores < −2; ¥ wasted from weight-for-length Z-scores (WLZ) < −2; ᵎ overweight from WLZ > +2; ¤ acute-malnutrition from mid-upper-arm-circumference Z-scores < −2; # macrocephalus from head circumference-for-age Z-scores > +2. Statistical analysis: Independent t-test was used for continuous normally distributed data and the Mann–Whitney U test was used for continuous † non-normally distributed data; Pearson's Chi-square test was used for categorical determine the differences in HEU and HUU infants; * p-value shows significant difference of <0.05.

Table 5.7. Maternal haemoglobin levels, anaemia status, and anthropometric measurements at antenatal care visits and delivery, stratified by maternal HIV status.

	ANC First trimester			ANC Second trimester			ANC Third trimester			At delivery		
	MLWH	**MnLWH**	p-value	**MLWH**	**MnLWH**	p-value	**MLWH**	**MnLWH**	p-value	**MLWH**	**MnLWH**	p-value
	n=86	n=95		n=86	n=95		n=86	n=95		n=86	n=95	
Haemoglobin levels (g/dL) mean ± SD	11.4 ± 1.4	11.9 ± 2.3	0.125	11.7 ± 2.1	11.7 ± 1.9	0.715	11.6 ± 1.1	11.8 ± 1.5	0.182	11.4 ± 2.3	11.0 ± 1.6	0.388
Anaemia n (%) †	18 (24.0)	22 (29.7)	0.430	27 (35.5)	20 (24.7)	0.138	15 (22.7)	15 (20.8)	0.788	18 (24.0)	22 (29.7)	0.556
Weight (kg) mean ± SD	69.1 ± 11.1	65.1 ± 11.7	0.009 *	72.1 ± 10.9	69.7 ± 11.7	0.084	74.0 ± 11.2	72.5 ± 11.5	0.208	71.3 ± 11.9	72.1 ± 11.1	0.692
Height (cm) mean ± SD ‡	159.4 ± 7.8	158.4 ± 7.1	0.136	159.4 ± 7.8	158.1 ± 6.9	0.077	159.1 ± 7.6	157.1 ± 9.3	0.061	158.1 ± 8.5	158.7 ± 7.7	0.328
BMI mean ± SD ‡	27.3 ± 5.0	26.0 ± 5.0	0.062	28.4 ± 4.5	28.0 ± 4.9	0.505	29.2 ± 4.3	29.8 ± 8.3	0.508	28.5 ± 5.1	28.8 ± 5.0	0.643
MUAC (cm) mean ± SD	28.4 ± 4.4	27.2 ± 4.1	0.059	29.1 ± 4.2 ‡	29.0 ± 3.8 ‡	0.749	31.3 ± 13.5	30.0 ± 3.6	0.203	29.6 ± 4.7 ‡	29.8 ± 5.1 ‡	0.847
Normal MUAC n (%) §	79 (95.2)	87 (95.6)	0.073	79 (95.2)	87 (95.6)	0.894	72 (98.6)	81 (97.6)	0.637	58 (90.6)	75 (93.8)	0.483

Abbreviations: MLWH: mothers living with HIV; MnLWH: mothers not living with HIV; BMI: body mass index; MUAC: Mid-upper-arm circumference; MAM: moderate acute malnutrition; SD: standard deviation.

† World Health Organization anaemia cut-offs: pregnant women: <10.9 g/dL; § MUAC cut offs for pregnant normal MUAC in mothers ≥23.0 cm; BMI categories at ANC a visits and delivery not performed due to lack of classifications in pregnant mothers; Mothers n without missing numbers for: haemoglobin levels at first trimester: MLWH=76 and MnLWH=81, at second trimester: MLWH=75 and MnLWH=74, at third trimester: MLWH=66 and MnLWH=72; MUAC at first trimester: MLWH=82 and MnLWH=93, at second trimester: MLWH=82 and MnLWH=87, at third trimester: MLWH=75 and MnLWH=91, and BMI at first trimester: MLWH=85 and MnLWH=95, at second trimester: MLWH=83 and MnLWH=94, at third trimester: MLWH=73 and MnLWH=87.

Statistical analysis: to determine the difference in continuous data between MLWH and MnLWH. Independent-t-test and Mann-Whitney U test (‡ non-normally distributed) were used; and for categorical data. Pearson's Chi-square test was used to determine the differences in MLWH and MnLWH; * p-value shows significant difference of <0.05.

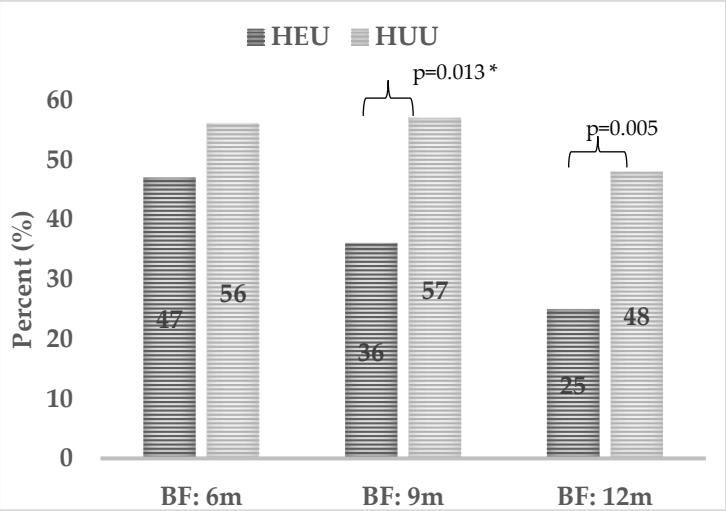


Figure 5.2. Infant breastfeeding practices (any breastfeeding) by HIV exposure status at 6, 9, and 12 months of life. Abbreviations: HEU: HIV-exposed-uninfected; HUU: HIV-unexposed-uninfected, Pearson's Chi-square test was used for categorical data to determine the differences in HEU and HUU infants; * p-value shows significant difference of <0.05.

5.6. REFERENCES

Abu-Ouf, N. M., & Jan, M. M. (2015). The impact of maternal iron deficiency and iron deficiency anemia on child's health. *Saudi medical journal*, *36*(2), 146.

Allali, S., Brousse, V., Sacri, A.-S., Chalumeau, M., & de Montalembert, M. (2017). Anemia in children: prevalence, causes, diagnostic work-up, and long-term consequences. *Expert review of hematology*, *10*(11), 1023-1028.

Appiah, C. A., Mensah, F. O., Hayford, F. E., Awuuh, V. A., & Kpewou, D. E. (2020). Predictors of undernutrition and anemia among children aged 6–24 months in a low-resourced setting of Ghana: a baseline survey. *Journal of Health Research*, *35*(1), 27-37.

Belperio, P. S., & Rhew, D. C. (2004). Prevalence and outcomes of anemia in individuals with human immunodeficiency virus: a systematic review of the literature. *The American journal of medicine*, *116*(7), 27-43.

Bennett, J. E., Dolin, R., & Blaser, M. J. (2019). *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases E-Book: 2-Volume Set*. Elsevier health sciences.

Berhane, K., Karim, R., Cohen, M. H., Masri-Lavine, L., Young, M., Anastos, K., Augenbraun, M., Watts, D. H., & Levine, A. M. (2004). Impact of highly active antiretroviral therapy on anemia and relationship between anemia and survival in a large cohort of HIV-infected women: Women's Interagency HIV Study. *JAIDS Journal of Acquired Immune Deficiency Syndromes*, *37*(2), 1245-1252.

Berhane, Y., Haile, D., & Tolessa, T. (2020). Anemia in HIV/AIDS Patients on antiretroviral treatment at Ayder Specialized Hospital, Mekele, Ethiopia: a case-control study. *Journal of Blood Medicine*, 379-387.

Chalashika, P., Essex, C., Mellor, D., Swift, J. A., & Langley-Evans, S. (2017). Birthweight, HIV exposure and infant feeding as predictors of malnutrition in Botswanan infants. *J Hum Nutr Diet*, *30*(6), 779-790. https://doi.org/10.1111/jhn.12517

Chen, J.-C., Zhang, Y., Rongkavilit, C., Wang, B., Huang, X.-M., Nong, Z., Liu, J., Zeng, D., & McGrath, E. (2019). Growth of HIV-exposed infants in Southwest China: a comparative study. *Global Pediatric Health*, *6*, 2333794X19854964.

da Silva, L. L. S., Fawzi, W. W., Cardoso, M. A., & Group, E. W. (2018). Factors associated with anemia in young children in Brazil. *PloS One*, *13*(9), e0204504.

Dalili, H., Baghersalimi, A., Dalili, S., Pakdaman, F., Rad, A. H., Kakroodi, M. A., Rezvany, S., & Koohmanaei, S. (2015). Is there any relation between Duration of breastfeeding and anemia? *Iranian journal of pediatric hematology and oncology*, *5*(4), 218.

Dryden-Peterson, S., Shapiro, R. L., Hughes, M. D., Powis, K., Ogwu, A., Moffat, C., Moyo, S., Makhema, J., Essex, M., & Lockman, S. (2011). Increased risk of severe infant anemia following exposure to maternal HAART, Botswana. *Journal of acquired immune deficiency syndromes (1999)*, *56*(5), 428.

Ejigu, Y., Magnus, J. H., Sundby, J., & Magnus, M. C. (2020). Differences in Growth of HIV-exposed Uninfected Infants in Ethiopia According to Timing of In-utero Antiretroviral Therapy Exposure. *Pediatr. Infect. Dis. J*, *39*(8), 730.

Faber, M. (2007a). Complementary foods consumed by 6 – 12-month-old rural infants in South Africa are inadequate in micronutrients. *Public Health Nutr*, *8*(4), 373-381. https://doi.org/10.1079/phn2004685

Faber, M. (2007b). Dietary intake and anthropometric status differ for anaemic and non-anaemic rural South African infants aged 6–12 months. *Journal of Health, Population, and Nutrition*, *25*(3), 285.

Fadnes, L. T., Engebretsen, I. M., Wamani, H., Semiyaga, N. B., Tylleskär, T., & Tumwine, J. K. (2009). Infant feeding among HIV-positive mothers and the general population mothers: comparison of two cross-sectional surveys in Eastern Uganda. *BMC Public Health*, *9*, 1-14.

Fakier, A., Petro, G., & Fawcus, S. (2017). Mid-upper arm circumference: A surrogate for body mass index in pregnant women. *South African Medical Journal*, *107*(7), 606-610.

Feleke, B. E. (2016). Maternal HIV status affects the infant hemoglobin level: A comparative cross-sectional study. *Medicine (Baltimore)*, *95*(31), e4372. https://doi.org/10.1097/MD.0000000000004372

Feleke, B. E. (2016). Maternal HIV status affects the infant hemoglobin level: A comparative cross-sectional study. *Medicine*, *95*(31), e4372.

Gemede, H. F., Kaba, F., & Dufera, M. (2021). Nutritional Knowledge, Practices, Nutritional Status and the Associated Factors Among HIV Positive Mothers On Antiretroviral Therapy: Evidence from Cross Sectional Survey in Abay Choman Health Centers, Western Ethiopia.

Goon, D. T., Ajayi, A. I., & Adeniyi, O. V. (2020). Reasons for the early introduction of complementary feeding to HIV-exposed infants in the Eastern Cape, South Africa: an exploratory qualitative study. *Medicina*, *56*(12), 703.

Guaraldi, G., Bonfanti, P., Di Biagio, A., Gori, A., Milić, J., Saltini, P., Segala, F. V., Squillace, N., Taramasso, L., & Cingolani, A. (2023). Evidence gaps on weight gain in people living with HIV: a scoping review to define a research agenda. *BMC Infectious Diseases*, *23*(1), 230.

Haile, D., Belachew, T., Berhanu, G., Setegn, T., & Biadgilign, S. (2015). Complementary feeding practices and associated factors among HIV positive mothers in Southern Ethiopia. *Journal of Health, Population and Nutrition*, *34*, 1-9.

Haryono, E., Slamet, M., & Septian, D. (2023). STATISTIKA SPSS 28.

Huo, J., Sun, J., Fang, Z., Chang, S., Zhao, L., Fu, P., Wang, J., Huang, J., Wang, L., & Begin, F. (2015). Effect of home-based complementary food fortification on prevalence of anemia among infants and young children aged 6 to 23 months in poor rural regions of China. *Food and nutrition bulletin*, *36*(4), 405-414. DOI:10.1177/0379572115616001

Joshi, N., Agho, K. E., Dibley, M. J., Senarath, U., & Tiwari, K. (2012). Determinants of inappropriate complementary feeding practices in young children in Nepal: secondary data analysis of Demographic and Health Survey 2006. *Maternal & child nutrition*, *8*, 45-59.

Kpewou, D. E., Poirot, E., Berger, J., Som, S. V., Laillou, A., Belayneh, S. N., & Wieringa, F. T. (2020). Maternal mid-upper arm circumference during pregnancy and linear growth among Cambodian infants during the first months of life. *Maternal & child nutrition*, *16*, e12951.

Lane, C., Adair, L., Bobrow, E., Ndayisaba, G. F., Asiimwe, A., & Mugwaneza, P. (2021). Longitudinal interrelationship between HIV viral suppression, maternal weight change, breastfeeding, and length in HIV-exposed and uninfected infants participating in the Kabeho study in Kigali, Rwanda. *Annals of Epidemiology*, *53*, 1-6. e1.

Le Roux, S. M., Abrams, E. J., Donald, K. A., Brittain, K., Phillips, T. K., Nguyen, K. K., Zerbe, A., Kroon, M., & Myer, L. (2019). Growth trajectories of breastfed HIV-exposed uninfected and HIV-unexposed children under conditions of universal maternal antiretroviral therapy: a prospective study. *The Lancet Child & Adolescent Health*, *3*(4), 234-244.

Levine, A. M., Berhane, K., Masri-Lavine, L., Sanchez, M. L., Young, M., Augenbraun, M., Cohen, M., Anastos, K., Newman, M., & Gange, S. J. (2001). Prevalence and correlates of anemia in a large cohort of HIV-infected women: Women's Interagency HIV Study. *JAIDS Journal of Acquired Immune Deficiency Syndromes*, *26*(1), 28-35.

Levy, T. S., Méndez-Gómez-Humarán, I., Ruán, M. d. C. M., Tapia, B. M., Hernández, S. V., & Avila, M. H. (2017). Validation of Masimo Pronto 7 and HemoCue 201 for hemoglobin determination in children from 1 to 5 years of age. *PloS One*, *12*(2), e0170990.

Li, H., Xiao, J., Liao, M., Huang, G., Zheng, J., Wang, H., Huang, Q., & Wang, A. (2020). Anemia prevalence, severity and associated factors among children aged 6–71 months in rural Hunan Province, China: a community-based cross-sectional study. *BMC Public Health*, *20*(1), 1-13.

Mahan, L. K., & Raymond, J. L. (2016). *Krause's food & the nutrition care process-e-book*. Elsevier Health Sciences.

Miniello, V. L., Verga, M. C., Miniello, A., Di Mauro, C., Diaferio, L., & Francavilla, R. (2021). Complementary feeding and iron status:"the unbearable lightness of being" infants. *Nutrients*, *13*(12), 4201.

Moraleda, C., de Deus, N., Serna-Bolea, C., Renom, M., Quintó, L., Macete, E., Menéndez, C., & Naniche, D. (2014). Impact of HIV exposure on health outcomes in HIV-negative infants born to HIV-positive mothers in Sub-Saharan Africa. *JAIDS Journal of Acquired Immune Deficiency Syndromes*, *65*(2), 182-189.

Mphasha, M., Makwela, M., Muleka, N., Maanaso, B., & Phoku, M. (2023). Breastfeeding and Complementary Feeding Practices among Caregivers at Seshego Zone 4 Clinic in Limpopo Province, South Africa. *Children*, *10*(6), 986.

Musuka, G. N., Dzinamarira, T., Cuadros, D. F., Murewanhema, G., Chingombe, I., Takavarasha, F., Herrera, H., & Mapingure, M. (2021). Mothers' HIV status and their children's nutritional status: Insights from secondary analysis of the Zimbabwe Demographic and Health Survey data (2015–2016). *Food Science & Nutrition*, *9*(10), 5509-5516.

Nabakwe, E., Ettyang, G. A., Egesah, O., & Mwangi, A. (2018). Anemia and nutritional status of HIV-exposed infants and HIV-infected mothers in Busia County, Western Kenya. *East African Medical Journal*, *95*(5), 1535-1547.

National Heart, L., & Institute, B. (2011). *Your guide to anemia*. https://www.nhlbi.nih.gov/health-topics/anemia

Nkrumah, B., Nguah, S. B., Sarpong, N., Dekker, D., Idriss, A., May, J., & Adu-Sarkodie, Y. (2011). Hemoglobin estimation by the HemoCue® portable hemoglobin photometer in a resource poor setting. *BMC clinical pathology*, *11*(1), 1-6.

Nyofane, M., Hoffman, M., Mulol, H., Botha, T., Vannevel, V., Pattinson, R., & Feucht, U. (2022). Early Childhood Growth Parameters in South African Children with Exposure to Maternal HIV Infection and Placental Insufficiency. *Viruses*, *14*(12), 2745.

O'Brien, K. O., Zavaleta, N., Abrams, S. A., & Caulfield, L. E. (2003). Maternal iron status influences iron transfer to the fetus during the third trimester of pregnancy. *The American journal of clinical nutrition*, *77*(4), 924-930.

Obeagu, E. I., Okwuanaso, C. B., Edoho, S. H., & Obeagu, G. U. (2022). Under-nutrition among HIV-exposed Uninfected Children: A Review of African Perspective. *Madonna University journal of Medicine and Health Sciences ISSN: 2814-3035*, *2*(3), 120-127.

Odhiambo, C., Zeh, C., Ondoa, P., Omolo, P., Akoth, B., Lwamba, H., Lando, R., Williamson, J., Otieno, J., & Masaba, R. (2015). Anemia and red blood cell abnormalities in HIV-infected and HIV-exposed breastfed infants: a secondary analysis of the kisumu breastfeeding study. *PloS One*, *10*(11), e0141599.

Osterbauer, B., Kapisi, J., Bigira, V., Mwangwa, F., Kinara, S., Kamya, M. R., & Dorsey, G. (2012). Factors associated with malaria parasitaemia, malnutrition, and anaemia among HIV-exposed and unexposed Ugandan infants: a cross-sectional survey. *Malaria journal*, *11*(1), 1-6.

Provan, D., Singer, C. R., & Baglin, T. (2009). *Oxford handbook of clinical haematology*. Oxford University Press, USA.

Ramlal, R. T., Tembo, M., Soko, A., Chigwenembe, M., Ellington, S., Kayira, D., King, C. C., Chasela, C., Jamieson, D., & Van Der Horst, C. (2012). Maternal Mid–Upper Arm Circumference Is Associated With Birth Weight Among HIV-Infected Malawians. *Nutrition in Clinical Practice*, *27*(3), 416-421.

Rosala-Hallas, A., Bartlett, J. W., & Filteau, S. (2017). Growth of HIV-exposed uninfected, compared with HIV-unexposed, Zambian children: a longitudinal analysis from infancy to school age. *BMC pediatrics*, *17*(1), 80.

Rossouw, M. E., Cornell, M., Cotton, M. F., & Esser, M. M. (2016). Feeding practices and nutritional status of HIV-exposed and HIV-unexposed infants in the Western Cape. *Southern African journal of HIV medicine*, *17*(1).

Rothman, M., Faber, M., Covic, N., Matsungo, T. M., Cockeran, M., Kvalsvig, J. D., & Smuts, C. M. (2018). Infant development at the age of 6 months in relation to feeding practices, Iron status, and growth in a peri-urban community of South Africa. *Nutrients*, *10*(1), 73.

Sanou, D., & Ngnie-Teta, I. (2012). Risk factors for anemia in preschool children in sub-Saharan Africa. In.

Santos, R. F. d., Gonzalez, E. S. C., Albuquerque, E. C. d., Arruda, I. K. G. d., Diniz, A. d. S., Figueroa, J. N., & Pereira, A. P. C. (2011). Prevalence of anemia in under five-year-old children in a children's hospital in Recife, Brazil. *Revista brasileira de hematologia e hemoterapia*, *33*, 100-104.

Sartorius, B. K., Chersich, M. F., Mwaura, M., Meda, N., Temmerman, M., Newell, M. L., Farley, T. M., & Luchters, S. (2013). Maternal anaemia and duration of zidovudine in antiretroviral regimens for preventing mother-to-child transmission: a randomized trial in three African countries. *BMC Infectious Diseases*, *13*(1), 1-14.

Siekmans, K., Receveur, O., & Haddad, S. (2014). Can an integrated approach reduce child vulnerability to anaemia? Evidence from three African countries. *PloS One*, *9*(3), e90108.

Smith, B. L., Zizzo, S., Amzel, A., Wiant, S., Pezzulo, M. C., Konopka, S., Golin, R., & Vrazo, A. C. (2018). Integration of Neonatal and Child Health Interventions with Pediatric HIV Interventions in Global Health. *Int J MCH AIDS*, *7*(1), 192-206. https://doi.org/10.21106/ijma.268

Smuts, C. M., Dhansay, M. A., Faber, M., van Stuijvenberg, M. E., Swanevelder, S., Gross, R., & Benade, A. S. (2005). Efficacy of multiple micronutrient supplementation for improving anemia, micronutrient status, and growth in South African infants. *The Journal of nutrition*, *135*(3), 653S-659S.

Stats, S. (2022). *Mid-Year Population Estimates 2022*

Stewart, A., Marfell-Jones, M., Olds, T., & de Ridder, H. (2012). *International standards for anthropometric assessment, 2011*.

Stiller, C. K., Golembiewski, S. K. E., Golembiewski, M., Mondal, S., Biesalski, H.-K., & Scherbaum, V. (2020). Prevalence of undernutrition and anemia among santal adivasi children, Birbhum District, West Bengal, India. *International journal of environmental research and public health*, *17*(1), 342.

Stires, H., LaMori, J., Chow, W., Zalewski, Z., Vidulich, A., Avina, M., Sloan, C., Hughes IV, R., & Hardy, H. (2021). Weight gain and related comorbidities following antiretroviral initiation in the 2000s: a systematic literature review. *AIDS Research and Human Retroviruses*, *37*(11), 834-841.

Tabrizi, F. M., & Barjasteh, S. (2015). Maternal hemoglobin levels during pregnancy and their association with birth weight of neonates. *Iranian journal of pediatric hematology and oncology*, *5*(4), 211.

Tam, P.-Y. I., Wiens, M. O., Kabakyenga, J., Kiwanuka, J., Kumbakumba, E., & Moschovis, P. P. (2018). Pneumonia in HIV-exposed and infected children and association with malnutrition. *The Pediatric infectious disease journal*, *37*(10), 1011.

Tantracheewathorn, S., & Lohajaroensub, S. (2005). Incidence and risk factors of iron deficiency anemia in term infants. *J Med Assoc Thai*, *88*(1), 45-51.

Teklemariam, Z., Mitiku, H., & Mesfin, F. (2015). Prevalence of anemia and nutritional status among HIV-positive children receiving antiretroviral therapy in Harar, eastern Ethiopa. *HIV/AIDS (Auckland, NZ)*, *7*, 191.

Teklemariam, Z., Mitiku, H., & Mesfin, F. (2015). Prevalence of anemia and nutritional status among HIV-positive children receiving antiretroviral therapy in Harar, eastern Ethiopa. *HIV/AIDS (Auckland, N.Z.)*, *7*, 191-196. https://doi.org/10.2147/HIV.S78845

Tshiambara, P., Hoffman, M., Legodi, H., Botha, T., Mulol, H., Pisa, P., & Feucht, U. (2023). Comparison of Feeding Practices and Growth of Urbanized African Infants Aged 6–12 Months Old by Maternal HIV Status in Gauteng Province, South Africa. *Nutrients*, *15*(6), 1500. https://doi.org/https://doi.org/10.3390/nu15061500

Turawa, E., Awotiwon, O., Dhansay, M. A., Cois, A., Labadarios, D., Bradshaw, D., & Pillay-van Wyk, V. (2021). Prevalence of anaemia, iron deficiency, and iron deficiency anaemia in women of reproductive age and children under 5 years of age in south africa (1997–2021): A systematic review. *International Journal of Environmental Research and Public Health*, *18*(23), 12799.

UNAIDS. (2022a). *AIDS statistics—Fact sheet| UNAIDS (2021)*.

UNAIDS. (2022b). *In Danger: UNAIDS Global AIDS Update 2022*. Joint United Nations Programme on HIV/AIDS Geneva, Switzerland.

Ververs, M.-t., Antierens, A., Sackl, A., Staderini, N., & Captier, V. (2013). Which anthropometric indicators identify a pregnant woman as acutely malnourished and predict adverse birth outcomes in the humanitarian context? *PLoS currents*, *5*.

Volberding, P. A., Levine, A. M., Dieterich, D., Mildvan, D., Mitsuyasu, R., Saag, M., & Group, A. i. H. W. (2004). Anemia in HIV infection: clinical impact and evidence-based management strategies. *Clinical infectious diseases*, *38*(10), 1454-1463.

White, M., Feucht, U. D., du Toit, L. d. V., Rossouw, T., & Connor, K. L. (2021). Understanding the Impact of Maternal HIV Infection on the Health and Well-Being of

Mothers and Infants in South Africa: Siyakhula Collaborative Workshop Report. *J. Multidiscip. Healthc*, *14*, 1987-1999.

Widen, E. M., Tsai, I., Collins, S. M., Wekesa, P., China, J., Krumdieck, N., Miller, J. D., Weiser, S. D., Onono, M., & Young, S. L. (2019). HIV infection and increased food insecurity are associated with adverse body composition changes among pregnant and lactating Kenyan women. *European journal of clinical nutrition*, *73*(3), 474-482.

World Health Organization. (1995). *The use and interpretation of anthropometry: WHO Technical Reports Series no. 854*.

World Health Organisation. (2001). *WHO: Iron deficiency anaemia: assessment, prevention, and control*.

World Health Organization. (2009). *WHO child growth standards and the identification of severe acute malnutrition in infants and children: joint statement by the World Health Organization and the United Nations Children's Fund*.

World Health Organization. (2016). *WHO recommendations on antenatal care for a positive pregnancy experience*. World Health Organization.

World Health Organization. *Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Geneva, Switzerland: WHO, 2011*. WHO/NMH/NHD/MNM/11.1. Vitamin and Mineral Nutrition Information System (VMNIS). Retrieved July 2019 from https://apps.who.int/iris/handle/10665/85839

World Health Organization. (2019). *UNICEF-WHO low birthweight estimates: levels and trends 2000-2015*. World Health Organization.

World Health Organization. (2020a). *Global anaemia reduction efforts among women of reproductive age: impact, achievement of targets and the way forward for optimizing efforts*.

World Health Organization. (2020b). *WHO Guidance Helps Detect Iron Deficiency and Protect Brain Development*.

World Health Organization. (2021a). *Indicators for assessing infant and young child feeding practices: definitions and measurement methods*.

World Health Organization (2021b). *Prevalence of anaemia in children aged 6–59 months* https://www.who.int/data/gho/data/indicators/indicator-details/GHO/prevalence-of-anaemia-in-children-under-5-years-(-)

Yisak, H., Ambaw, B., Walle, Z., Alebachew, B., & Ewunetei, A. (2020). Minimum acceptable diet and associated factors among HIV-exposed children aged 6–24 months in Debre Tabor town, Ethiopia. *HIV/AIDS-Research and Palliative Care*, 639-645.

CHAPTER 6: GENERAL SUMMARY

Main research findings, strengths, limitations, implication, conclusion and recommendations

6.1. INTRODUCTION

This general discussion is divided into three sections – the first section focuses on the main research findings, the second section summarises the strengths and limitations of the research, the third section presents the public health implications of this project, conclusion and recommendations (shown in Table 6.1).

Table 6.1. Summary of key findings of this study

Title of the article/manuscript	Objective	Main findings	Future studies
Comparison of Feeding Practices and Growth of Urbanized African Infants Aged 6–12 Months Old by Maternal HIV Status in Gauteng Province, South Africa	To determine differences in infant feeding practices and anthropometric measures by HIV exposure status at 6, 9, and 12 months in the Siyakhula study.	• Inappropriate feeding practices in the complementary feeding phase, commonly with too early introduction of solid foods • Breastfeeding rates were lower in HEU than HUU infants • Poorer growth in HEU than HUU infants	• Determine dietary intake of HEU and HUU infants at 6-12 months. • Determine the breastmilk composition of trace elements fed to HEU and HUU infants.
Dietary intake in the complementary feeding phase and growth of infants by maternal HIV status in an urban setting in Gauteng Province, South Africa	To compare the dietary intake, micronutrient composition of breastmilk, and growth of HIV-exposed-uninfected (HEU) and HIV-unexposed-uninfected (HUU) infants aged 6 and 12 months in an urban setting.	• Lower Z-scores in HEU infants as compared to HUU infants • Non-breastfed HUU infants had lower dietary intakes than HEU infants • Flesh food consumption was lower in HUU than HEU infants • Dietary diversity was low in both HEU and HUU infants •	• Determine the anaemia status, body composition, and growth in mother-infant pairs. • Determine the association between haemoglobin levels and growth of the infants aged 6–12 months.
	To compare the micronutrient composition of breastmilk fed to HIV-exposed-uninfected (HEU) and HIV-unexposed-uninfected (HUU) infants aged 6 and 12 months in an urban setting.	• Micronutrient breastmilk composition was similar between MLWH and MnLWH	• Determine the body composition of mothers • Include 9 months visit data and • Bigger sample size

Haemoglobin level and growth of South African infants, aged 6–12 months, exposed to maternal HIV infection	To determine the differences in haemoglobin levels and growth parameters between HEU and HUU infants, with a further determination of correlations between haemoglobin levels and growth in 6–12-month-old infants in the Siyakhula study in South Africa.	• MLWH had lower haemoglobin levels and were more anaemic than MnLWH in the postpartum phase • HEU and HUU infants had similar haemoglobin levels, but HEU infants had higher percentages of infants who were anaemic • Positive correlations were found between haemoglobin levels and WAZ of HEU infants at 9 and 12 months	• Associate growth and haemoglobin. • Intervention studies focusing on the effectiveness of nutrition education in improving growth of HEU and HUU infants.

Abbreviations: HEU: HIV-exposed-uninfected; HUU: HIV-unexposed-uninfected; NCDs: non-communicable diseases; MLWH: mothers living with HIV; MnLWH: mothers not living with HIV.

6.2. MAIN RESEARCH FINDINGS

The first article, presented in Chapter 3 and published in the Nutrients journal (see Appendix M), compared feeding practices and growth in HIV-exposed-uninfected (HEU) and HIV-unexposed-uninfected (HUU) infants between 6–12 months of age in urbanised African infants in South Africa. HEU infants had lower Z-scores than HUU infants at birth, 6, 9 and 12 months which is consistent to other findings in Africa (Evans, Chasekwa, Ntozini, Majo, Mutasa, Tavengwa *et al*., 2020; Lane, Widen, Collins & Young, 2020). Although the HEU infants had poor growth, they were able to catch-upon growth by 12 months (LAZ and HCZ were similar ($p>0.05$)), supported by other African studies (Le Roux, Abrams, Donald, Brittain, Phillips, Nguyen *et al*., 2019; Slogrove, Esser, Cotton, Speert, Kollmann, Singer *et al*., 2017). The result supports the notion of successful PMTCT and ART program in South Africa, with high HIV prevalence in SSA.

Breastfeeding rates were lower in HEU than HUU infants, especially at 9 and 12 months, and this is similar to other studies (Budree, Goddard, Brittain, Cader, Myer & Zar, 2017; Rossouw, Cornell, Cotton & Esser, 2016). Even though there is progress in the breastfeeding rates in this setting, lack of knowledge on the importance of breastfeeding and the composition of breastmilk, lack of family support and fear of on vertical transmission are some of the reasons leading to lower rates of breastfeeding (World Health Organization, 2019). Strengthening the counselling provided during antenatal and postnatal care in health facilities needs to be done together with education on the composition of breastmilk as some mothers do not believe that breastmilk is sufficient for the first six months and beyond.

Early introduction to complementary foods is common in Africa and was the case in this setting (Esubalew, Atenafu, Abebe, 2018; Sayed, Schönfeldt, 2020). Inappropriate feeding practices such as early or late introduction of solids food may lead to undernutrition, increasing the risk of poor growth and development in infants aged 6–12 months. From six months on, as infants shift from exclusive breastfeeding to supplemental feeding, their nutritional needs increase. (Dewey, 2013).

The second manuscript, presented in Chapter 4 and to be submitted in the South African Journal of Clinical Nutrition (see Appendix N for journal submission guidelines), compared the dietary intake, growth, and the micronutrient composition of breastmilk fed to HEU and HUU infants aged 6 and 12 months in an urban setting. The Z-scores of HEU infants were lower than those of HUU infants.

HEU infants had smaller Z-scores than HUU infants. Inadequate dietary intake is an immediate cause of undernutrition and low intakes of dietary fats were found in HEU and HUU infants. Dietary intakes of iron, calcium and vitamin A intakes were lower in HUU than HEU infants in line with other studies (Álvarez-Zaragoza, Vásquez-Garibay, Sánchez Ramírez & Larrosa Haro, 2023; Lane *et al*., 2017; Parker *et al*., 2019).

HUU infants had lower consumption of flesh food products than HEU infants at 12 months, similar to other African studies (Faber, 2007; Williams, Chantry, Geubbels, Ramaiya, Shemdoe, Tancredi *et al*., 2016). Flesh products are a rich source of heme iron needed for oxygen transportation, but they are expensive. Financial constraints, poverty, or food insecurity affects the consumption of these products (Mahan & Raymond, 2016; Pienaar, van Rooyen & Walsh, 2017). The comparison between HEU and HUU infants is challenging due to limited published data on dietary intake and growth.

Micronutrient trace elements breastmilk composition was similar in MLWH and MnLWH, supporting that all mothers can safely and adequately breastfeed their infants even in the context of HIV while on treatment (Mulol & Coutsoudis, 2016; WHO, 2021). Other research, however, showed disparities in the composition of breastmilk, which could be attributed to differences in analytical methods, larger sample sizes, geographical areas, and population groups. (Bzikowska-Jura, Sobieraj, Michalska-Kacymirow & Wesołowska, 2021; Nakamori, Ninh, Isomura, Yoshiike, Hien, Nhug et al., 2009).

In manuscript 3, presented in Chapter 5 and to be submitted to the Maternal and Child Health journal (see Appendix O for submission guidelines), the multiple cross-sectional analysis determined the differences in haemoglobin levels and growth parameters between HEU and HUU infants, with further determination of correlations between haemoglobin levels and growth in 6, 9, and 12-month-old infants. HEU and HUU presented similar mean haemoglobin levels and anaemia status at 6, 9 and 12 months, similar to Mozambique (Moraleda, de Deus, Serna-Bolea, Renom, Quintó, Macete *et al*., 2014). No correlations were found between HEU and HUU infants haemoglobin and Z-scores indices in this study and is line with another study in Uganda (Osterbauer, Kapisi, Bigira, Mwangwa, Kinara, Kamya & Dorsey, 2012).

6.3. STRENGTHS AND LIMITATIONS

The strengths and limitations for the published article and two manuscripts of this PhD thesis were included in the individual discussions. However, strengths and limitations as they apply to the overall research project include the following.

6.3.1. Strengths

- The major strength of the research was the study of dietary intake, haemoglobin levels and growth, with known birth information and measurements serving as a baseline in high HIV-prevalence settings.
- The detailed feeding practices information, including age, type of complementary foods introduced and given to the infants, food frequently consumed, and dietary intake of the infants given by mothers is an important contribution.
- The further experiential analysis of trace elements composition in breastmilk of MLWH and MnLWH adds to better understanding.
- Using the World Health Organization (WHO) growth Z-scores that are corrected for gestational age and sex-specific at 6–12 months.
- Having similar numbers in HEU versus HUU infants in the groups provided for better interpretation of the results.
- All measurements and information collected at each visit were done by the same research assistants with years of experience in data collection, who were trained by health professionals, including nutritionists for dietary intake and food consumption, and retired nurses who collected the blood and gave immunisations.

6.3.2. Limitations

- This study had a smaller sample size to warrant for multiple linear regression on categorical variables such as nutritional classifications of the infants and food groups.
- In this study, dietary analysis was purposely limited to certain macro- and micronutrients that play a role in optimising nutritional status of the infants, and dietary adequacy could not be determined due to the unmeasured breastmilk intake of breastfed infants.
- The use of single 24-hour recalls with potential recall bias was not ideal, but it was not practical to have more than one recall due to the nature of the study.
- The impact of COVID-19 resulted in loss of follow up of some of the infants, as 15 of the infants had relocated by 9-month visit.

- The cross-sectional study design, limiting the generalisation and causation of this PhD study.

6.4. PUBLIC HEALTH IMPLICATIONS AND FUTURE RESEARCH

Undernutrition is a major cause of death in children under five, contributing to neonatal and infant mortality, and stunted growth (WHO, 2021). Maternal undernutrition leads to poor birth outcomes, including infant stunting, which is exacerbated by inappropriate feeding practices, more especially in the context of HIV (Masilela & Modjadji, 2023; WHO, 2021). Current findings support HEU infants a higher risk of experiencing hostile birth outcomes, including stunting. that HEU infants are at a higher risk of poor birth outcomes, including stunting, putting emphasis of the significance of nutrition throughout the first 1000 days in this context.

Nutrition interventions, including specific interventions tailored to address immediate cause of undernutrition, can be beneficial in these settings by promoting breastfeeding and appropriate complementary feeding (WHO, 2021). Inappropriate feeding practice with early introduction of solids foods, lack of continued breastfeeding and not achieving the dietary diversity score raises concerns in this setting, especially with other prevalent risk factors, like the high maternal HIV prevalence (Stats SA, 2022; WHO, 2016). Therefore, investigating the mother's dietary intake will be essential and to also educate mothers and caregivers on the locally available and affordable micronutrient rich sources that are important for growth and development of HEU and HUU infants.

Consumption of miscellaneous food products such as chips and sodas which that are rich in calories may lead to overweight and obesity, and discouraging the consumption of these products will be beneficial and educating mothers on the appropriate feeding practices using the infant and young child feeding (IYCF) policy (Faber 2007; WHO, 2021). Coordinated efforts to address the nutrition sensitive interventions, including food security programmes, will assist in reducing stunting and help mothers in these settings to meet the adequate intakes while meeting the minimal acceptable diet (WHO, 2021).

HIV increases the risk of anaemia and this was also the case in this study in terms of maternal postpartum anaemia. Adherence to ART treatment and good nutrition is important in decreasing the prevalence of anaemia. Infants' haemoglobin was not associated with mothers' haemoglobin which might signify that PMTCT and ART programmes are successfully implemented in these

settings. Monitoring and evaluation with the encouragement and routine testing of HIV is essential in this setting during antenatal and postnatal periods, with the inclusion of nutrition education and counselling on the importance of micronutrient supplementation and dietary diversity. Coordinated campaigns for expecting moms can emphasise the significance of proper nutrition throughout the initial 1000 days to prevent anaemia in both HEU and HUU infants residing in resources limited areas with high prevalence of HIV.

6.5. CONCLUSION

In conclusion, the research in this thesis aimed to compare the growth, feeding practices, and haemoglobin levels of HEU and HUU infants aged 6–12 months stratified by maternal HIV status. Although HEU infants had suboptimal growth parameters at birth, they were able to catch up on growth by 12 months (their LAZ was similar to their HUU counterparts). With adherence to ART by mothers and continuous counselling and education by healthcare workers, HVTP is protective against stunting. Nutrition as an integral part of HVTP cannot be underplayed in the prevention of anaemia and stunting.

The HEU infants were found to have lower rates of breastfeeding than HUU infants. The infant and young child feeding policy needs to be taught and followed. Support from family and community members to breastfeeding mothers is required. Continuous nutrition education encompassing the importance and benefits of breastfeeding, and the composition of breastmilk will be essential in increasing breastfeeding rates in MLWH. The HEU infants had better dietary intakes and DDS than HUU infants, signifying the progress made with the HVTP and nutrition education and counselling. A holistic approach towards optimal nutrition of both HEU and HUU infants is important in securing better health for the future of these infants even before reaching their first birthday. Nutrition counselling and education can improve DDS and thereby prevent micronutrient deficiencies including iron and zinc, which are critical for cognitive development, and strengthening the immune system.

6.6. RECOMMENDATIONS

The results of this research and recommendations will be further disseminated to healthcare professionals, policymakers, academics, and community members through publication in peer-reviewed research journals (Nutrients, South African Journal of Clinical Nutrition, and Maternal

& Child Nutrition Journals), conferences (nutrition society of South Africa, Child health priorities and international AIDS conference) and social media (linked in, Facebook and X (Twitter)).

Future studies on the growth of HEU infants can:

i) Use a longitudinal study design to determine the growth patterns of HEU infants over 24 months with a bigger sample size to better understand the relationship between the growth and nutrition of these infants and children.

ii) Conduct a pediatric food-based dietary guidelines knowledge and attitude surveys for mothers, caregivers, and community health workers focusing on the knowledge and awareness of the South African Paediatric Food based Dietary Guidelines in order to establish the attitude leading to inappropriate feeding practices in the community.

iii) Use of a complete full blood count assessment to determine the iron deficiency anaemia status of HEU and HUU infants.

iv) Conduct intervention studies at the community level with training the trainer including community health workers, nurses, doctors, and non-governmental organizations on nutrition messages aimed at promoting, protecting, and supporting breastfeeding (breastmilk composition) as well as appropriate complementary feeding including food planning and preparation from six months and breastfeeding support group initiation.

In summary therefore this body of research shows that maternal HIV infection influences infant growth and feeding practices, but that better infant feeding counselling and implementation need to be prioritized for all South African mothers, irrespective of the HIV status.

6.7. REFERENCES

Álvarez-Zaragoza, C., Vásquez-Garibay, E.M., Sánchez Ramírez, C.A. and Larrosa Haro, A., 2023. Anthropometric Indicators and Dietary Intake in Toddlers Aged from 12 to 24 Months Who Attended Private Clinics in the Metropolitan Area of Guadalajara. *Children*, *10*(7), p.1259.

Budree, Goddard, Brittain, Cader, Myer, & Zar. 2017. Infant feeding practices in a South African birth cohort—A longitudinal study. *Maternal and chil nutrition*. 13, e12371.

Bzikowska-Jura A, Sobieraj P, Michalska-Kacymirow M & Wesołowska A. 2021. Investigation of iron and zinc concentrations in human milk in correlation to maternal factors: An observational pilot study in poland. *Nutrients*. 13(2):303.

Dewey, K.G. 2013. The challenge of meeting nutrient needs of infants and young children during the period of complementary feeding: An evolutionary perspective. *The Journal of nutrition*. 143(12)**:**2050-4.

Esubalew, F., Atenafu, A. and Abebe, Z. 2018. Feeding practices according to the WHO-recommendations for HIV exposed children in northwest Ethiopia: A cross-sectional study. *Clinical nutrition ESPEN*. 28114-20.

Evans, C., Chasekwa, B., Ntozini, R., Majo, F.D., Mutasa, K., Tavengwa, N., et al. 2020. Mortality, HIV transmission and growth in children exposed to hiv in rural Zimbabwe. *Clinical infectious diseases*. 72586-94.

Faber, M. 2007. Complementary foods consumed by 6 – 12-month-old rural infants in south africa are inadequate in micronutrients. *Public Health Nutrition*. 8(4)**:**373-81.

Lane, C., Widen, E., Collins, S. and Young, S. 2020. HIV-exposed, uninfected infants in uganda experience poorer growth and body composition trajectories than HIV-unexposed infants. *Journal of Acquired Immune Deficiency Syndromes*. 4(Supplement_2)**:**1023-.

Le Roux S, M, Abrams EJ, Donald KA, Brittain. K, Phillips. TK, Nguyen. KK, et al. Growth trajectories of breastfed HIV-exposed uninfected and HIV-unexposed children under conditions of universal maternal antiretroviral therapy: A prospective study. *The Lancet Child & Adolescent Health.* 2019; 3(4):234-44.

Mahan, L.K. and Raymond, J.L. 2016. Krause's food & the nutrition care process-e-book, Elsevier Health Sciences.

Masilela, L.N. and Modjadji, P., 2023. Child Nutrition Outcomes and Maternal Nutrition-Related Knowledge in Rural Localities of Mbombela, South Africa. *Children*, 10(8), p.1294.

Moraleda, C., de Deus, N., Serna-Bolea, C., Renom, M., Quintó, L., Macete, E., Menéndez, C. and Naniche, D., 2014. Impact of HIV exposure on health outcomes in HIV-negative infants born to HIV-positive mothers in Sub-Saharan Africa. *JAIDS Journal of Acquired Immune Deficiency Syndromes*, *65*(2), pp.182-189.Nakamori M, Ninh NX, Isomura H, Yoshiike N, Hien VTT, Nhug BT, et al. 2009. Nutritional status of lactating mothers and their breast milk concentration of iron, zinc and copper in rural vietnam. *J Nutr Sci Vitaminol*. 55(4):338-45.

Osterbauer, B., Kapisi, J., Bigira, V., Mwangwa, F., Kinara, S., Kamya, M. R., & Dorsey, G. (2012). Factors associated with malaria parasitaemia, malnutrition, and anaemia among HIV-exposed and unexposed Ugandan infants: a cross-sectional survey. *Malaria journal*, 11(1), 1-6.

Parker, M.E., Tembo, M., Adair, L., Chasela, C., Piwoz, E.G., Jamieson, D.J., Ellington, S., Kayira, D., Soko, A., Mkhomawanthu, C. and Martinson, F., 2013. The health of HIV-exposed children after early weaning. *Maternal & child nutrition*, *9*(2), pp.217-232.

Pienaar, M., Van Rooyen, F.C. and Walsh, C.M. 2017. Household food security and hiv status in rural and urban communities in the free state province, south africa. *Journal of Social Aspects of HIV/AIDS*. 14(1)**:**118-31.

Rossouw, M.E.; Cornell, M.; Cotton, M.F.; Esser, M.M. 2016. Feeding practices and nutritional status of HIV-exposed and HIV-unexposed infants in the Western Cape. *South african Journal of HIV Medicine*. 17, 398.

Sayed, N. and Schönfeldt, H.C. 2020. A review of complementary feeding practices in south africa. *South African Journal of Clinical Nutrition*. 33(2)**:**36-43.

Slogrove AL, Esser MM, Cotton MF, Speert DP, Kollmann TR, Singer J. and Bettinger JA. A prospective cohort study of common childhood infections in south african HIV-exposed

uninfected and HIV-unexposed infants. *The Pediatric infectious disease journal*. 2017; 36(2):e38.

Stats, S. 2022. Mid-year population estimates 2022. Stats SA: Pretoria, South Africa.

Williams, A.M., Chantry, C., Geubbels, E.L., Ramaiya, A.K., Shemdoe, A.I., Tancredi, D.J. and Young, S.L. 2016. Breastfeeding and complementary feeding practices among HIV-exposed infants in coastal Tanzania. *Journal of Human Lactation*. 32(1)**:**112-22.

World Health Organization. *Advocacy brief: Breastfeeding and HIV: Global breastfeeding collective*. World Health Organization. 2019. Available from: https://www.who.int/nutrition/publications/infantfeeding/breastfeeding-and-hiv-advocacybrief/en/.

World Health Organization. *UNICEF/WHO/the world bank group joint child malnutrition estimates: Levels and trends in child malnutrition: Key findings of the 2020 edition*. Geneva, WHO, Switzerland, 2021.

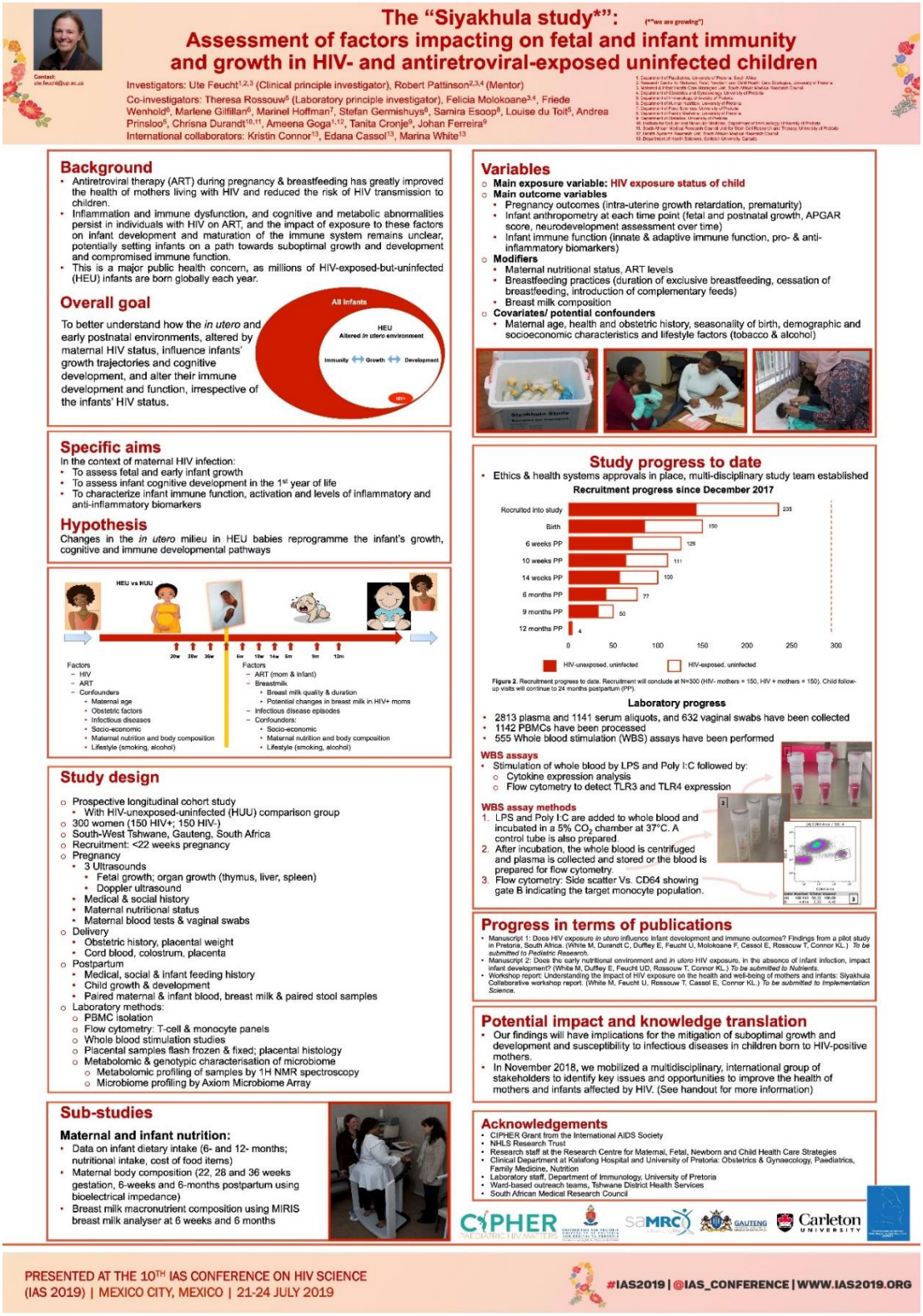
Jeremiah 29:13

"You will seek me and find me, when you seek me with all your heart."

May this thesis shows that God is alive and good.

7. APPENDICES

APPENDIX A: Siyakhula study poster presentation by the PI



APPENDIX B: INFORMED CONSENT

PATIENT / PARTICIPANT'S INFORMATION LEAFLET & INFORMED CONSENT FORM FOR A NON-INTERVENTION STUDY

STUDY TITLE: Assessment of factors impacting on foetal and infant immunity, growth, and neurodevelopment in HIV- and antiretroviral-exposed uninfected children

STUDY NAME: Siyakhula Study

SPONSOR: The International AIDS Society

Principal Investigator: Prof Ute Feucht

Institution: University of Pretoria

MRC

DAYTIME AND AFTER HOURS TELEPHONE NUMBER(S):

Daytime numbers: 012 373 1082

Afterhours: 083 368 4995

DATE AND TIME OF FIRST INFORMED CONSENT DISCUSSION:

dd	**mmm**	**ivy**

:
Time

Dear Patient

Dear Ms. / Mrs. .....

1) INTRODUCTION

We invite you to participate in a research study. We are doing research on factors that may influence the immune system (these are the cells of the body that fight infection), growth and development of children born to HIV-negative women compared to HIV-positive women. I am going to give you information about the study and invite you to be part of this research. If there is anything that you do not understand please ask me to explain. You should not agree to take part unless you are completely happy about all the procedures involved.

2) THE NATURE AND PURPOSE OF THIS STUDY

The aim of the research is to understand how mother's HIV infection influences the growth of the foetus (unborn baby) during pregnancy compared to HIV negative women. We also want to follow-up your baby after birth to learn about the immune function, growth and brain development of babies from both HIV-negative and HIV-positive mothers.

3) EXPLANATION OF PROCEDURES TO BE FOLLOWED

We are inviting all pregnant women from the Southwest Tshwane, with a pregnancy before 22 weeks, to participate in the research. We are looking for HIV-negative women and HIV-positive women on treatment who are able to follow up at the clinic with their babies for 2 years after delivery. We will pay for your transportation to the clinic for the study.

If you agree to participate in the research, we will ask you to come for three (3) visits for a sonar to Kalafong Hospital during your pregnancy. You will deliver your baby at Kalafong or Pretoria West Hospital. After delivery, we will ask to see you and your baby for 8 visits at Kalafong Hospital until the child is 2-years-old. The following procedures will be done during pregnancy, delivery and after delivery.

3.1 The procedures for the mother

3.1.1 During pregnancy

- We routinely do one ultrasound (sonar) to see how far pregnant you are. More sonars are done if there are problems with the pregnancy. In this research, you will have a total of three (3) sonars to look at any abnormalities and to see how the baby is growing.
- We will ask you questions about your health and social circumstances.
- The routine antenatal care clinical examinations and tests will be done as always.
- At each visit when you have a sonar done and around time of delivery, another test will be done with a specialised machine, which looks like a big scale. This test is done to see how your body changes and it measures how much fat and muscle you have. This test will also be done after your baby is born, when you are able to stand upright when you come for your 6 weeks visit. This measurement is not painful at all and it is not harmful to you or the baby.
- A small amount of blood, 30 millilitres (about 2 tablespoons), will be collected from your arm with a syringe, at 28 and 36 weeks. The blood will be sent for tests to look for markers of inflammation and other related biological factors. If you are HIV infected, blood will also be sent for antiretroviral drug levels – this is to see how much medicine is in your blood.
- We will also take vaginal swabs at 28 and 36 weeks. This sample will be tested to look for markers of inflammation and infections and other biological markers important for your health.
- An oral glucose tolerance test (a test to look for abnormal blood sugar levels) is usually done in patients who have a high risk of diabetes. In this study, we will do this test in all women because, if the mother is diabetic, this can affect the growth of the unborn baby.

3.1.2 At delivery

- At delivery or just after birth, we will collect another 30 millilitres (about 2 tablespoons) of blood to look for markers of inflammation and other biological factors. In women who are HIV-infected, we will also test the amount of virus in your blood.

- After your baby is born we will use a small needle to take blood from the umbilical cord to test for inflammation and other related biological factors important for the development of the baby, such as infection markers and growth factors.
- We will also take a few small pieces of the placenta after delivery and we will test factors that are important for the development of the baby.
- Before you leave the hospital, we will ask you to express some breastmilk (about one tablespoon), so that we can measure substances in the breastmilk that are important for the newborn.
- We will also ask you to give a stool sample, if at all possible.

3.1.3 The next two years

- We will ask you to come to Kalafong Hospital with your baby for eight (8) visits when the baby is 6, 10 and 14 weeks old, and at 6, 9, 12, 18 and 24 months old. These visits are part of the routine follow-up care for you and your baby and will replace your usual clinic visits.
- At these visits, we will ask you to answer some questions about you and your baby's health, diet and how you feed your baby.
- We will take 30 millilitres (about 2 tablespoons) of blood to look for markers of inflammation and infections and other related biological markers important for your health.
- At each visit, we will also ask you to express some breastmilk (about 6 tablespoons) so that we can measure substances in the breast milk that are important for the development of your baby.
- We will ask for a stool sample at 6 weeks and again at 3, 6 and 12 months. We will provide you with a container so you can do this at home, if you so prefer.

3.2 The procedures for the child

3.2.1 Newborn

- The routine measurements of the newborn, such as length, weight and the size of the head, will be taken.
- In addition, we will collect stool from the newborn to look at the organisms in the stool.
- For babies born to HIV infected mothers, 5 millilitres (one teaspoon) of blood will be taken on the baby for HIV birth PCR test as part of routine newborn care.

3.2.2 Child visits

- The child visits will be when the baby is 6, 10 and 14 weeks old, and 6, 9, 12, 18 and 24 months old.
- At these visits we will weigh and measure your baby's length and head size and to look at his or her Road to Health chart.
- We will do an assessment of your child's brain development at these time points by looking if he or she can do the usual things expected of a child at that age.
- At these visits, we will take 10 millilitres (about 2 teaspoons) of blood from your baby to check for low iron levels (anaemia) and to look at biological factors important for the growth and health of the baby.
- We will collect stool from your child at 6 weeks and 3, 6 and 12 months.
- If there are any problems with the child's development or if they have anaemia, your child will be referred for further care.
- We will also offer the childhood immunisations at all time points as required by the National Immunisation Programme and this will replace your regular clinic visits.

3.3 Testing of samples

Most of the tests will be done at the Department of Immunology at the University of Pretoria. We will also send a small amount of blood, vaginal swab, breastmilk, placenta and stool overseas for testing at the Department of Health Sciences at Carleton University in Canada. We also ask your permission to store all the left-over samples that we have collected for future testing. We will first get approval from the Faculty of Health Sciences Research Ethics Committee, University of Pretoria and the Research Ethics Board at Carleton University before doing any more tests on these samples.

4) RISK AND DISCOMFORT INVOLVED.

The main inconvenience for you will be your doctor visits will be longer than usual. There is only minimal risk or possible discomfort involved with providing blood, breastmilk or stool samples, or having the vaginal swab, or measuring your child's growth and development. Taking blood can sometimes be painful and could cause bruising afterwards.

5) POSSIBLE BENEFITS OF THIS STUDY.

The benefits during pregnancy are that you will be seen by a specialist and you will have detailed sonars by a skilled specialist in this field. If there are any complications, you will receive treatment immediately.

The benefits for your baby are that a specialist will do the routine visits. Your child will receive additional screening for growth and brain development. We will be able to diagnose anaemia and any possible problems with development early and your child can get treatment. Your child will also get all required immunisations, which means that your child will not have to go to the clinic as well.

6) VOLUNTARY PARTICIPATION

Your participation in this research is entirely voluntary. It is your choice whether to participate or not. Whether you choose to participate or not, all the necessary services at this clinic or hospital will continue and nothing will change. If you choose not to participate in this research project you will be offered the treatment that is routinely offered in this clinic or hospital. You are allowed to withdraw from the study at any time. Any information or samples we collect from you as part of the study before you withdraw will remain part of the study. There will be no further information or samples collected from you if you withdraw from the study.

7) I understand that if I and my baby do not want to participate in this study, I will still

receive standard treatment for my illness.

8) I may at any time withdraw from this study.

9) REIMBURSEMENTS

There are no direct financial benefits to you, but we will give you money to pay for your transport to the hospital during pregnancy and for the follow-up visits. The amount will be based on the distance you stay from the clinic.

10) HAS THE STUDY RECEIVED ETHICAL APPROVAL?

This Protocol was submitted to the Faculty of Health Sciences Research Ethics Committee, University of Pretoria, telephone numbers 012 3563084 / 012 3563085 and

written approval has been granted by that committee. This protocol was also submitted to the Carleton University Research Ethics Board, and written approval has been granted. The study has been structured in accordance with the Declaration of Helsinki (last update: October 2013), which deals with the recommendations guiding doctors in biomedical research involving human/subjects. A copy of the Declaration may be obtained from the investigator, should you wish to review it.

11) INFORMATION If you have any questions concerning this study, you should contact:

1. Dr Felicia Molokoane: 083 368 4995
2. Prof Mphele Mulaudzi: 083 258 8705
3. Prof Ute Feucht: 072 428 0465

12) CONFIDENTIALITY

The information that we collect from this research project will be kept confidential. Participants will be identified for study purposes with a unique study number. Your personal identifying information will not be connected to the information collected for this research study. Information collected about you and your baby during the research will be stored safely and will only be available to the approved researchers.

13) CONSENT TO PARTICIPATE IN THIS STUDY

I have read or had read to me in a language that I understand the above information before signing this consent form. The content and meaning of this information have been explained to me. I have been given the opportunity to ask questions and am satisfied that they have been answered satisfactorily. I understand that if I do not participate, it will not alter my management in any way. I hereby volunteer to take part in this study.

I have received a signed copy of this informed consent agreement.

.....	.....
Patient name	Date
.....	.....
Patient signature	Date
.....	.....
Investigator name	Date
.....	.....
Investigator signature	Date
.....	.....
Witness name and signature	Date

VERBAL PATIENT INFORMED CONSENT (applicable when patients cannot read or write)

I, the undersigned, Dr ……..…, have read and have explained fully to the patient, named …….. and/or his/her relative, the patient information leaflet, which has indicated the nature and purpose of the study in which I have asked the patient to participate. The explanation I have given has mentioned both the possible risks and benefits of the study and the alternative treatments available for his/her illness. The patient indicated that he/she understands that he/she will be free to withdraw from the study at any time for any reason and without jeopardizing his/her treatment.

I hereby certify that the patient has agreed to participate in this study.

Investigator's Name _____
(Please print)

Investigator's Signature _____ Date _____

Patient's Name _____
(Please print)

Patient's Signature _____ Date _____

Witness's Name _____ Witness's Signature _____ Date _____
_____
(Please print)

(Witness - sign that he/she has witnessed the process of informed consent)

APPENDIX C: MATERNAL AND INFANT POSTPARTUM QUESTIONNAIRE

Maternal and infant postpartum questionnaire **Study Nr_____ Date:_____**

BREASTFEEDING

1. Did you ever breastfeed or try to breastfeed your baby, even if only for a single feed?

□ Yes → Skip to question 3

□ No

□ Prefer not to answer → Skip to question 5

2. If no, why was this? Select all that apply

□ Personal choice → Skip to question 11

□ Personal circumstances (e.g., other demands, return to work) → Skip to question 11

□ You were unwell → Skip to question 11

□ Baby was too small or unwell → Skip to question 11

□ Didn't think you had enough milk → Skip to question 11

□ Lack of support/resources → Skip to question 11

□ Other reason: Please specify:_____ → Skip to question 11

□ Prefer not to answer → Skip to question 13

3. How soon after birth was your baby first put to the breast? ***(SKIP IF NO TO question 1)***

_____ minutes or _____ hours after birth

□ Never (baby was fed pumped milk)

□ Prefer not to answer

4. Has your baby ever been fed breastmilk from a bottle?

□ Yes

□ No

□ Prefer not to answer

5a. At how many weeks or months after birth is this follow-up visit occurring?

□ 6 weeks postnatal *or* □ 14 weeks postnatal *or*

□ 6 months postnatal *or* □ 9 months postnatal *or*

□ 12 months postnatal *or* □ 24 months postnatal

5b. Thinking about the time between when your baby was born and now (this visit), how did you feed your baby from birth until now? (For example, if this visit is occurring at approximately 14 weeks after your baby was born, how did you feed your baby from birth until 14 weeks of age?)

□ Breastmilk only directly from the breast (*no* expressed breastmilk and *no* formula feeding) from birth to baby's current age

□ Breastmilk only with some feeding directly from the breast and some from expressed breastmilk (e.g.: expressed using your hand or a pump) before baby's current age, but *no* formula feeding up to baby's current age

□ Breastmilk and formula feeding (baby received some formula before his/her current age, but still received some direct or expressed breastmilk at his/her current age)

□ Formula feeding only (baby did not receive any breastmilk between birth and his/her current age)

6. Are you currently breastfeeding your baby or giving your baby expressed breast milk?

□ Yes

□ No → Skip to question 8

□ Prefer not to answer → Skip to question 13

If yes, is your baby currently receiving breastmilk only?

□ Yes → Skip to question 13

□ No, my baby receives both breastmilk and formula

□ Prefer not to answer

7. Which scenario best describes your baby's feeding?

□ My baby receives infant formula most (80–100%) of the time.

□ My baby receives breastmilk most (80–100%) of the time.

□ My baby receives both breastmilk and formula equally.

□ Prefer not to answer

8. How old was your baby when you stopped breastfeeding?

_____ days or _____weeks

□ Prefer not to answer

9. How old was your baby when you introduced formula?

_____ days or _____weeks

□ Prefer not to answer

10. What was the main reason for introducing formula?

□ Breastfeeding took too long or was too tiring

□ Needed to return to work

□ Convenience or to allow others to feed

□ To try and get baby to sleep through the night

□ Insufficient milk to satisfy the baby

□ Baby wouldn't suck because unwell or low birth weight

□ Baby wouldn't suck for no apparent reason

□ Baby irritable or colicky

□ Baby not gaining weight

□ Painful breasts or sore nipples

□ Mastitis or breast abscess

□ Milk dried up

□ The right time/age to change

□ Other reason → (Please specify:_____)

□ Prefer not to answer

11. What type of formula do you usually feed your baby?

□ Cow's milk-based formula

□ Lactose-free cow's milk-based formula

□ Soy-based formula

□ Other →(Please specify:_____)

□ Prefer not to answer

What is the specific brand and type of formula that you usually feed your baby? Indicate all that apply

_____

_____

12. What form of formula do you usually use?

□ Liquid ready-to-use

□ Powder concentrate (add water)

□ Prefer not to answer

13a. Has your baby had any liquids other than breastmilk or formula since his/her birth (even if it was a temporary supplement)? Other liquids include water, glucose water, evaporated milks, goat's milk, cow's milk, tea, rooibos tea or any other drink (including muthi). Any solids, like porridge, vegetables?

□ Yes → (if yes, please specify: _____)

□ No

□ Prefer not to answer

13b. How old was your baby when you gave this drink for the first time? (in months) _____

At the moment, does your baby get any semi-solid or solid food (with a spoon)?

□ Yes

□ No

□ Prefer not to answer

13c. What was the first semi-solid or solid food (with a spoon) that your baby ate?

_____

_____

How old was your baby when you first gave semi-solid food? (in months) _____

14. Does your baby receive any vitamins or supplement drops?

□ Yes

□ No

□ Prefer not to answer

15. If yes, which of the following? How often are you giving the vitamins or supplements?

□ Vitamin D drops → _____ times per (day, week, month)

□ Other (Please specify:_____) → _____ times per (day, week, month)

□ Prefer not to answer

16. Has your baby ever taken any prescribed medications?

□ Yes → If yes, please specify: _____

□ No

□ Prefer not to answer

MOTHER'S DIETARY SUPPLEMENTS

Now we would like to ask some general questions about your health and lifestyle since your baby was born.

17. How would you currently rate your general health?

□ Excellent

□ Very good

□ Good

□ Fair

□ Poor

□ Prefer not to answer

18. What is your current weight? _____kilograms

□ Prefer not to answer

19. Since your baby was born, have you taken any vitamins, minerals or other dietary supplements?

□ Yes

□ No → Skip to question 20

□ Prefer not to say → Skip to question 20

	How often have you used this *since you gave birth*?	
Prenatal vitamin (before baby is born and possibly during breastfeeding)	□ Never □ Less than 1 per month	If Yes, please list the brand name and specific type:

	□ 1–3 days per month □ 1–3 days per week □ 4–6 days per week □ Every day □ Prefer not to say	_____ _____
Note: Some pregnant women are prescribed a Pregamal Tablet and asked to take 2 tablets per day during pregnancy and breastfeeding.	□ Has this dose and intake routine been prescribed by your health care provider? Are you adhering to this prescription (are you following the instructions)? □ Yes □ No □ Don't know □ Prefer not to say	
Multivitamin	□ Never □ Less than 1 per month □ 1–3 days per month □ 1–3 days per week □ 4–6 days per week □ Every day □ Prefer not to say	If Yes, please list the brand name and specific type: _____ _____ Does your multivitamin contain minerals (like iron, zinc, etc.)? □ Yes □ No □ Prefer not to say
Folic acid or folate (NOT as part of a multivitamin or prenatal multivitamin)	□ Never □ Less than 1 per month □ 1–3 days per month	<u>Dose:</u> □ less than 400 mcg (0.4 mg) □ 400–799 mcg □ 800–999 mcg

	□ 1–3 days per week □ 4–6 days per week □ Every day □ Prefer not to say	□ 1000 (1 mg) or more, up to 4000 mcg (4 mg) □ 5000 (5 mg) □ Don't know □ Prefer not to say
Iron (NOT as part of a multivitamin or prenatal multivitamin)	□ Never □ Less than 1 per month □ 1–3 days per month □1–3 days per week □ 4–6 days per week □ Every day □ Prefer not to say	<u>Dose:</u> □ Less than 10 mg □ 10–14 mg □ 15–39 mg □ 40 mg or more □ Don't know □ Prefer not to say
Note: Some pregnant women are prescribed a Gulf Ferrous Sulphate Compound Tablet and asked to take 2 tablets per day during pregnancy and breastfeeding.	□ Has this dose and intake routine been prescribed by your health care provider? Are you adhering to this prescription (are you following the instructions)? □ Yes □ No □ Don't know □ Prefer not to say	
Calcium supplements or calcium containing antacids (NOT as part of a multivitamin or prenatal multivitamin)	□ Never □ Less than 1 per month □ 1–3 days per month □ 1–3 days per week	<u>Dose:</u> □ less than 500 mg □ 500–599 mg □ 600–999 mg □ 1000 or more □ Don't know □ Prefer not to say

	□ 4–6 days per week □ Every day □ Prefer not to say	
Other, Please specify: (Examples Vitamin C, Zinc, Vitamin D, Probiotics, Cod liver oil, other Fish oil or Omega 3 fatty acids Name of supplement: _____ _____	□ Never □ Less than 1 per month □ 1–3 days per month □ 1–3 days per week □ 4–6 days per week □ Every day □ Prefer not to say	Dose: _____ _____

FOOD SECURITY

The following questions ask about your access to food over the past 12 months.

20. Which of the following statements best describes the food eaten in your household in the past 12 months?

□ You and other household members always had enough of the kinds of food you wanted to eat.

□ You and other household members had enough to eat, but not always the kinds of food you wanted.

□ Sometimes you and other household members did not have enough to eat.

□ Often you and other household members didn't have enough to eat.

□ Don't know

□ Prefer not to answer

The following statements may be used to describe the food situation for a household. Please indicate if the statement was often true, sometimes true, or never true for you and other household members in the past 12 months.

21. You and other household members worried that food would run out before you got money to buy more. Was that often true, sometimes true, or never true in the past 12 months?

□ Often true
□ Sometimes true
□ Never true
□ Don't know
□ Prefer not to answer

22. The food that you and other household members bought just didn't last, and there wasn't any money to get more. Was that often true, sometimes true, or never true in the past 12 months?

□ Often true
□ Sometimes true
□ Never true
□ Don't know
□ Prefer not to answer

23. You and other household members couldn't afford to eat balanced meals. In the past 12 months was that often true, sometimes true, or never true?

□ Often true
□ Sometimes true
□ Never true
□ Don't know
□ Prefer not to answer

If the participant responds "often true" or "sometimes true" to ANY ONE of question 20–22 OR "Sometimes" or "Often" to question 19, then continue to question 23; otherwise, skip to the next section (question 29).

The following questions are about the food situation in the past 12 months for you or any other adults in your household.

24. In the past 12 months, did you or other adults in your household ever cut the size of your meals or skip meals because there wasn't enough money for food?

□ Yes
□ No → Skip to question 28
□ Don't know
□ Prefer not to answer

25. How often did this happen?

□ Almost every month
□ Some months but not every month
□ Only 1 or 2 months
□ Don't know
□ Prefer not to answer

26. In the past 12 months, did you personally ever eat less than you felt you should have because there wasn't enough money to buy food?

□ Yes
□ No
□ Don't know
□ Prefer not to answer

27. In the past 12 months, did you personally lose weight because you didn't have enough money for food?

□ Yes
□ No
□ Don't know
□ Prefer not to answer

If the participant responded "yes" to question 24, 26 or 27, continue to 28; otherwise, skip to the next section (question 30).

28. In the past 12 months, did you or other adults in your household ever not eat for a whole day because there wasn't enough money for food?

□ Yes

□ No

□ Don't know

□ Prefer not to answer

29. How often did this happen?

□ Almost every month

□ Some months but not every month

□ Only 1 or 2 months

□ Don't know

□ Prefer not to answer

SMOKING AND ALCOHOL

30. How many cigarettes do you smoke each day now?

_____ number of cigarettes

□ Do not smoke → Skip to question 32

□ Prefer not to answer

31. Do you smoke inside your home?

□ Yes

□ No

□ Prefer not to answer

32. Does any member of your household smoke cigarettes (even if not inside your home)?

□ Yes

□ No

□ Prefer not to answer

33. How often are you usually exposed to other people's tobacco smoke inside your home?

□ Every day
□ Almost every day
□ At least once a week
□ At least once a month
□ Less than once a month
□ Never
□ Don't know
□ Prefer not to answer

34. During leisure time outside of your home, how often are you usually exposed to other people's tobacco smoke?

□ Every day
□ Almost every day
□ At least once a week
□ At least once a month
□ Less than once a month
□ Never
□ Don't know
□ Prefer not to answer

35. Since your baby was born, how often do you drink alcohol?

□ 6 to 7 times a week
□ 4 to 5 times a week
□ 2 to 3 times a week
□ Once a week
□ 2 to 3 times a month → skip to next section
□ About once a month → skip to next section
□ Less than monthly → skip to next section
□ Never → skip to next section
□ Don't know → skip to next section
□ Prefer not to answer → skip to next section

36. Since your baby was born, how often do you have four (4) or more alcoholic drinks at the same sitting or occasion?

□ 6 to 7 times a week
□ 4 to 5 times a week
□ 2 to 3 times a week
□ Once a week
□ 2 to 3 times a month
□ About once a month
□ 6 to 11 times a year
□ 1 to 5 times a year
□ Never
□ Don't know
□ Prefer not to answer

EMOTIONAL HEALTH

As you have recently had a baby, we would like to ask you some questions about how you have been feeling lately. If there are any questions you do not want to answer please skip the question and move on to the next question.

Please check the answer that comes closest to how you have felt **over the last week** only, not just how you feel today.

In the past 7 days:

37. I have been able to laugh and see the funny side of things

□ As much as I always could
□ Not quite so much now
□ Definitely not so much now
□ Not at all

38. I have looked forward with enjoyment to things

□ As much as I ever did
□ Rather less than I used to
□ Definitely less than I used to

□ Hardly at all

39. I have blamed myself unnecessarily when things went wrong

□ Yes, most of the time

□ Yes, some of the time

□ Not very often

□ No, never

40. I have been anxious or worried for no good reason

□ No, not at all

□ Hardly ever

□ Yes, sometimes

□ Yes, very often

41. I have felt scared or panicky for no very good reason

□ Yes, quite a lot

□ Yes, sometimes

□ No, not much

□ No, not at all

42. Things have been getting on top of me

□ Yes, most of the time I haven't been able to cope at all

□ Yes, sometimes I haven't been coping as well as usual

□ No, most of the time I have coped quite well

□ No, I have been coping as well as ever

43. I have been so unhappy that I have had difficulty sleeping

□ Yes, most of the time

□ Yes, sometimes

□ Not very often

□ No, not at all

44. I have felt sad or miserable

□ Yes, most of the time

□ Yes, quite often

□ Not very often

□ No, not at all

45. I have been so unhappy that I have been crying

□ Yes, most of the time

□ Yes, quite often

□ Only occasionally

□ No, never

46. The thought of harming myself has occurred to me

□ Yes, quite often

□ Sometimes

□ Hardly ever

□ Never

APPENDIX D: INFANT FOLLOW-UP FORM

INFANT FOLLOW-UP FORM **Date:_____**

Study Nr: _____ **Infant Hospital file Nr: _____**

Infant Data of Birth_____ **Infant Age in months_____**

Infant visit	6 months		12 months		
Immunisation given today	OPV	Rotavirus	Hexaxim	PCV	Measles
Infant study blood done?	Yes / No / Not indicated		Comment:		
Breast milk sample taken?	Yes / No / Not indicated		Comment:		

Maternal information

Maternal date of birth & age (yrs)	DOB:		Age:	
Maternal weight & MUAC	Weight (kg):	Height (cm):	MUAC (cm):	
Maternal body composition measurements	Done (Yes / No)			
Maternal HIV-status:	Positive / Negative / Unknown/HIV-negative		Date:	
– Latest CD4:			Date:	
– Latest Viral load:			Date:	
– Current ART:			Date:	
In the last week has she missed any dose?	Yes / No	If yes, how many doses	_____	Not sure
– Current Bactrim:			Date:	
– Current other: Diflucan, etc			Date:	
Maternal TB status:	Current / Previous / never tested / Unknown		Date:	
– Current INH prophylaxis:				

– Current TB treatment:		
– Type TB		

Other infections during post pregnancy:	Yes/No	When	Treatment	Results
–				
–				
–				

Complications/illnesses post-partum	Yes / No (Check exclusions) (If yes: What, when & treatment)	
What?	When	Treatment
–		
–		
–		

Medication use after pregnancy	Yes / No / Unsure (If yes: Check exclusions) (If yes: What & when)	
– Antibiotics		Date:
– Iron supplement		Date:
–		Date:
–		Date:

PHQ-2

Now I want to ask you specifically about how you have been feeling. Over the past two weeks, how often have you been bothered by any of the following problems?

11	Little interest or pleasure in doing things?
12	Feeling down, depressed or hopeless?

1	Not at all	2	Several days	3	More than half the days	4	Nearly every day

1	Not at all	2	Several days	3	More than half the days	4	Nearly every day

13 Have you ever received treatment (psychotherapy or medication) for depression? If yes, when was this?

1	No	2	After child was born	3	Before child was born

Additional Questionnaires

Infant information:

Previous illnesses (This information is collected from mother's history and also information documented on RTHB, or any other patient health record like discharge summaries)

a. Was there any perinatal illnesses? This refers to illness in the first week of life. ***This will only be asked at 6 weeks visit unless the 6 weeks visit was missed***	1	Yes	2	No	3	N/A

b. If yes describe

How old was the baby in days?		
What was the diagnosis		
What was the treatment received?		

c. Has your child had malnutrition/Kwashiorkor or has a health provider told you that he/she was not growing as well as expected.	1	Yes	2	No
d. Has your child had diarrhea? Date……..	1	Yes	2	No

e. Has your child had difficulty in breathing? Date……	1	Yes	2	No
f. Was your child admitted for any illness in the hospital?	1	Yes	2	No
g. If yes describe ……. When (month/year)…….				
h. Did you visit any health care facility because your child was ill?	1	Yes	2	No
i. If yes describe ……. Date…….				
j. Does your child currently need or use medicine prescribed by a doctor or nurse?	1	Yes	2	No
k. If yes, describe:				
l. Does your child have any chronic illness or medical condition?	1	Yes	2	No
m. If yes, describe:				
n. Is your child limited in any way in his or her ability to do the things most children of the same age can do?	1	Yes	2	No
o. If yes describe:				
p. Does your child have any kind of developmental problem, disability for which he/she needs or gets special treatment or stimulation?	1	Yes	2	No
q. If yes describe?				

r. Do you currently access any child care grant assistance because of your child's condition/illness (care dependency grant)?	1	Yes	2	No
s. Do you currently receive child social grant for this child?	1	Yes	2	No
t. Do you receive a foster care grant for this child	1	Yes	2	No

Infant symptoms:

Is the baby Admitted to neonatal unit?	**YES**	**NO**
Baby clinical examination abnormal	**YES**	**NO**
		If NO, infant symptoms section is Not Applicable

INFANT SYMPTOMS			
Infant anthropometry:	Weight (kg):	Length(cm)	MUAC (cm):
	HC (cm)		

Failure to thrive	≤ 2.5 kg	YES	NO	**Please add extra notes and barcodes, if needed:**	
	< -2 z-score	YES	NO		
Anaemia	YES	NO	Value:	___ ___ ___ ___	
Thrombocytopaenia	YES	NO	Value:	___ ___ ___ ___	

Signs of RDS	YES	NO

RR > 60bpm	YES	NO	*Retractions*	YES	NO	
Grunting	YES	NO	*Apnoea*	YES	NO	
Alar flaring	YES	NO	*Cyanosis*	YES	NO	

Central cyanosis	YES	NO	% SATS RA	

CNS signs	Convulsions/lethargy/↓ LOC	YES	NO	

Hepatomegaly	YES	NO	Below costal margin = cm	

Splenomegaly	YES	NO		
LN > 1 cm	YES	NO	Anatomical sites:	

Any other perinatal problem?	RDS/HMD	Congenital pneumonia	Neonatal sepsis
	Jaundice	Convulsions	Other: _____

NVP started?	YES	NO	**Dose?**	**Still given?**	
AZT started?	YES	NO	**Dose?**	**Still given?**	

Other drugs started?		Time/s given	_____h_____
Iron supplementation			
Iron treatment			

GMCD (scores 0-3)
Expressive Language
Receptive Language
Gross Movements
Fine Movements
Relating
Play activities
Self-help activities
Bayleys (from 6 month visit onwards)

NEXT APPT DATE	**DD/MM/YYYY**

BLOOD TAKEN	YES	NO	**BIRTH PCR RESULT**	POS	NEG	

Other PCR tests done?	

IF POS – DATE THAT MOTHER INFORMED OF RESULT	**DD/MM/YYYY**
IF POS - DATE & TIME OF ARV INITIATION	**DD/MM/YYYY _____h_____**

DRUGS AND DOSAGES USED FOR INITIATION	*1*		*DOSE*
	2		*DOSE*
	3		*DOSE*

The neurodevelopmental assessments done?			
Infant developmental screening done? (GMCD)	Yes	No	
Bayley's Developmental Assessment done	Yes	No	
Lifestyle & dietary questionnaire done?	Yes	No	

Form completed by: _____

APPENDIX E: 24 HOUR RECALL

24-hr DIETARY RECALL

File number: ____

What day is it today?	1 = Monday	2 = Tuesday	3 = Wednesday	4 = Thursday	5 = Friday	

Greetings!

Thank you for giving up your time to participate in this study. I hope you are enjoying it so far. Here we want to find out what your baby is eating and drinking. This information is important to know as it will tell us how much and what types of food babies in the area are eating.

There are no right or wrong answers.

Everything you tell me is confidential.

Is there anything you want to ask now? Are you willing to go on with the questions?

I want to find out about everything your baby ate or drank yesterday, including breast milk and water. Please tell me everything your baby ate from the time he/she woke up yesterday, throughout the day and during the night. I will also ask you where your baby ate the food and how much he/she ate.

File number:

Time of day	What food and drink	How was it prepared? What was added?	How much was eaten	How much left?
Waking up to about 9 o'clock (breakfast time)				
Mid-morning (09h00 – 12h00)				
Lunch time (12h00 – 14h00)				
Afternoon (14h00 - 17h00)				
Supper time (17h00 – sunset)				
After supper; during the night				

Would you describe the food that your baby ate yesterday as typical of his/her usual food intake?	1 = Yes	2 = No	
If NO, please give the reason:			

APPENDIX F: FOOD FREQUENCY QUESTIONNAIRE

FOOD FREQUENCY

Baby's code: | | | | |

Ask for each food item, one at a time, how often the child usually eats the specific food item. The last week (or last seven days) should be taken as guideline, therefore the frequency that the child ate the food item during the last week. Make a cross on the option that describes the mother's answer the best). The options are as follows:

Every day
Most days: not every day, but at least 4 times per week
Once a week: less than 4 times per week, but at least once per week
Never

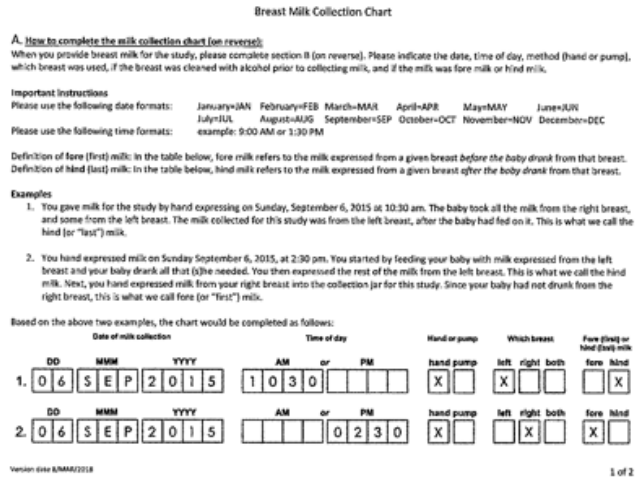
Food item	*Frequency of intake during the last week*			
	Month 6	Month 12	Month 18	Month 24
Date (dd/mm/yyyy):				
Fieldworker's code:				
Breastmilk	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never
Formula milk *If formula milk was used, please give name of the formula milk:*	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never .....
Cow's milk / amasi / maas Milk powder e.g. Klim, Nespray	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never
Yoghurt / danone	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never
Baby foods in a jar e.g. Purity	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never
Infant cereals or infant porridge e.g. Nestum, Cerelac, Cream of Maize, Baby Mabele	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never
Porridge made with maize meal (soft, stiff or crumbly)	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never

Cooked porridge, other than maize meal porridge e.g. oats, mabele	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never
Instant porridge, e.g. instant Maize, Mabele	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never
Bread	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never
Rice	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never
Potatoes	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never
Vegetables, any type (NOT potatoes) *If vegetables were eaten, please name the type of vegetables eaten mostly:*	1___Every day 2___Most days 3___Once a week 4___Never 1 ..... 2 ..... 3	1___Every day 2___Most days 3___Once a week 4___Never 1 ..... 2 ..... 3	1___Every day 2___Most days 3___Once a week 4___Never 1 ..... 2 ..... 3	1___Every day 2___Most days 3___Once a week 4___Never 1. ..... 2. ..... 3. .....
Fruit juice (includes juice squeezed from the fruit)	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never
Fresh fruit (any type) *If fruit were eaten, please name the type of fruit eaten mostly:*	1___Every day 2___Most days 3___Once a week 4___Never 1 ..... 2 ..... 3	1___Every day 2___Most days 3___Once a week 4___Never 1 ..... 2 ..... 3	1___Every day 2___Most days 3___Once a week 4___Never 1 ..... 2 ..... 3	1___Every day 2___Most days 3___Once a week 4___Never 1. ..... 2. ..... 3. .....
Eggs	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never

Red meat (beef, pork, mutton) / stew / sausage / mince meat	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never
Chicken / poultry	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never
Liver (e.g. chicken liver, beef liver, sheep liver etc)	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never
Fish (fresh or canned)	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never
Sweets / Chocolates	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never
Chips / Cheese curls / Niknaks	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never
Fizzy cold drink e.g. Coke	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never
Juice concentrate, mix with water e.g. Oros	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never
Rooibos	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never
Tea, normal	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never
Salt (added to food)	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never

How often did you use oil when preparing the baby's food?	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never
How often did you use margarine when preparing the baby's food? [any type of margarine]	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never
How often did you use peanut butter when preparing the baby's food?	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never	1___Every day 2___Most days 3___Once a week 4___Never

APPENDIX G: BREASTMILK COLLECTION CHART



Breast Milk Collection Chart

A. How to complete the milk collection chart (on reverse):
When you provide breast milk for the study, please complete section B (on reverse). Please indicate the date, time of day, method (hand or pump), which breast was used, if the breast was cleaned with alcohol prior to collecting milk, and if the milk was fore milk or hind milk.

Important instructions
Please use the following date formats: January=JAN February=FEB March=MAR April=APR May=MAY June=JUN July=JUL August=AUG September=SEP October=OCT November=NOV December=DEC
Please use the following time formats: example: 9:00 AM or 1:30 PM

Definition of **fore** (first) milk: In the table below, fore milk refers to the milk expressed from a given breast *before the baby drank* from that breast.
Definition of **hind** (last) milk: In the table below, hind milk refers to the milk expressed from a given breast *after the baby drank* from that breast.

Examples

1. You gave milk for the study by hand expressing on Sunday, September 6, 2015 at 10:30 am. The baby took all the milk from the right breast, and some from the left breast. The milk collected for this study was from the left breast, after the baby had fed on it. This is what we call the hind (or "last") milk.

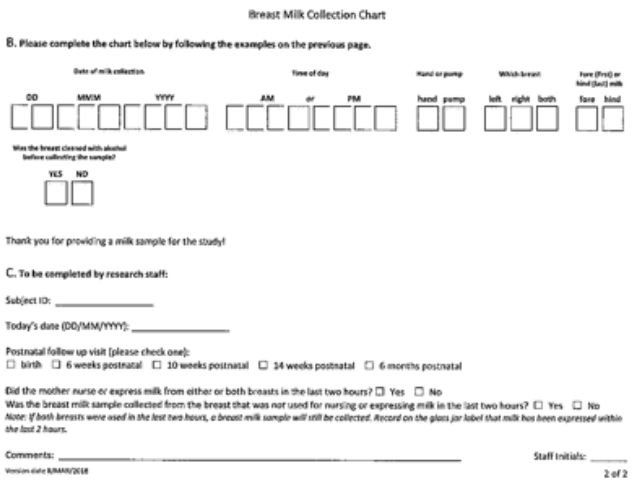
2. You hand expressed milk on Sunday September 6, 2015, at 2:30 pm. You started by feeding your baby with milk expressed from the left breast and your baby drank all that (s)he needed. You then expressed the rest of the milk from the left breast. This is what we call the hind milk. Next, you hand expressed milk from your right breast into the collection jar for this study. Since your baby had not drunk from the right breast, this is what we call fore (or "first") milk.

Based on the above two examples, the chart would be completed as follows:

	Date of milk collection			Time of day			Hand or pump		Which breast			Fore (first) or hind (last) milk	
	DD	MMM	YYYY	AM	or	PM	hand	pump	left	right	both	fore	hind
1.	06	SEP	2015	1030			X		X				X
	DD	MMM	YYYY	AM	or	PM	hand	pump	left	right	both	fore	hind
2.	06	SEP	2015			0230	X			X		X	

Version date 8/MAR/2018

1 of 2



Breast Milk Collection Chart

B. Please complete the chart below by following the examples on the previous page.

Date of milk collection			Time of day			Hand or pump		Which breast			Fore (first) or hind (last) milk	
DD	MMM	YYYY	AM	or	PM	hand	pump	left	right	both	fore	hind

Was the breast cleaned with alcohol before collecting the sample?
YES NO

Thank you for providing a milk sample for the study!

C. To be completed by research staff:

Subject ID: _____

Today's date (DD/MM/YYYY): _____

Postnatal follow up visit (please check one):
☐ birth ☐ 6 weeks postnatal ☐ 10 weeks postnatal ☐ 14 weeks postnatal ☐ 6 months postnatal

Did the mother nurse or express milk from either or both breasts in the last two hours? ☐ Yes ☐ No
Was the breast milk sample collected from the breast that was not used for nursing or expressing milk in the last two hours? ☐ Yes ☐ No
Note: If both breasts were used in the last two hours, a breast milk sample will still be collected. Record on the glass jar label that milk has been expressed within the last 2 hours.

Comments: _____ Staff Initials: _____

Version date 8/MAR/2018

2 of 2

APPENDIX H: SIYAKHULA STUDY APPROVAL CERTIFICATES

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences

The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.

- FWA 00002567, Approved dd 22 May 2002 and Expires 03/20/2022.
- IRB 0000 2235 IORG0001762 Approved dd 22/04/2011 and Expires 03/14/2020

17 May 2019

Approval Certificate
Amendment

Ethics Reference No.: 294-2017
Title: Assessment of factors impacting on foetal and infant immunity, growth, and neurodevelopment in HIV- and antiretroviral-exposed uninfected children (the Siyakhula study)

Dear Dr UD Feucht

The **Amendment** as supported by documents received between 2019-05-02 and 2019-05-17 for your research, was approved by the Faculty of Health Sciences Research Ethics Committee on its quorate meeting of 2019-05-15.

Please note the following about your ethics approval:

- Please remember to use your protocol number (294-2017) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

Dr R Sommers
MBChB MMed (Int) MPharmMed PhD
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 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, Second Edition 2015 (Department of Health).

Research Ethics Committee
Room 4-60 Level 4 Tswaipoort Building
University of Pretoria, Private Bag X323
Arcadia 0007 South Africa
Tel +27 (0)12 356 3084
E-mail deepeks.behan@up.ac.za
www.up.ac.za

Fakulteit Gesondheidswetenskappe
Lefapha la Disaense tša Maphelo

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

Enquiries: Dr. Lufuno Razwiedani
Tel: +27 12 451 9036
E-mail: lufuno.razwiedani@gauteng.gov.za

TSHWANE RESEARCH COMMITTEE: CLEARANCE CERTIFICATE

MEETING: 06/2017
PROJECT NUMBER: 93/2017
NHRD REFERENCE NUMBER: GP_201710_002

TOPIC: Assessment of factors impacting on foetal and infant immunity, growth, and neurodevelopment in HIV- and antiretroviral-exposed uninfected children

Name of the Researcher:

Felicia Molokoane	Andrea Prinsloo
Ameena Goga	Louise du Toit
Mphele Mulaudzi	Helen Steel
Robert Pattinson	Marlene Gilfillan
Theuns Avenant	Ute Feucht
Jennifer Makin	Theresa Rossouw

Facility: Kalafong Tertiary Hospital
Pretoria West Hospital

Name of the Department: University of Pretoria

NB: THIS OFFICE REQUEST A FULL REPORT ON THE OUTCOME OF THE RESEARCH DONE AND

NOTE THAT RESUBMISSION OF THE PROTOCOL BY RESEARCHER(S) IS REQUIRED IF THERE IS DEPARTURE FROM THE PROTOCOL PROCEDURES AS APPROVED BY THE COMMITTEE.

DECISION OF THE COMMITTEE: APPROVED

.....
Dr. R. Oyedipe
Acting Chairperson: Tshwane Research Committee
Date: 09/11/2017

.....
Ms. M. Lerutla
Acting Chief Director: Tshwane District Health
Date: 9/11/17

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Permission to conduct research and access records / files / database at Kalafong Hospital

To: Chief Executive Officer/Information Officer
Kalafong Hospital
Dr Letebele

From: The Investigator
Prof U Feucht

Re: Permission to do research at Kalafong Hospital

Proff/Drs Pattinson, Avenant, Molokoane, Mulaudzi and I are researchers working at the MRC Unit and Department of Obstetrics and Gynaecology and the Department of Paediatrics at Kalafong Hospital. I am requesting permission on behalf of all of us to conduct a study on the Kalafong Hospital grounds that involves patient follow-up and access to patient records.

The request is lodged with you in terms of the requirements of the Promotion of Access to Information Act. No. 2 of 2000.

The title of the study is: Assessment of factors impacting on foetal and infant immunity, growth, and neurodevelopment in HIV- and antiretroviral-exposed uninfected children.

The researchers request access to the following information: Clinical files, record books and databases.

We intend to publish the findings of the study in a professional journal and/ or at professional meeting like symposia, congresses, or other meetings of such a nature.

We intend to protect the personal identity of the patients by assigning each patient a random code number.

We undertake not to proceed with the study until we have received approval from the Faculty of Health Sciences Research Ethics Committee, University of Pretoria.

Yours sincerely

Jeuch

Permission to do the research study at this hospital and to access the information as requested, is hereby approved.

Chief Executive Officer

Kalafong Hospital

Dr K. E. Letebele - Kankell

Signature of the CEO

Date: 04/10/2017

GAUTENG PROVINCIAL GOVERNMENT
KALAFONG HOSPITAL / HOSPITAAL
04 OCT 2017
PRIVATE BAG / PRIVAATSAK X081 PRETORIA 0001
SUPERINTENDENT'S OFFICE

Hospital Official Stamp

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

DECLARATION OF INTENT FROM THE PRIMARY HEALTH CARE MANAGER, TSHWANE

18 September 2017

Prof/Drs Pattinson, Avenant, Molokoane, Mulaudzi and I are researchers working at the MRC Unit and Department of Obstetrics and Gynaecology and the Department of Paediatrics at Kalafong Hospital. I am requesting permission on behalf of all of us to recruit patients at Primary Health Care facilities in the Tshwane District Health services. The title of the study is: **Assessment of factors impacting on foetal and infant immunity, growth, and neurodevelopment in HIV- and antiretroviral-exposed uninfected children.**

The researchers request the following: Access to potential study participants for recruitment.

The study follow-up will be conducted at Kalafong Hospital. We intend to protect the personal identity of the patients by assigning each patient a random code number. We undertake not to proceed with the study until we have received approval from the Faculty of Health Sciences Research Ethics Committee, University of Pretoria.

Yours sincerely

Prof UD Feucht

I give preliminary permission for the research project: **Assessment of factors impacting on foetal and infant immunity, growth, and neurodevelopment in HIV- and antiretroviral-exposed uninfected children.**

The final approval will be from the Tshwane Research Ethics Committee and that this is only to indicate that the Tshwane Provincial office is willing to assist.

Other comments or conditions prescribed: For issues affecting Kalafong hospital, please contact the office below:

Enquiries: Innocentia Mcena
Directorate: Office of CEO – Kalafong Provincial Tertiary Hospital
Tel: +27 (0)12 3186501[Number]
Fax: +27 (0)12 318 6791

Signature & Date 18 09 2017
Primary Health Care, Tshwane

APPENDIX I: SUB-STUDY/PHD STATISTICAL SUPPORT LETTER

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA
Denkleiers • Leading Minds • Dikgopolo tša Dihlalefi

DEPARTMENT OF STATISTICS

LETTER OF STATISTICAL SUPPORT

Date: 13th February 2020

This letter is to confirm that ***Phumudzo Mamphwe*** studying at the University of Pretoria, discussed the project with the title "***Growth, feeding and heamoglobin levels of infants aged 6-12 months exposed and unexposed to HIV in a peri-urban area in Gauteng Province, South Africa***" with me.

I hereby confirm that I am aware of the project and also undertake to assist with the statistical analysis of the data generated from the project. The aim of the proposed study is to determine the changes and compare the growth patterns, feeding practices and heamoglobin levels of infants ages 6-12 months exposed and unexposed to HIV.

The sample will consist of a prospective longitudinal cohort study of 100 HEU (HIV exposed and uninfected) and 100 HUU (HIV unexposed and uninfected) infants.

The data analysis will consist of descriptive statistics such as mean, median, standard deviations, frequencies, proportions etc. to describe the results and graphical representations can be made were applicable to assist in visualizing aspects of the data.

The primary outcomes will be to compare the results between the HEU and HUU infants. The procedures may include tests like the Chi-squared test, Mann-Whitney U test or the independent t test. Regression analysis may also be considered, in order to evaluate the effect that the different characteristics have on the primary outcome.

Cronje

Tanita Cronje
Department of Statistics
Internal Statistical Consultation Service
tanita.cronje@up.ac.za

APPENDIX J: SUB-STUDY/PHD APPROVAL CERTIFICATES

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Natural and Agricultural Sciences
Ethics Committee

E-mail: ethics.nas@up.ac.za

14 April 2020

ETHICS SUBMISSION: LETTER OF APPROVAL

Ms P Mamphwe
Department of Consumer and Food Sciences
Faculty of Natural and Agricultural Science
University of Pretoria

Reference number: NAS063/2020
Project title: GROWTH, INFANT FEEDING AND HAEMOGLOBIN LEVELS IN 6 TO 12-MONTH OLD INFANTS EXPOSED AND UNEXPOSED TO MATERNAL HIV INFECTION IN A PERI-URBAN AREA IN GAUTENG PROVINCE, SOUTH AFRICA

Dear Ms P Mamphwe,

We are pleased to inform you that your submission conforms to the requirements of the Faculty of Natural and Agricultural Sciences Research Ethics committee.

Please note the following about your ethics approval:

- Please use your reference number (NAS063/2020) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
- Please note that ethical approval is granted for the duration of the research (e.g. Honours studies: 1 year, Masters studies: two years, and PhD studies: three years) and should be extended when the approval period lapses.
- The digital archiving of data is a requirement of the University of Pretoria. The data should be accessible in the event of an enquiry or further analysis of the data.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.
- **Applications using Animals:** NAS ethics recommendation does not imply that AEC approval is granted. The application has been pre-screened and recommended for review by the AEC. Research may not proceed until AEC approval is granted.

Post approval submissions including application for ethics extension and amendments to the approved application should be submitted online via the Ethics work centre.

We wish you the best with your research.

Yours sincerely,

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences

Institution: The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.

- FWA 00002567. Approved dd 22 May 2002 and Expires 03/20/2022.
- IORG # IORG0001762. OMB No. 0990-0279 Approved for use through February 28, 2022 and Expires: 03/04/2023.

4 June 2020

Approval Certificate
New Application

Ethics Reference No.: NAS063/2020
Title: GROWTH, INFANT FEEDING AND HAEMOGLOBIN LEVELS IN 6 TO 12-MONTH OLD INFANTS EXPOSED AND UNEXPOSED TO MATERNAL HIV INFECTION IN A PERI-URBAN AREA IN GAUTENG PROVINCE, SOUTH AFRICA

Dear Dr AMP Hoffman

The **New Application** as supported by documents received between 2020-04-16 and 2020-05-27 for your research, was approved by the Faculty of Health Sciences Research Ethics Committee on its quorate meeting of 2020-05-27.

Please note the following about your ethics approval:

- Ethics Approval is valid for 1 year and needs to be renewed annually by 2021-06-04.
- Please remember to use your protocol number (NAS063/2020) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

Dr R Sommers
MBChB MMed (Int) MPharmMed PhD
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 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, Second Edition 2015 (Department of Health)

Research Ethics Committee
Room 4-60, Level 4, Tswaipolo Building
University of Pretoria, Private Bag x323
Gezina 0031, South Africa
Tel +27 (0)12 356 3084
Email: deepeka.behari@up.ac.za
www.up.ac.za

Fakulteit Gesondheidswetenskappe
Lefapha la Disaense tša Maphelo

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Natural and Agricultural Sciences
Ethics Committee

E-mail: ethics.nas@up.ac.za

13 May 2021

ETHICS SUBMISSION: EXTENSION LETTER

Dr AMP Hoffman
Department of Consumer and Food Sciences
Faculty of Natural and Agricultural Science
University of Pretoria

Reference number: NAS063/2020
Project title: Growth patterns in relation to feeding practices and haemoglobin levels of infants exposed to maternal HIV infection in a South African peri-urban area

Dear Dr AMP Hoffman,

We are pleased to inform your application for ethics extension conforms to the requirements of the Faculty of Natural and Agricultural Sciences Research Ethics Committee.

Please note the following about your ethics approval:

- Please use your reference number (NAS063/2020) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.
- Please note that ethical approval is granted for the duration of the research (e.g. Honours studies: 1 year, Masters studies: two years, and PhD studies: three years) and should be extended when the approval period lapses.
- The digital archiving of data is a requirement of the University of Pretoria. The data should be accessible in the event of an enquiry or further analysis of the data.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.
- **Applications using GM permits:** If the GM permit expires before the end of the study, please make an amendment to the application with the new GM permit before the old one expires.
- **Applications using Animals:** NAS ethics recommendation does not imply that Animal Ethics Committee (AEC) approval is granted. The application has been pre-screened and recommended for review by the AEC. Research may not proceed until AEC approval is granted.

Post approval submissions including application for ethics extension and amendments to the approved application should be submitted online via the ethics work centre.

We wish you the best with your research.

Yours sincerely,

Prof VJ Maharaj
Chairperson: NAS Ethics Committee

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences

Institution: The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.
- FWA 00002567, Approved dd 22 May 2002 and Expires 03/20/2022.
- IORG #: IORG0001762 OMB No. 0990-0279 Approved for use through February 28, 2022 and Expires: 03/04/2023.

Faculty of Health Sciences **Research Ethics Committee**

23 June 2021

Approval Certificate
Annual Renewal

Dear Dr AMP Hoffman

Ethics Reference No.: NAS063/2020
Title: Growth patterns in relation to feeding practices and haemoglobin levels of infants exposed to maternal HIV infection in a South African peri-urban area

The **Annual Renewal** as supported by documents received between 2021-05-25 and 2021-06-17 for your research, was approved by the Faculty of Health Sciences Research Ethics Committee on 2021-06-17 as resolved by its quorate meeting.

Please note the following about your ethics approval:
- Renewal of ethics approval is valid for 1 year, subsequent annual renewal will become due on 2022-06-23.
- Please remember to use your protocol number (NAS063/2020) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:
- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

On behalf of the FHS REC, Dr R Sommers
MBChB, MMed (Int), MPharmMed, PhD
***Deputy Chairperson** of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria*

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 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, Second Edition 2015 (Department of Health)

Research Ethics Committee
Room 4-80, Level 4, Tswelopele Building
University of Pretoria, Private Bag x323
Gezina 0031, South Africa
Tel +27 (0)12 356 3084
Email: deepeka.behari@up.ac.za
www.up.ac.za

Fakulteit Gesondheidswetenskappe
Lefapha la Disaense tša Maphelo

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences

Faculty of Health Sciences **Research Ethics Committee**

23 March 2023

Approval Certificate
Annual Renewal

Dear Dr AMP Hoffman,

Ethics Reference No.: NAS063/2020 – Line 3
Title: Growth patterns in relation to feeding practices and haemoglobin levels of infants exposed to maternal HIV infection in a South African peri-urban area

The **Annual Renewal** as supported by documents received between 2023-02-21 and 2023-03-15 for your research, was approved by the Faculty of Health Sciences Research Ethics Committee on 2023-03-15 as resolved by its quorate meeting.

Please note the following about your ethics approval:
- Renewal of ethics approval is valid for 1 year, subsequent annual renewal will become due on 2024-03-23.
- Please remember to use your protocol number (NAS063/2020) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:
- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

On behalf of the FHS REC, Professor C Kotzé
MBChB, DMH, MMed(Psych), FCPsych, Phd
***Acting Chairperson**: Faculty of Health Sciences Research Ethics Committee*

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 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, Second Edition 2016 (Department of Health)

Research Ethics Committee
Room 4-60, Level 4, Tswelopele Building
University of Pretoria, Private Bag x323
Gezina 0031, South Africa
Tel +27 (0)12 356 3084
Email: deepika.bohani@up.ac.za
www.up.ac.za

Fakulteit Gesondheidswetenskappe
Lefapha la Disaense tša Maphelo

nutrients

MDPI

Article

Comparison of Feeding Practices and Growth of Urbanized African Infants Aged 6–12 Months Old by Maternal HIV Status in Gauteng Province, South Africa

Phumudzo Tshiambara [1,2,3,4,*], Marinel Hoffman [2,3,4], Heather Legodi [1], Tanita Botha [5], Helen Mulol [3,4,6], Pedro Pisa [1] and Ute Feucht [3,4,6]

[1] Department of Human Nutrition, Faculty of Health Sciences, University of Pretoria, Prinshof Campus, Pretoria 0084, South Africa
[2] Department of Consumer and Food Sciences, Faculty of Natural and Agricultural Sciences, University of Pretoria, Hatfield Campus, Pretoria 0028, South Africa
[3] Research Centre for Maternal, Fetal, Newborn and Child Health Care Strategies, University of Pretoria, Kalafong Provincial Tertiary Hospital, Pretoria 0001, South Africa
[4] Research Unit for Maternal and Infant Health Care Strategies, South African Medical Research Council, Pretoria 0001, South Africa
[5] Department of Statistics, Faculty of Natural and Agricultural Sciences, University of Pretoria, Hatfield Campus, Pretoria 0028, South Africa
[6] Department of Paediatrics, Faculty of Health Sciences, University of Pretoria, Prinshof Campus, Pretoria 0084, South Africa
* Correspondence: phumudzo.mamphwe@up.ac.za or phumumamphwe@gmail.com; Tel.: +27-12-356-3205

Abstract: Appropriate feeding practices are protective against malnutrition and poor growth. We compared feeding practices and growth in HIV-exposed-uninfected (HEU) and HIV-unexposed-uninfected (HUU) between 6-12 months of age in urbanized African infants in South Africa. A repeated cross-sectional analysis was used to determine differences in infant feeding practices and anthropometric measures by HIV exposure status at 6, 9, and 12 months in the Siyakhula study. The study included 181 infants (86 HEU; 95 HUU). Breastfeeding rates were lower in HEU vs. HUU infants at 9 (35.6% vs. 57.3%; $p = 0.013$) and 12 months (24.7% vs. 48.0%; $p = 0.005$). Introduction to early complementary foods was common (HEU = 16.2 ± 11.0 vs. HUU = 12.8 ± 9.3 weeks; $p = 0.118$). Lower weight-for-age Z-scores (WAZ) and head circumference-for-age Z-scores (HCZ) were found in HEU infants at birth. At 6 months, WAZ, length-for-age Z-scores (LAZ), HCZ, and mid-upper-arm circumference-for-age Z-scores (MUACAZ) were lower in HEU vs. HUU infants. At 9 months, lower WAZ, LAZ, and MUACAZ were found in HEU vs. HUU infants. At 12 months, lower WAZ, MUACAZ, and weight-for-length Z-scores (−0.2 ± 1.2 vs. 0.2 ± 1.2; $p = 0.020$) were observed. HEU infants had lower rates of breastfeeding and poorer growth compared to HUU infants. Maternal HIV exposure affects the feeding practices and growth of infants.

Keywords: HIV exposure; infants; anthropometry; growth; feeding practices; breastfeeding; nutrition

check for updates

Citation: Tshiambara, P.; Hoffman, M.; Legodi, H.; Botha, T.; Mulol, H.; Pisa, P.; Feucht, U. Comparison of Feeding Practices and Growth of Urbanized African Infants Aged 6–12 Months Old by Maternal HIV Status in Gauteng Province, South Africa. *Nutrients* **2023**, *15*, 1500. https://doi.org/10.3390/nu15061500

Academic Editor: Kingsley E. Agho

Received: 15 February 2023
Revised: 15 March 2023
Accepted: 19 March 2023
Published: 21 March 2023

1. Introduction

Globally 38.4 million people were living with HIV, and 28.7 million were accessing antiretroviral therapy (ART) in the year 2022 [1]. In the high-prevalence country of South Africa, 8.2 million people were living with HIV, including 23.9% of all women of reproductive age in 2021 [2]. The country introduced public health HIV programs, including the prevention of mother-to-child transmission (PMTCT) program in 2002 and the ART program in 2004, to reduce mortality and prevent viral transmission [3,4]. The success of these programs has led to large numbers of infants being born with in utero exposure to maternal HIV infection while remaining HIV-exposed-uninfected (HEU) [5,6]. In Sub-Saharan Africa (SSA) alone, there are more than one million annual births of HEU children [7].

Sub-optimal infant feeding practices negatively impact childhood growth. Therefore the Infant and Young Child Feeding (IYCF) policy emphasizes breastfeeding as a cornerstone for health and survival, including in the context of HIV [8]. This is also in line with the World Health Organization (WHO) recommendations for breastfeeding for at least the first 24 months of life [9], preventing malnutrition, including stunting, underweight, and wasting [10–12]. Many women living with HIV still fear that breastfeeding may lead to vertical HIV transmission [13,14], even though this risk is significantly lowered within the context of ART provision [3,15].

To ensure optimal growth, safe and nutritious food should be introduced to infants after the age of 6 months, in addition to continued breastfeeding [16]. However, early introduction of complementary feeding is a very common practice in South Africa (45–87.5%) [17–22], which is an important public health concern [23], with stunting (30.5%), wasting (30.3%) and underweight (33.2%) reported already in 4–5-month-old infants [24].

Infants who are HEU are known to be at greater risk of adverse birth outcomes, morbidity, and infections, affecting their growth and development [25,26]. These sub-optimal growth outcomes include being underweight, stunting, or even wasting [10,27]. In addition, HEU infants are also smaller at birth in terms of the mean weight and length [28,29], which may contribute to their poor growth observed as early as three months of age, as documented in multiple African countries (Zambia: lower mean weight 2.9 kg vs. 3.0 kg between 1–16 weeks, lower length in HEU infants at 6 months (64.3 cm ± 1.1) [30]; Zimbabwe: the likelihood of stunting (25%), underweight (55%), and wasting (58%) high in HEU infants [31]; Ethiopia: risk of stunting of 51.9 per 100 person-years infants HIV exposed from conception [32]; and South Africa:10% HEU stunted [33]). Limited research is available on HEU infants that focuses on the complementary feeding introduction phase, especially in terms of having an appropriate HIV-unexposed-uninfected (HUU) comparison group [11,28,34–37]. Therefore, this study aimed to compare the feeding practices and growth of HEU and HUU infants between 6–12 months of age in Tshwane District, Gauteng Province, South Africa.

2. Materials and Methods

2.1. Study Design and Setting

This study is a sub-study of the longitudinal Siyakhula cohort study [37], which aims to better understand how the in-utero and early postnatal environments, altered by maternal HIV infection and the treatment thereof, influence infants' growth trajectories and cognitive development and alter their immune development and function, irrespective of the infants' own HIV status. For this study, mother-infant dyads who attended the 6 (n = 181), 9 (n = 166), and 12 (n = 155) month follow-up visits were included, with declining numbers due to loss to follow-up and relocation.

2.2. Data Collection

All study-related information was collected at the central study site at Kalafong Provincial Tertiary Hospital, Gauteng Province, South Africa [37]. After obtaining informed consent, questionnaires were administered in the participants' preferred local languages by trained fieldworkers. Socio-demographic information was collected using a structured questionnaire and included maternal age, marital status, level of education, employment status, as well as the infants' age, sex, and HIV exposure status (with all women living with HIV self-reporting use of ART during and after pregnancy, with the first-line regimen at the time of study being a once-daily fixed-dose combination of tenofovir, emtricitabine, and efavirenz).

Infant growth was assessed by documenting weight (calibrated digital scale; Seca 354, Seca, Hamburg, Germany), length (mechanical infantometer; Seca 416, Seca, Hamburg, Germany), head circumference, and mid-upper-arm-circumference (MUAC) (non-stretchable tape measure; KDS measure, model F10-02DM 2m, Kyoto, Japan), wearing minimal cloth-

ing. These measurements were available for the time of birth (except MUAC) for baseline purposes and then at 6, 9 and 12 months as part of study-related procedures. Z-score indices, including weight-for-age (WAZ), length-for-age (LAZ), weight-for-length (WLZ), HC-for-age (HCZ) & MUAC-for-age (MUACZ), were computed using the Intergrowth-21st and WHO Anthro child growth standards v3.2.2 according to sex, with correction for gestational age [38,39]. Nutritional classifications of underweight, stunting and wasting were defined as Z-scores below −2 standard deviations (SD) for WAZ, LAZ, and WLZ, respectively, and for overweight WLZ above +2 SD of the median values of the reference data [40].

Infant feeding information, including breastfeeding and complementary feeding practices, was collected using maternal recall following the WHO global feeding practices indicators [38]. Collected information included duration and type of feeding, age of introduction of complementary feedings, and type of food [38]. The history of the usual food consumption was also collected using the unquantified food frequency questionnaire (FFQ) previously used in similar settings [18,19,41,42], where mothers were asked about the usual infant food consumption during the past seven days.

2.3. Data Processing and Statistical Analysis

The Research Electronic Data Capture v8.3.5 was used to capture the data [43]. Z-scores were computed using the Intergrowth-21st and the WHO Anthro [38,39], and values <−3 and >+3 were excluded from analysis due to implausibility from clinical settings. Descriptive statistics were used to present the socio-demographic information, anthropometric measurements, feeding practices and infant HIV exposure. All continuous data were presented as means and standard deviations, with the categorical data represented as frequencies and percentages. The normality of the data was assessed using the Shapiro–Wilk test. Comparisons between HEU and HUU groups were performed using the independent *t*-test (or its non-parametric equivalent Mann–Whitney U test) for continuous variables or the Pearson Chi-squared test for categorical variables. All statistical analyses were performed using R version 4.1.2 program [44] and performed at a 5% level of significance.

2.4. Ethical Consideration

The Faculty of Health Sciences Research Ethics Committee (Ref. no.: 294/2017) at the University of Pretoria approved the Siyakhula study. All relevant information was shared with the mothers prior to data collection. Mothers gave consent for themselves and their infants for each study visit, and the Declaration of Helsinki guidelines were followed. This sub-study was approved by the Faculty of Natural and Agricultural Sciences and the Faculty of Health Sciences Research Ethics Committee and the (Ref. no.: NAS063/2020) at the same university.

3. Results

3.1. Description of the Study Population

A total of 181 mother-infant dyads (86 HEU; 95 HUU) were included in this study. The maternal socio-demographic characteristics are presented in Table 1. Mothers living with and without HIV were similar in terms of employment status, social grants, and access to electricity, while significant differences were found in terms of the mean maternal age (36.9 years ± 8.6 vs. 31.3 ± 6.3 years; $p < 0.001$) and education level ($p < 0.001$). There were no significant differences in the maternal socio-demographic characteristics when comparing the mothers in the overall Siyakhula study and this sub-study).

Table 1. Socio-demographic characteristics of the study mothers according to HIV status.

		Study Population	Mothers Living with HIV	Mothers Not Living with HIV	*p*-Value
		(*n* = 181)	(*n* = 86)	(*n* = 95)	
Age (years) mean ± SD [1]		33.9 ± 7.9	36.9 ± 8.6	31.3 ± 6.3	*<0.001*
Education *n* (%) [2]	Formal education, but without school completion [3]	86 (48.6)	55 (66.3)	31 (33.0)	*<0.001*
	Completed secondary schooling	58 (32.8)	19 (22.9)	39 (41.5)	
	Tertiary education	33 (18.6)	9 (10.8)	24 (25.5)	
Employment *n* (%) [2]	Yes	84 (47.5)	41 (49.4)	43 (45.7)	0.738
Child support grant *n* (%) [2]	Yes	136 (76.8)	62 (74.7)	74 (78.7)	0.649
Marital status *n* (%) [2]	Single	134 (75.7)	60 (72.3)	74 (78.7)	0.412
	Married	43 (24.3)	23 (27.7)	20 (21.3)	
Access to water *n* (%) [2]	Communal tap	40 (22.6)	21 (25.3)	19 (20.2)	0.534
	Inside yard	88 (49.7)	42 (50.6)	46 (48.9)	
	Inside house	49 (27.7)	20 (24.1)	29 (30.9)	
Access to electricity *n* (%) [2]	Yes	165 (93.2)	76 (91.6)	89 (94.7)	0.601
Access to toilet *n* (%) [2]	None [4]	2 (1.1)	2 (2.4)	0 (0)	0.816
	Pit latrine	60 (33.9)	29 (34.9)	31 (33.0)	
	Flush toilet	115 (65.0)	52 (62.7)	63 (67.0)	

Values in *italic* font indicate significant *p*-values ($p < 0.05$); [1] non-normal distributed data; [2] excludes missing numbers; [3] formal education = includes any primary and secondary schooling; [4] none: not considered in the calculation. Mann–Whitney U test was used for continuous non-normally distributed data; Pearson's Chi-square test was used for categorical data to determine the differences in mothers living with HIV and mothers not living with HIV.

3.2. Birth Characteristics of the Infants

The birth characteristics of the HEU vs. HUU infants are presented in Table 2. More HEU vs. HUU infants were males, but the difference was not statistically significant. Significant differences were found in the mean birth weight (2.84 ± 0.49 kg vs. 3.06 ± 0.51 kg; $p = 0.005$); WAZ (−0.7 ± 0.9 vs. −0.2 ± 1.0; $p = 0.003$); head circumference (33.8 ± 1.8 cm vs. 34.5 ± 1.6 cm; $p = 0.013$) and HCZ (0.3 ± 1.3 vs. 0.7 ± 1.2; $p = 0.038$) of HEU vs. HUU infants. No significant differences were found between HEU vs. HUU infants in terms of the mean length (49.1 ± 4.1 cm vs. 49.9 ± 3.4 cm; $p = 0.184$) and LAZ (0.6 ± 1.4 vs. 0.7 ± 1.5; $p = 0.804$) at birth. In addition, no significant differences were found in the birth characteristics of the HEU vs. HUU infants when comparing the mothers in the overall Siyakhula study and this sub-study.

3.3. Feeding Practices

Breastfeeding and complementary feeding of HEU vs. HUU infants are further presented in Table 3. No significant differences were found in the early initiation of breastfeeding between HEU and HUU infants who were breastfed within one hour or after one hour after delivery ($p = 0.297$), based on maternal recall at the time of the study visit. The mean age of breastfeeding cessation was similar in both HEU vs. HUU infants (18.9 ± 15.8 vs. 18.1 ± 15.8 weeks; $p = 0.778$) before 6 months, and the mean age of formula milk introduction (18.4 ± 15.9 vs. 17.4 ± 15.4 weeks; $p = 0.770$). Early introduction of complementary foods before 6 months was common in both groups (16.2 ± 11.0 vs. 12.8 ± 9.3; $p = 0.118$).

Table 2. Gestational age, sex, and anthropometric measurements and indices of HIV-exposed-uninfected and HIV-unexposed-uninfected infants at birth.

		Study Population	HEU Infants	HUU Infants	*p*-Value
		(*n* = 181)	(*n* = 86)	(*n* = 95)	
Gestational age (weeks) [1,2]		38.2 ± 1.7	38.2 ± 1.5	38.3 ± 1.8	0.293
Infant sex *n* (%)	Male	105 (58.0)	54 (62.8)	51 (53.7)	0.276
Birth body measurements [1]	Weight (kg) [3]	2.95 ± 0.51	2.84 ± 0.49	3.06 ± 0.51	*0.005*
	Length (cm) [2]	49.5 ± 3.8	49.1 ± 4.1	49.9 ± 3.4	0.184
	Head circumference (cm) [2]	34.1 ± 1.7	33.8 ± 1.8	34.5 ± 1.6	*0.013*
Birth Z-scores indices [1,3,4]	Weight-for-age	−0.5 ± 1.0	−0.7 ± 0.9	−0.2 ± 1.0	*0.003*
	Length-for-age	0.6 ± 1.5	0.6 ± 1.4	0.7 ± 1.5	0.804
	Head circumference-for-age	0.5 ± 1.3	0.3 ± 1.3	0.7 ± 1.2	*0.038*

Values in *italic* font indicate significant *p*-values ($p < 0.05$). Abbreviations: HEU: HIV-exposed-uninfected (born to mothers living with HIV); HUU: HIV-unexposed-uninfected (born to mothers not living with HIV); [1] data presented as mean ± SD; [2] non-normal distributed data; [3] normal distributed data; [4] the birth Z-scores indices sex-normalized were computed using INTERGROWTH-21st software, using gestation-adjusted age for preterm infants. Independent t-test was used for continuous normally distributed data, and the Mann–Whitney U test was used for continuous non-normally distributed data; Pearson's Chi-square test was used for categorical data to determine the differences in HEU and HUU infants.

Table 3. Feeding practices of HEU and HUU infants before 6 months based on maternal recall.

		HEU Infants	HUU Infants	*p*-Value
		n = 86	*n* = 95	
Initiation of breastfeeding *n* (%) [1,2]	<1 h after birth	40 (46.5)	55 (57.9)	0.297
	>1 h after birth	39 (45.3)	33 (34.7)	
	Never breastfed	7 (8.2)	7 (7.4)	
Baby received liquids/foods other than breastmilk/formula milk before age 6 months *n* (%) [1,2]	Yes	60 (69.8)	76 (80.9)	0.120
Breastfeeding cessation age (weeks) [1,2,3]		18.9 ± 15.8	18.1 ± 15.8	0.778
Formula milk introduction mean age (weeks) [1,2,3]		18.4 ± 15.9	17.4 ± 15.4	0.770
Type of formula milk *n* (%) [1,2]	Commercial cow's milk-based formula	55 (93.2)	49 (84.5)	n/a
	Others [4]	4 (6.8)	9 (15.5)	
Main reason for introducing formula milk *n* (%) [1,2]	Return to work	17 (31.5)	29 (43.3)	0.281
	Insufficient milk/baby not growing	15 (27.8)	17 (25.4)	
	Convenience	6 (11.1)	10 (14.9)	
	Baby/mother unwell	16 (29.6)	11 (16.4)	
Complementary feeding introduction (weeks) [1,2,3]		16.2 ± 11.0	12.8 ± 9.3	0.118
First liquid introduced *n* (%) [1,2]	Water	71 (91.0)	85 (92.4)	0.966
	Others [5]	7 (9.0)	7 (7.6)	
First solid food introduced *n* (%) [1,2]	Mabelle/maize meal soft porridge	72 (83.7)	72 (75.8)	0.256
	Others [6]	14 (16.3)	23 (24.2)	

Abbreviations: HEU: HIV-exposed-uninfected; HUU: HIV-unexposed-uninfected; n/a: not applicable (no comparisons were performed due to one of the groups having less than five count leading to volatile results. [1] result based on maternal recall and *n* numbers vary as mothers with missing information were excluded; [2] data presented as mean ± SD; [3] non-normal distributed data; [4] others include lactose-free cow's milk and soy-based formula; [5] others include tea and juice; [6] others include baby cereal and instant porridge; Mann–Whitney U test used for continuous non-normally distributed data; Pearson's Chi-square test used for categorical data to determine the differences in HEU and HUU infants. Significant *p*-values were defined as $p < 0.05$.

The breastfeeding practices amongst HEU and HUU infants at 6, 9, and 12 months are shown in Figure 1. Similar percentages of EBF at birth and breastfeeding at 6 months were observed in the HEU and HUU infants, but significant differences were found at 9 (35.6% vs. 57.3%; $p = 0.013$) and 12 months (24.7% vs. 48.0%; $p = 0.005$).

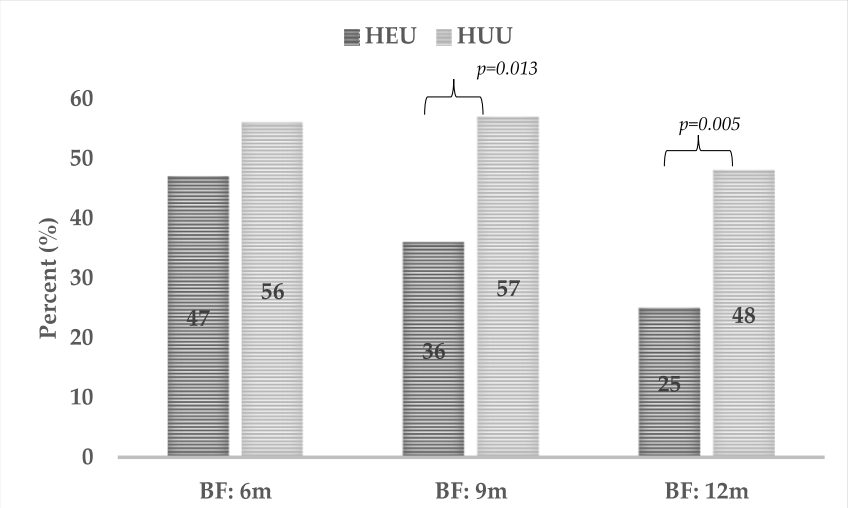


Figure 1. Infant breastfeeding practices by HIV exposure status in the first 12 months of life. Values in *italic* font indicate significant *p*-values ($p < 0.05$). Abbreviations: HEU: HIV-exposed-uninfected; HUU: HIV-unexposed-uninfected. The mixed feeding category was separately calculated, and no comparison tests were performed due to one group having <5 count, which led to volatile results. Pearson's Chi-square test was used for categorical data to determine the differences in HEU and HUU infants.

The foods frequently consumed by infants at 6 and 12 months are presented in Table 4. At 6 months, maizemeal soft porridge (11.4% vs. 10.1%), infant cereal (52.9% vs. 56.2%), and baby food in a jar (34.3% vs. 29.2%) were consumed at least four days per week by HEU vs. HUU infants. At 12 months, carbonated/fizzy drinks (9.3% vs. 14.3%) and sweets/chocolates (22.7% vs. 11.7%) were consumed at least four days per week by HEU vs. HUU infants.

3.4. Growth of HEU vs. HUU Infants

The anthropometric measurements, Z-score indices, and nutritional classification of the infants at 6, 9, and 12 months of age by HIV exposure status are presented in Table 5. HEU infants had a significantly lower mean weight compared to HUU infants at 6 months (7.3 ± 0.9 kg vs. 7.8 ± 1.0 kg; $p = 0.001$), and at 9 months (8.3 ± 1.0 vs. 8.8 ± 1.1 kg; $p = 0.002$), also at 12 months the mean weight was lower although this did not reach statistical significance (9.1 ± 1.2 kg vs. 9.4 ± 1.3 kg; $p = 0.106$). The mean WAZ was significantly lower in HEU as compared to HUU infants at 6 (-0.6 ± 1.1 vs. 0.1 ± 1.2; $p < 0.001$), 9 (-0.4 ± 1.1 vs. 0.1 ± 1.1; $p = 0.003$) and 12 months (-0.3 ± 1.1 vs. 0.1 ± 1.2; $p = 0.022$). No significant difference was found in the underweight classification of infants at 6, 9, and 12 months.

Table 4. Usual intake of food items by 6 and 12 months old HEU compared to HUU infants determined by quantified food frequency questionnaire (%).

Foods	Consumption at Age 6 Months (%) [1]						Consumption at Age 12 Months (%) [2]					
	Most Days [3]		Once a Week		Never		Most Days [3]		Once a Week		Never	
	HEU Infants	HUU Infants	HEU Infants	HUU Infants	HEU Infants	HUU Infants	HEU Infants	HUU Infants	HEU Infants	HUU Infants	HEU Infants	HUU Infants
Starches												
Maizemeal porridge—soft	11.4	10.1	7.1	3.4	81.4	86.5	49.3	42.9	6.7	15.6	44.0	41.6
Infant cereal	52.9	56.2	2.9	6.7	44.3	37.1	38.7	46.8	9.3	6.5	52.0	46.8
Instant porridge	10.0	11.2	2.9	0	87.1	88.8	28.0	16.9	14.7	13.0	57.3	70.1
Bread	4.3	0	1.4	4.5	94.3	95.5	56.0	54.5	28.0	27.3	16.0	18.2
Rice	1.4	0	2.9	1.1	95.7	98.9	17.3	18.2	42.7	37.7	40.0	44.2
Potato	15.7	11.2	15.7	13.5	68.6	75.3	54.7	42.9	36.0	44.2	9.3	13.0
Dairy products												
Fresh, fermented or powder milk	0	0	0	2.2	100	97.8	13.3	19.5	22.7	15.6	64.0	64.9
Yoghurt/dairy snack for baby	7.1	1.1	4.3	7.9	88.6	91.0	33.3	31.2	26.7	39.0	40.0	29.9
Animal foods/meat products												
Red meat	0	0	2.9	0	97.1	100	13.3	10.4	28.0	32.5	58.7	57.1
Liver	0	0	2.9	3.4	97.1	96.6	10.7	11.7	33.3	37.7	56.0	50.6
Chicken	0	5.6	4.3	3.4	95.7	91.0	36.0	40.3	32.0	27.3	32.0	32.5
Fish	1.4	0	2.9	2.2	95.7	97.8	12.0	11.7	34.7	32.5	53.3	55.8
Eggs	4.3	3.4	2.9	6.7	92.9	89.9	38.7	27.3	33.3	44.2	28.0	28.6
Vegetables												
Any vegetables [4]	9.0	8.7	14.5	10.1	76.8	80.9	48.0	51.9	40.0	31.2	12	16.9
• Orange [5]	87.5	88.2	n/a	n/a	n/a	n/a	70.7	68.8	n/a	n/a	n/a	n/a
• Dark-green leafy [6]	6.3	11.8	n/a	n/a	n/a	n/a	17.3	12.9	n/a	n/a	n/a	n/a
• Red/yellow [7]	6.2	0	n/a	n/a	n/a	n/a	0	0	n/a	n/a	n/a	n/a
Fruits												
Any fruits [8]	11.4	6.8	11.4	9.1	77.1	84.1	64.0	59.17	24.0	31.2	12.0	9.1
• Orange [9]	28.6	18.8	n/a	n/a	n/a	n/a	58.7	63.8	n/a	n/a	n/a	n/a
• Green [10]	28.6	12.5	n/a	n/a	n/a	n/a	29.3	36.2	n/a	n/a	n/a	n/a
• Red/yellow [11]	42.8	68.7	n/a	n/a	n/a	n/a	0	0	n/a	n/a	n/a	n/a

Table 4. *Cont.*

Foods	Consumption at Age 6 Months (%) [1]						Consumption at Age 12 Months (%) [2]					
	Most Days [3]		Once a Week		Never		Most Days [3]		Once a Week		Never	
	HEU Infants	HUU Infants	HEU Infants	HUU Infants	HEU Infants	HUU Infants	HEU Infants	HUU Infants	HEU Infants	HUU Infants	HEU Infants	HUU Infants
Food items added to porridge												
Salt	5.7	4.5	2.9	3.4	91.4	92.1	62.7	54.5	9.3	14.3	28.0	31.2
Oil	7.1	5.6	5.7	5.6	87.1	88.8	50.7	37.7	20.0	22.1	29.3	40.3
Margarine	8.6	7.9	10.0	6.7	81.4	85.4	42.7	29.9	26.7	28.6	30.7	41.6
Peanut butter	4.3	3.4	2.9	6.7	92.9	89.9	29.3	35.1	20.0	16.9	50.7	48.1
Miscellaneous												
Sweets/chocolates	1.4	0	7.1	5.6	91.4	94.4	22.7	11.7	29.3	29.9	48.0	58.4
Kids tea	4.3	2.2	4.3	3.4	91.4	94.4	46.7	35.1	18.7	15.6	34.7	49.4
Black/English tea	0	0	0	0	100	100	10.7	6.5	4.0	7.8	85.3	85.7
Chips	1.4	2.2	12.9	4.5	85.7	93.3	50.7	46.8	34.7	32.5	14.7	20.8
Carbonated/fizzy drinks	1.4	0	2.9	4.5	95.7	95.5	9.3	14.3	32.0	18.2	58.7	67.5
Fruit juice	4.3	5.7	7.2	8.0	88.5	86.3	21.3	36.4	34.7	28.6	44.0	35.1
Baby food in a jar/pureed	34.3	29.2	11.4	14.6	54.3	56.2	45.3	48.1	33.3	22.1	21.3	29.9
Juice concentrate	1.4	1.1	2.9	3.4	95.7	95.5	16.0	6.5	18.7	24.7	65.3	68.8

Abbreviations: HEU: HIV-exposed-uninfected; HUU: HIV-unexposed-uninfected; no comparisons were performed due to one of the groups having less than five counts leading to volatile results. [1] 6-months: HEU = 70, HUU = 89; [2] 12 months: HEU = 75, HUU = 77; [3] %: the categories 'every day' and 'most days' are grouped together; n/a: not applicable; [4] excludes infants who did not consume any vegetables; [5] orange colored vegetables (carrots, butternut, pumpkin, sweet potato); [6] dark-green-leafy colored vegetables (spinach, butternut leaves, cabbage); [7] red/yellow colored vegetables (tomatoes, beetroot, corn); [8] excludes infants who did not consume any fruits; [9] orange colored fruits (mangoes, peaches, oranges, mandarins); [10] green colored fruits (apples, grapes, avocado, pear) and [11] red/yellow colored fruits (banana, pineapple, watermelon, strawberry).

Table 5. Anthropometric measurements, Z-score indices, and nutritional classifications of the infants between 6–12 months of life by HIV exposure status.

	Age 6 Months			Age 9 Months			Age 12 Months		
	HEU Infants	HUU Infants	*p*-Value	HEU Infants	HUU Infants	*p*-Value	HEU Infants	HUU Infants	*p*-Value
	(*n* = 86)	(*n* = 95)		(*n* = 80)	(*n* = 86)		(*n* = 75)	(*n* = 80)	
Anthropometric measurements									
Weight (kg) [1]	7.3 ± 0.9 [2]	7.8 ± 1.0 [2]	*0.001*	8.3 ± 1.0 [3]	8.8 ± 1.1 [3]	*0.002*	9.1 ± 1.2 [2]	9.4 ± 1.3 [2]	0.106
Length (cm) [1]	65.3 ± 3.5 [2]	66.6 ± 2.8 [2]	*0.014*	70.1 ± 3.1 [3]	71.2 ± 2.8 [3]	*0.012*	74.4 ± 3.1 [3]	74.5 ± 2.7 [3]	0.704
Head circumference (cm) [1]	43.5 ± 1.6 [2]	43.9 ± 1.6 [2]	0.106	45.3 ± 1.4 [3]	45.4 ± 1.7 [3]	0.621	46.5 ± 1.6 [3]	46.6 ± 1.6 [3]	0.655
Mid-upper-arm-circumference (cm) [1]	14.6 ± 1.3 [2]	15.2 ± 1.1 [2]	*0.002*	15.2 ± 1.2 [2]	15.7 ± 1.5 [2]	*0.026*	15.6 ± 1.2 [2]	16.0 ± 1.3 [2]	0.075

Table 5. *Cont.*

	Age 6 Months			Age 9 Months			Age 12 Months		
	HEU Infants	**HUU Infants**	***p*-Value**	**HEU Infants**	**HUU Infants**	***p*-Value**	**HEU Infants**	**HUU Infants**	***p*-Value**
	(*n* = 86)	**(*n* = 95)**		**(*n* = 80)**	**(*n* = 86)**		**(*n* = 75)**	**(*n* = 80)**	
Z-score indices									
Weight-for-age Z-score [1,4]	−0.6 ± 1.1 [3]	0.1 ± 1.2 [3]	*<0.001*	−0.4 ± 1.1 [3]	0.1 ± 1.1 [3]	*0.003*	−0.3 ± 1.1 [2]	0.1 ± 1.2 [2]	*0.022*
Length-for-age Z-score [1,4]	−0.8 ± 1.4 [3]	−0.1 ± 1.2 [3]	*<0.001*	−0.5 ± 1.4 [3]	0.0 ± 1.3 [3]	*0.023*	−0.4 ± 1.3 [3]	−0.2 ± 1.1 [3]	0.308
Weight-for-length Z-score [1,4]	−0.1 ± 1.2 [3]	0.2 ± 1.1 [3]	0.074	−0.1 ± 1.2 [3]	0.2 ± 1.1 [3]	0.098	−0.2 ± 1.2 [3]	0.2 ± 1.2 [3]	*0.020*
Head circumference-for-age Z-score [1,4]	0.5 ± 1.2 [3]	0.9 ± 1.2 [3]	*0.019*	0.6 ± 1.2 [3]	0.8 ± 1.0 [3]	0.331	0.6 ± 1.2 [3]	0.9 ± 1.1 [3]	0.069
Mid-upper-arm-circumference-for-age Z-score [1,4]	0.5 ± 1.1 [3]	1.0 ± 0.9 [3]	*<0.001*	0.7 ± 1.0 [3]	1.1 ± 1.1 [3]	*0.013*	0.8 ± 1.1 [3]	1.3 ± 1.1 [3]	*0.025*
Nutritional classifications									
Underweight *n* (%) [5]	7 (8.9)	3 (3.4)	n/a	7 (8.8)	2 (2.4)	n/a	4 (5.5)	4 (5.1)	n/a
Stunted *n* (%) [6]	12 (15.0)	4 (4.6)	n/a	10 (12.5)	6 (7.1)	0.297	9 (12.3)	3 (3.8)	n/a
Wasted *n* (%) [7]	3 (3.7)	2 (2.3)	n/a	5 (6.2)	3 (3.6)	n/a	4 (5.5)	3 (3.9)	n/a
Overweight *n* (%) [8]	4 (4.9)	6 (6.8)	n/a	4 (5.0)	3 (3.6)	n/a	4 (5.5)	6 (7.8)	n/a
Acute malnutrition *n* (%) [9]	1 (1.3)	0 (0)	n/a	1 (1.2)	0 (0)	n/a	1 (1.4)	0 (0)	n/a
Macrocephalus *n* (%) [10]	8 (10.0)	17 (19.3)	0.140	10 (12.5)	10 (12.2)	>0.999	10 (13.7)	13 (16.9)	0.753

Values in *italics* font indicate significant *p*-values. Abbreviations: HEU: HIV-exposed-uninfected; HUU: HIV-unexposed-uninfected; n/a: not applicable (no comparisons were performed due to one of the groups having less than five counts leading to volatile results. [1] data presented as mean ± SD; [2] non-normal distributed data; [3] normal distributed data; [4] sex-normalized Z-scores indices at age 6–12 months were computed using World Health Organization Anthro software of 2010, using gestation-adjusted age for preterm infants; [5] underweight from weight-for-age Z-scores < −2; [6] stunted from length-for-age Z-scores < −2; [7] wasted from weight-for-length Z-scores (WLZ) < −2; [8] overweight from WLZ > +2; [9] acute-malnutrition from mid-upper-arm-circumference Z-scores < −2; [10] macrocephalus from head circumference-for-age Z-scores > +2. Independent t-test was used for continuous normally distributed data, and the Mann–Whitney U test was used for continuous non-normally distributed data; Pearson's Chi-square test was used for categorical determine the differences in HEU and HUU infants ($p < 0.05$).

The mean length was significantly lower in HEU vs. HUU infants at 6 months (65.3 ± 3.5 cm vs. 66.6 ± 2.8 cm; $p = 0.014$) and 9 months (70.1 ± 3.1 cm vs. 71.2 ± 2.8 cm; $p = 0.012$). The mean LAZ was also significantly lower in HEU vs. HUU infants at 6 months (−0.8 ± 1.4 vs. −0.1 ± 1.2; $p < 0.001$) and 9 months (−0.5 ± 1.4 vs. 0.0 ± 1.3; $p = 0.023$). Furthermore, HEU infants were at a higher risk of being stunted as compared to HUU infants at age 6 months (15.0% vs. 4.6%), although we could not perform significance tests due to low counts in the HUU group. The mean WLZ was significantly lower in HEU infants as compared to HUU infants at 12 months (−0.2 ± 1.2 vs. 0.2 ± 1.2; $p = 0.020$).

4. Discussion

Our study showed significant differences in terms of breastfeeding practices and growth between HEU and HUU infants in the second half of the first year of life, with inappropriate infant feeding practices identified. Appropriate feeding practices especially continued breastfeeding, is important from 6 months when a transition occurs from EBF to continued breastfeeding with complementary feeding, making this a critical time for growth monitoring and promotion. Furthermore, breastmilk is the best source of nutrition for infants [3], with inappropriate feeding practices potentially resulting in malnutrition, leading to well-documented increased morbidity and mortality risk [45].

Early cessation of breastfeeding was found in our study, especially in the HEU infants. Lack of knowledge and mothers' education level is possible contributory reasons for too early cessation of breastfeeding, as reported in another South African study [46]. Our study found the early introduction of complementary foods before 6 months in both groups, with HUU infants given complementary foods at an earlier age than HEU infants, although this difference was not significant. This was similar to another South African study which found the introduction of solids in HEU infants as early as 6 weeks of age [47], while an Ethiopian study found that 58% of HEU infants were not introduced to complementary feeding at the recommended age of 6 months [48].

Almost half (47%) of HEU infants were breastfed at 6 months, signifying the progress of the ART program in terms of breastfeeding promotion [47]. Breastfeeding, however, decreased over time in our study, with mothers stopping breastfeeding their infants at a mean age of 18 weeks, with reasons including the need to return to work (31.4% vs. 43.3%) and insufficient milk or not growing (27.7% vs. 25.4%) in HEU vs. HUU infants. Poor breastfeeding rates in our study may result from cultural practices, lack of knowledge, and previous provision of formula milk through PMTCT programs [49].

Lower breastfeeding rates were found in HEU vs. HUU infants at 9 months, lower than in Kenya [50] but higher than in the South African study in which no HEU infants were breastfed in the year 2009 [49]. Lack of knowledge and HIV stigma need to be addressed to increase the breastfeeding rates in the ART context [51]. The IYCF policy needs to be used to promote, protect and support breastfeeding, even in the context of HIV [52].

The unquantified food frequency questionnaire is simple and quick to administer and can be used to determine the foods frequently consumed [18,53], which may affect the growth of infants. Our results show that HEU (34.3%) and HUU (29.2%) infants were mostly consuming baby food in a jar/pureed at 6 months of age which also increased at 12 months to 45.3% vs. 48.1%, which is similar to other South African data [17,18]. Baby food purée in a jar has been reported to contain insufficient nutrients, such as iron and zinc, which are important for good growth and development [54]. HEU infants consumed more miscellaneous junk food products, such as chips (50.7% vs. 46.8%) and juice concentrate diluted with water (16.0% vs. 6.5%) at least 4 days per week at 12 months, similar to other South African studies [18,47]. The IYCF policy is, thus, very important in emphasizing the correct and timely introduction of complementary foods, which may improve the growth of HEU infants [9].

Our study found poor growth in HEU infants for the first 12 months of their life; similar findings have been reported in HEU infants who experience slower growth than HUU infants over the first 12 months of life resulting from a high risk of opportunistic infections,

such as pneumonia and lower respiratory tract infections [5,29,50,55]. The mechanism of higher morbidity in these infants has been suggested to be associated with multifactorial reasons, such as maternal socioeconomic factors, abnormal immunological factors, and maternal nutrition, which have been identified as possible causes of the increased morbidity in HEU infants [56,57]. Furthermore, the maternal use of ART affects the growth of HEU infants [31].

In our study, a lower mean LAZ was found in HEU infants at 6 months, which is similar to another South African study [58], and a lower mean HCZ was also reported in Zimbabwe at 6 months [59]. A lower mean LAZ was also observed at 9 months in HEU infants as compared to HUU infants, similar to other studies in Uganda [60], Rwanda [61], and Botswana [10]. HEU infants were at a higher risk of being stunted (12.5% vs. 7.1%) at 9 months as compared to HUU infants. A higher risk of stunting (20% vs. 10%; $p < 0.01$) was also found in Kenya at 9 months [50]. Stunting has irreversible consequences, yet it is important to identify it at an early age [9,62].

Our study differs from a study conducted in Botswana which found significant differences in HEU vs. HUU infants in terms of the underweight and stunting status at 6–24 months [10]. However, the study only included 37.2% of HEU infants. We found significantly lower mean WLZ in HEU infants as compared to HUU infants at 12 months. Similar findings were reported in another South African study at the age of 12 months [33]. A higher risk of being overweight in infancy might result in higher rates of obesity later in life, which increases the risk of non-communicable diseases [62]. This study found that maternal HIV exposure affects the feeding practices and growth of infants. Breastfeeding practices decreased with age.

The strength of our study lies in detailed infant feeding practices and anthropometric measurements collected by trained field workers to ensure quality control and validity. Further strengths include the repeated anthropometric measurements in infants at 6, 9, and 12 months, a control group (HUU infants), and a similar sample size in the HEU and HUU groups. Limitations of this study include uneven numbers across the time points due to loss of follow-up (some mothers moved away from the study site due to the SA national lockdown during the COVID-19 pandemic, amongst other reasons) and limited sample size. A further limitation is a potential recall bias as the unquantified FFQ asks for food consumed in the past seven days, and no associations were investigated due to the small number of counts in the different consumption frequencies in both the HEU and HUU groups. In addition, our results may only be partially generalizable, taking into account the sociodemographic and geographical context of this study. In addition, the results need to be interpreted with care as adjustment for confounders, such as maternal age, employment status, and education level, which could not be performed due to the low sample size.

5. Conclusions

In this study, we compared the feeding practices and growth of infants aged 6 and 12 months exposed and unexposed to maternal HIV infection. HEU infants had lower rates of breastfeeding at 9 and 12 months, and the breastfeeding rates decreased with age. HEU infants had lower LAZ, WAZ, and MUACZ at 6, 9, and 12 months and higher stunting rates compared to the HUU infants. Current findings will inform nutrition policy interventions aimed at educating mothers and caregivers about appropriate complementary feeding in order to promote optimal growth, even in the context of HIV. For future studies, a bigger sample size will be more beneficial, as well as using structured questionnaires to determine nutrient intake from 6 months of age in HEU children.

Author Contributions: Conceptualization, P.T., M.H. and U.F.; methodology, P.T., M.H. and U.F.; formal analysis, T.B. and P.T.; data curation, P.T. and H.M.; writing—original draft preparation, P.T.; review and editing P.T., M.H., H.L., H.M., P.P. and UF.; visualization, P.T.; supervision, M.H., H.L. and U.F.; project administration, P.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the University of Pretoria Department of Research and Innovation UCDP, the National Research Foundation (NRF): new Generation of Academics Program grant ID: NGAP_RDG190128411410, and the Collaborative Initiative for Paediatrics HIV Education and Research (CIPHER) of the International AIDS Society grant ID: 2017/560_FEU.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of the University of Pretoria (protocol code NAS063/2020 on 4 June 2020).

Informed Consent Statement: Informed consent was obtained from all participants involved in the study.

Data Availability Statement: Data are available on request from the corresponding author, due to the University of Pretoria policy on data publication.

Acknowledgments: The authors would like to thank all the staff and research assistants (Maryjane Ntima, Sheilla Sono, Lidisa Mothiba, Sicebile Sibiya, and Kedibone Matshai) for their assistance in the data collection of the Siyakhula study. Special thanks to the mothers and infants who participated in the study.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design, analysis, or writing of this article or in the decision to publish the results. Any opinions, findings, and conclusions are, therefore, of the authors.

References

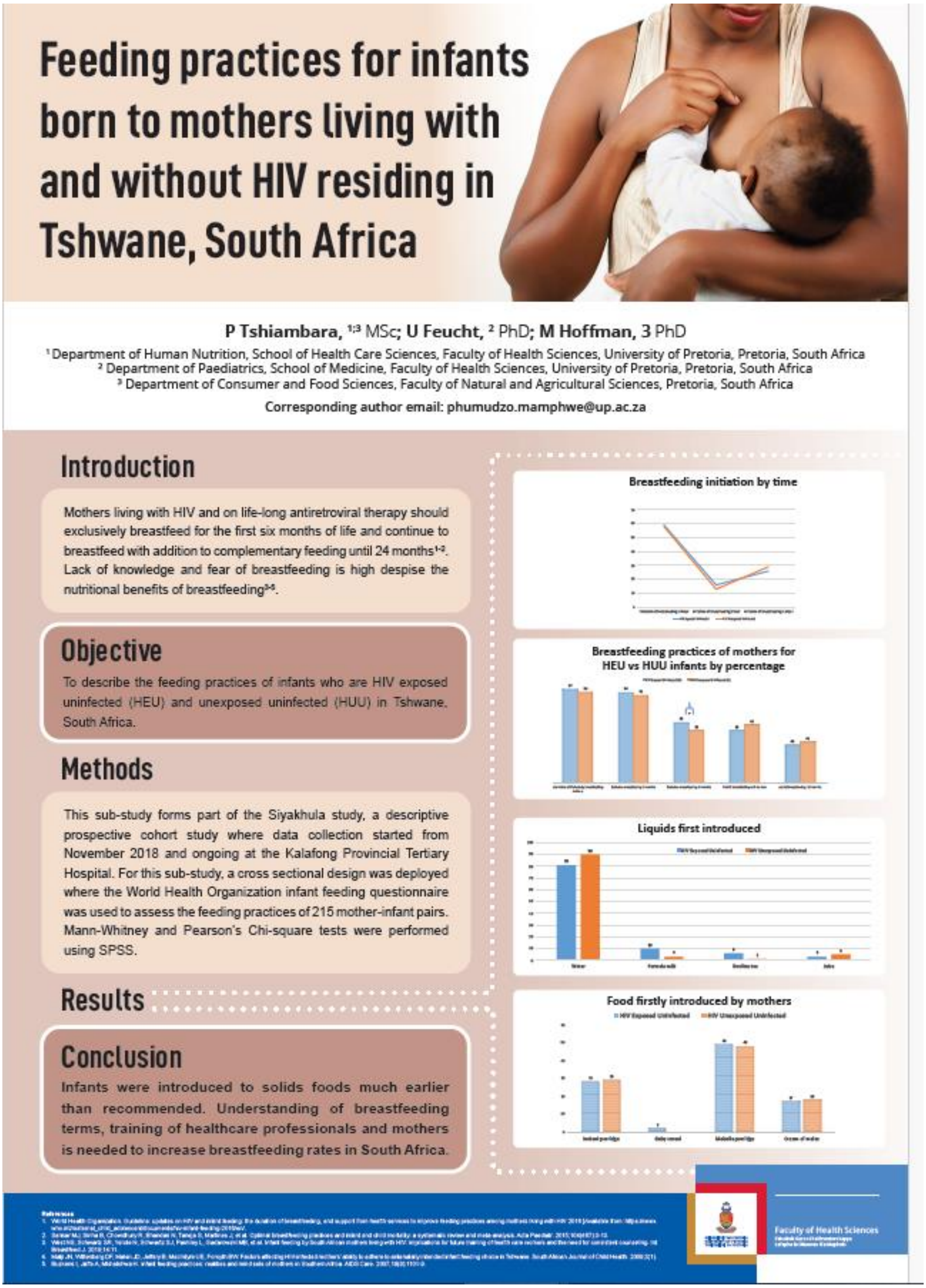
1. UNAIDS. *Danger: UNAIDS Global AIDS Update 2022*; Joint United Nations Programme on HIV/AIDS: Geneva, Switzerland, 2022.
2. Release, S. *Mid-Year Population Estimates 2021*; Statistics South Africa: Pretoria, South Africa, 2021; Volume 302. Available online: http://www.statssa.gov.za/publications (accessed on 15 September 2021).
3. World Health Organization. *Advocacy Brief: Breastfeeding and HIV: Global Breastfeeding Collective*; World Health Organization: Geneva, Switzerland, 2019.
4. UNAIDS. *Countdown to ZERO: Global Plan towards the Elimination of New HIV Infections among Children by 2015 and Keeping Their Mother Alive*; UNAIDS: Geneva, Switzerland, 2011.
5. Sugandhi, N.; Rodrigues, J.; Kim, M.; Ahmed, S.; Amzel, A.; Tolle, M.; Dziuban, E.J.; Kellerman, S.E.; Rivadeneira, E.; Child Survival Working Group of the Interagency Task Team on the Prevention and Treatment of HIV infection in Pregnant Women, Mothers and Children. HIV-exposed infants: Rethinking care for a lifelong condition. *AIDS* **2013**, *27* (Suppl. 2), S187–S195. [CrossRef] [PubMed]
6. Evans, C.; Chasekwa, B.; Ntozini, R.; Majo, F.D.; Mutasa, K.; Tavengwa, N.; Mutasa, B.; Mbuya, M.N.; Smith, L.E.; Stoltzfus, R.J. Mortality, HIV transmission and growth in children exposed to HIV in rural Zimbabwe. *Clin. Infect. Dis.* **2020**, *72*, 586–594. [CrossRef] [PubMed]
7. UNAIDS. *Update Miles to Go—Closing Gaps, Breaking Barriers, Righting Injustices*; UNAIDS: Geneva, Switzerland, 2018; Available online: http://aidsinfo.unaids.org (accessed on 25 February 2021).
8. World Health Organization. *Global Breastfeeding Scorecard, 2019: Increasing Commitment to Breastfeeding through Funding and Improved Policies and Programmes*; World Health Organization: Geneva, Switzerland, 2019.
9. World Health Organization. *Guideline: Updates on HIV and Infant Feeding: The Duration of Breastfeeding, and Support from Health Services to Improve Feeding Practices among Mothers Living with HIV*; 924154970X; World Health Organization: Geneva, Switzerland, 2016.
10. Chalashika, P.; Essex, C.; Mellor, D.; Swift, J.A.; Langley-Evans, S. Birthweight, HIV exposure and infant feeding as predictors of malnutrition in Botswanan infants. *J. Hum. Nutr. Diet.* **2017**, *30*, 779–790. [CrossRef]
11. Becquet, R.; Bland, R.; Leroy, V.; Rollins, N.C.; Ekouevi, D.K.; Coutsoudis, A.; Dabis, F.; Coovadia, H.M.; Salamon, R.; Newell, M.L. Duration, pattern of breastfeeding and postnatal transmission of HIV: Pooled analysis of individual data from West and South African cohorts. *PLoS ONE* **2009**, *4*, e7397. [CrossRef] [PubMed]
12. Goga, A.E.; Doherty, T.; Jackson, D.J.; Sanders, D.; Colvin, M.; Chopra, M.; Kuhn, L. Infant feeding practices at routine PMTCT sites, South Africa: Results of a prospective observational study amongst HIV exposed and unexposed infants-birth to 9 months. *Int. Breastfeed. J.* **2012**, *7*, 4. [CrossRef]
13. West, N.S.; Schwartz, S.R.; Yende, N.; Schwartz, S.J.; Parmley, L.; Gadarowski, M.B.; Mutunga, L.; Bassett, J.; Van Rie, A. Infant feeding by South African mothers living with HIV: Implications for future training of health care workers and the need for consistent counseling. *Int. Breastfeed. J.* **2019**, *14*, 11. [CrossRef]
14. Matji, J.N.; Wittenberg, D.F.; Makin, J.D.; Jeffery, B.; MacIntyre, U.E.; Forsyth, B.W. Factors affecting HIV-infected mothers' ability to adhere to antenatally intended infant feeding choice in Tshwane. *S. Afr. J. Child Health* **2009**, *3*, 20–23.

15. Coutsoudis, A.; Pillay, K.; Kuhn, L.; Spooner, E.; Tsai, W.-Y.; Coovadia, H.M.; South African Vitamin A Study Group. Method of feeding and transmission of HIV-1 from mothers to children by 15 months of age: Prospective cohort study from Durban, South Africa. *AIDS* **2001**, *15*, 379–387. [CrossRef]
16. Dewey, K. Guiding principles for complementary feeding of the breastfed child. In *Guidelines for an Integrated Approach to the Nutritional Care of HIV-Infected Children (6 Months–14 Years). Geneva: World Health Organization; 2009. Appendix V, Guiding Principles for Complementary Feeding of the Breastfed Child*; World Health Organization: Geneva, Switzerland, 2003.
17. Faber, M.; Laubscher, R.; Berti, C. Poor dietary diversity and low nutrient density of the complementary diet for 6- to 24-month-old children in urban and rural KwaZulu-Natal, South Africa. *Matern. Child Nutr.* **2016**, *12*, 528–545. [CrossRef]
18. Faber, M. Complementary foods consumed by 6–12-month-old rural infants in South Africa are inadequate in micronutrients. *Public Health Nutr.* **2007**, *8*, 373–381. [CrossRef]
19. Rothman, M.; Faber, M.; Covic, N.; Matsungo, T.M.; Cockeran, M.; Kvalsvig, J.D.; Smuts, C.M. Infant development at the age of 6 months in relation to feeding practices, Iron status, and growth in a peri-urban community of South Africa. *Nutrients* **2018**, *10*, 73. [CrossRef]
20. Matsungo, T.M.; Kruger, H.S.; Faber, M.; Rothman, M.; Smuts, C.M. The prevalence and factors associated with stunting among infants aged 6 months in a peri-urban South African community. *Public Health Nutr.* **2017**, *20*, 3209–3218. [CrossRef]
21. Sayed, N.; Schönfeldt, H.C. A review of complementary feeding practices in South Africa. *S. Afr. J. Clin. Nutr.* **2020**, *33*, 36–43. [CrossRef]
22. Mugware, A.; Motadi, S.A.; Mushaphi, L.F. Feeding practices and micronutrient status of children aged 0–36 months in Thulamela Municipality, Limpopo province. *Health SA Gesondheid* **2022**, *27*, 9. [CrossRef]
23. Stewart, C.P.; Iannotti, L.; Dewey, K.G.; Michaelsen, K.F.; Onyango, A.W. Contextualising complementary feeding in a broader framework for stunting prevention. *Matern. Child Nutr.* **2013**, *9*, 27–45. [CrossRef]
24. Masuke, R.; Msuya, S.E.; Mahande, J.M.; Diarz, E.J.; Stray-Pedersen, B.; Jahanpour, O.; Mgongo, M. Effect of inappropriate complementary feeding practices on the nutritional status of children aged 6–24 months in urban Moshi, Northern Tanzania: Cohort study. *PLoS ONE* **2021**, *16*, e0250562. [CrossRef]
25. Department of Health. National consolidated guidelines for the prevention of mother-to-child transmission of HIV (PMTCT) and the management of HIV in children, adolescents and adults. Africa, 2014. Available online: https://www.knowledgehub.org.za/system/files/elibdownloads/2019-07/National%2520consolidated%2520guidelines%25202015.pdf (accessed on 26 January 2021).
26. Slogrove, A.L.; Johnson, L.F.; Powis, K.M. Population-level Mortality Associated with HIV Exposure in HIV-uninfected Infants in Botswana and South Africa: A Model-based Evaluation. *J. Trop. Pediatr.* **2018**, *65*, 373–379. [CrossRef]
27. Wu, J.; Li, J.; Li, Y.; Loo, K.K.; Yang, H.; Wang, Q.; Duan, R.; Xiao, X.; Song, X.; Yang, S.; et al. Neurodevelopmental outcomes in young children born to HIV-positive mothers in rural Yunnan, China. *Pediatr. Int.* **2018**, *60*, 618–625. [CrossRef]
28. Rollins, N.C.; Ndirangu, J.; Bland, R.M.; Coutsoudis, A.; Coovadia, H.M.; Newell, M.L. Exclusive breastfeeding, diarrhoeal morbidity and all-cause mortality in infants of HIV-infected and HIV uninfected mothers: An intervention cohort study in KwaZulu Natal, South Africa. *PLoS ONE* **2013**, *8*, e81307. [CrossRef]
29. Kidzeru, E.B.; Hesseling, A.C.; Passmore, J.-A.S.; Myer, L.; Gamieldien, H.; Tchakoute, C.T.; Gray, C.M.; Sodora, D.L.; Jaspan, H.B. In-utero exposure to maternal HIV infection alters T-cell immune responses to vaccination in HIV-uninfected infants. *AIDS* **2014**, *28*, 1421. [CrossRef]
30. Rosala-Hallas, A.; Bartlett, J.W.; Filteau, S. Growth of HIV-exposed uninfected, compared with HIV-unexposed, Zambian children: A longitudinal analysis from infancy to school age. *BMC Pediatr.* **2017**, *17*, 80. [CrossRef] [PubMed]
31. Evans, C.; Humphrey, J.H.; Ntozini, R.; Prendergast, A.J. HIV-exposed uninfected infants in Zimbabwe: Insights into health outcomes in the pre-antiretroviral therapy era. *Front. Immunol.* **2016**, *7*, 190. [CrossRef] [PubMed]
32. Ejigu, Y.; Magnus, J.H.; Sundby, J.; Magnus, M.C. Differences in Growth of HIV-exposed Uninfected Infants in Ethiopia According to Timing of In-utero Antiretroviral Therapy Exposure. *Pediatr. Infect. Dis. J.* **2020**, *39*, 730. [CrossRef] [PubMed]
33. Le Roux, S.M.; Abrams, E.J.; Donald, K.A.; Brittain, K.; Phillips, T.K.; Nguyen, K.K.; Zerbe, A.; Kroon, M.; Myer, L. Growth trajectories of breastfed HIV-exposed uninfected and HIV-unexposed children under conditions of universal maternal antiretroviral therapy: A prospective study. *Lancet Child Adolesc. Health* **2019**, *3*, 234–244. [CrossRef]
34. Fadnes, L.T.; Engebretsen, I.M.; Wamani, H.; Semiyaga, N.B.; Tylleskar, T.; Tumwine, J.K. Infant feeding among HIV-positive mothers and the general population mothers: Comparison of two cross-sectional surveys in Eastern Uganda. *BMC Public Health* **2009**, *9*, 124. [CrossRef]
35. Kindra, G.; Coutsoudis, A.; Esposito, F.; Esterhuizen, T. Breastfeeding in HIV exposed infants significantly improves child health: A prospective study. *Matern. Child Health J.* **2012**, *16*, 632–640. [CrossRef]
36. Iliyasu, Z.; Galadanci, H.S.; Iliyasu, M.L.; Babashani, M.; Gajida, A.U.; Nass, N.S.; Aliyu, M.H. Determinants of Infant Feeding Practices Among HIV-Infected Mothers in Urban Kano, Nigeria. *J. Hum. Lact.* **2019**, *35*, 592–600. [CrossRef]
37. White, M.; Feucht, U.D.; du Toit, L.d.V.; Rossouw, T.; Connor, K.L. Understanding the Impact of Maternal HIV Infection on the Health and Well-Being of Mothers and Infants in South Africa: Siyakhula Collaborative Workshop Report. *J. Multidiscip. Healthc.* **2021**, *14*, 1987–1999. [CrossRef]
38. World Health Organization. *Indicators for Assessing Infant and Young Child Feeding Practices: Part 2: Measurement*; World Health Organization: Geneva, Switzerland, 2010.

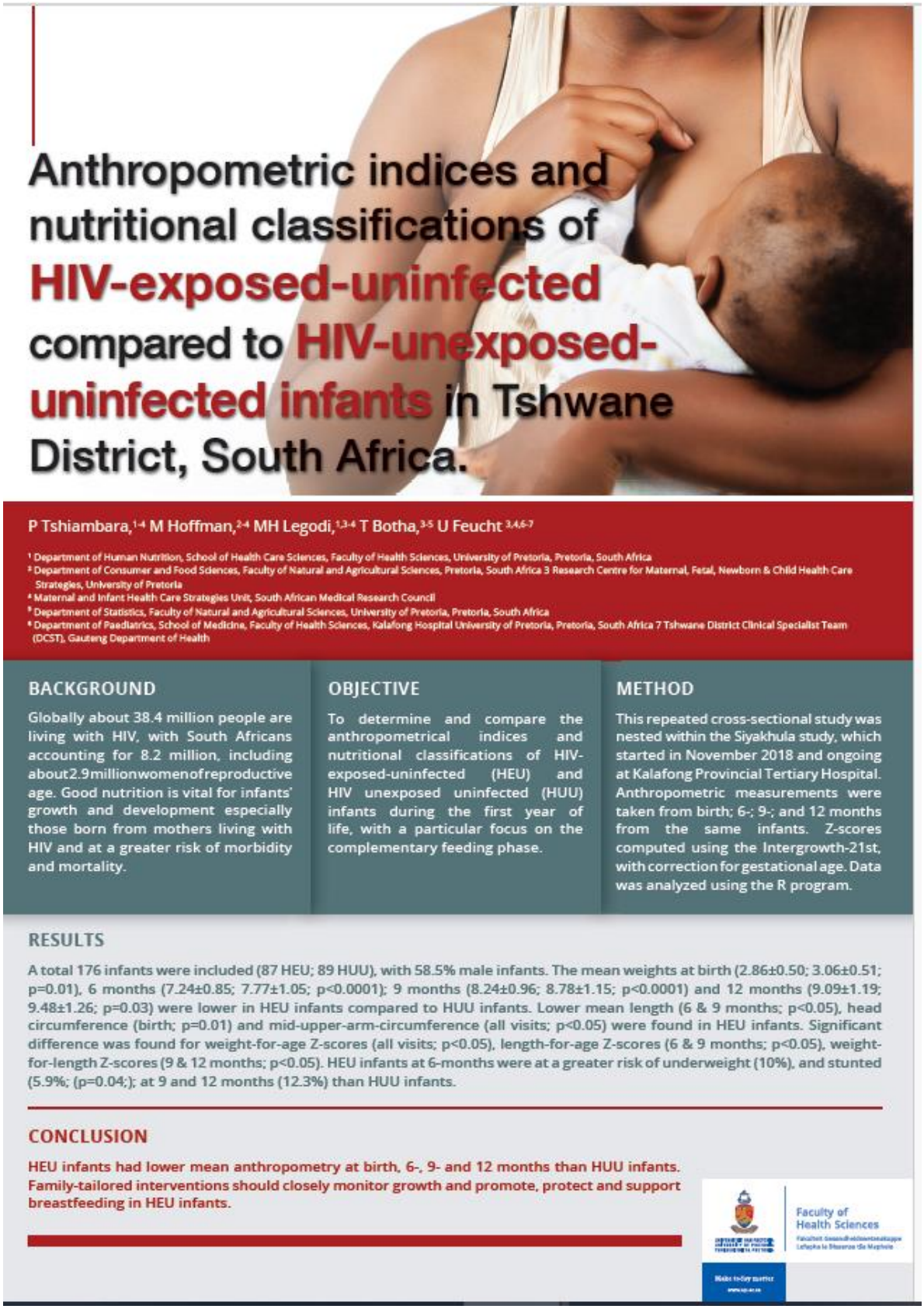
39. Villar, J.; Cheikh Ismail, L.; Victora, C.G.; Ohuma, E.O.; Bertino, E.; Altman, D.G.; Lambert, A.; Papageorghiou, A.T.; Carvalho, M.; Jaffer, Y.A. International Fetal and Newborn Growth Consortium for the 21st Century (INTERGROWTH-21st). International standards for newborn weight, length, and head circumference by gestational age and sex: The Newborn Cross-Sectional Study of the INTERGROWTH-21st Project. *Lancet* **2014**, *384*, 857–868.
40. World Health Organization. *WHO Child Growth Standards: Length/Height-for-Age, Weight-for-Age, Weight-for-Length, Weight-for-Height and Body Mass Index-for-Age: Methods and Development*; 924154693X; World Health Organization: Geneva, Switzerland, 2006.
41. Smuts, C.M.; Dhansay, M.A.; Faber, M.; van Stuijvenberg, M.E.; Swanevelder, S.; Gross, R.; Benade, A.S. Efficacy of multiple micronutrient supplementation for improving anemia, micronutrient status, and growth in South African infants. *J. Nutr.* **2005**, *135*, 653S–659S. [CrossRef]
42. Faber, M.; Benade, A. Nutritional status and dietary practices of 4–24-month-old children from a rural South African community. *Public Health Nutr.* **1999**, *2*, 179–185. [CrossRef]
43. Patridge, E.F.; Bardyn, T.P. Research electronic data capture (REDCap). *JMLA* **2018**, *106*, 142. [CrossRef]
44. Team, R.C. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2013; Available online: http://www.R-project.org/ (accessed on 5 January 2023).
45. UNICEF. *Adopting Optimal Feeding Practices Is Fundamental to a Child's Survival, Growth and Development, but too Few Children Benefit*; UNICEF: New York, NY, USA, 2015.
46. Jackson, D.; Swanevelder, S.; Doherty, T.; Lombard, C.; Bhardwaj, S.; Goga, A. Changes in rates of early exclusive breast feeding in South Africa from 2010 to 2013: Data from three national surveys before and during implementation of a change in national breastfeeding policy. *BMJ Open* **2019**, *9*, e028095. [CrossRef]
47. Budree, S.; Goddard, E.; Brittain, K.; Cader, S.; Myer, L.; Zar, H.J. Infant feeding practices in a South African birth cohort—A longitudinal study. *Matern. Child Nutr.* **2017**, *13*, e12371. [CrossRef]
48. Haile, D.; Belachew, T.; Berhanu, G.; Setegn, T.; Biadgilign, S. Complementary feeding practices and associated factors among HIV positive mothers in Southern Ethiopia. *J. Health Popul. Nutr.* **2015**, *34*, 5. [CrossRef]
49. Rossouw, M.E.; Cornell, M.; Cotton, M.F.; Esser, M.M. Feeding practices and nutritional status of HIV-exposed and HIV-unexposed infants in the Western Cape. *S. Afr. J. HIV Med.* **2016**, *17*, 398. [CrossRef]
50. Neary, J.; Langat, A.; Singa, B.; Kinuthia, J.; Itindi, J.; Nyaboe, E.; Ng'anga, L.W.; Katana, A.; John-Stewart, G.C.; Mcgrath, C.J. Higher prevalence of stunting and poor growth outcomes in HIV-exposed uninfected than HIV-unexposed infants in Kenya. *AIDS* **2021**, *36*, 605–610. [CrossRef]
51. Lang'at, P.C.; Ogada, I.; Steenbeek, A.; MacDonald, N.E.; Ochola, S.; Bor, W.; Odinga, G. Infant feeding practices among HIV-exposed infants less than 6 months of age in Bomet County, Kenya: An in-depth qualitative study of feeding choices. *Arch. Dis. Child* **2018**, *103*, 470–473. [CrossRef]
52. World Health Organization. *Indicators for Assessing Infant and Young Child Feeding Practices: Definitions and Measurement Methods*; World Health Organization: Geneva, Switzerland, 2021.
53. Cape, W.; Faber, M.; Benadé, S. Breastfeeding, complementary feeding and nutritional status of 6–12-month-old infants in rural KwaZulu-Natal. *S. Afr. J. Clin. Nutr.* **2007**, *20*, 16–24. [CrossRef]
54. Mir-Marqués, A.; González-Masó, A.; Cervera, M.L.; de la Guardia, M. Mineral profile of Spanish commercial baby food. *Food Chem.* **2015**, *172*, 238–244. [CrossRef]
55. Slogrove, A.L. It is a question of equity: Time to talk about children who are HIV-exposed and "HIV-free". *J. Int. AIDS Soc.* **2021**, *24*, e25850. [CrossRef] [PubMed]
56. Evans, C.; Jones, C.E.; Prendergast, A.J. HIV-exposed, uninfected infants: New global challenges in the era of paediatric HIV elimination. *Lancet Infect. Dis.* **2016**, *16*, e92–e107. [CrossRef] [PubMed]
57. Ruck, C.E.; Smolen, K.K. Effect of maternal HIV infection on infant development and outcomes. *Front. Virol.* **2022**, *2*, 885246. [CrossRef]
58. Pillay, L.; Moodley, D.; Emel, L.M.; Nkwanyana, N.M.; Naidoo, K. Growth patterns and clinical outcomes in association with breastfeeding duration in HIV exposed and unexposed infants: A cohort study in KwaZulu Natal, South Africa. *BMC Pediatr.* **2021**, *21*, 183. [CrossRef]
59. Evans, C.; Chasekwa, B.; Ntozini, R.; Humphrey, J.H.; Prendergast, A.J. Head circumferences of children born to HIV-infected and HIV-uninfected mothers in Zimbabwe during the preantiretroviral therapy era. *AIDS* **2016**, *30*, 2323. [CrossRef]
60. Lane, C.; Widen, E.; Collins, S.; Young, S. HIV-exposed, uninfected infants in Uganda experience poorer growth and body composition trajectories than HIV-unexposed infants. *J. Acquir. Immune Defic. Syndr.* **2020**, *4*, 1023.
61. Lane, C.E.; Bobrow, E.A.; Ndatimana, D.; Ndayisaba, G.F.; Adair, L.S. Determinants of growth in HIV-exposed and HIV-uninfected infants in the Kabeho Study. *Matern. Child Nutr.* **2019**, *15*, e12776. [CrossRef]
62. World Health Organization. *UNICEF/WHO/The World Bank Group Joint Child Malnutrition Estimates: Levels and Trends in Child Malnutrition: Key Findings of the 2020 Edition*; World Health Organization: Geneva, Switzerland, 2020.

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

APPENDIX K: POSTER PRESENTATION FOR RESEARCH DAY



APPENDIX L: POSTER FOR THE GROW GREAT SUMMIT 2022 PRESENTATIONS



APPENDIX M: *NUTRIENTS* AUTHOR GUIDELINES

https://www.mdpi.com/journal/nutrients

Please see Author Guidelines for submission to Nutrients

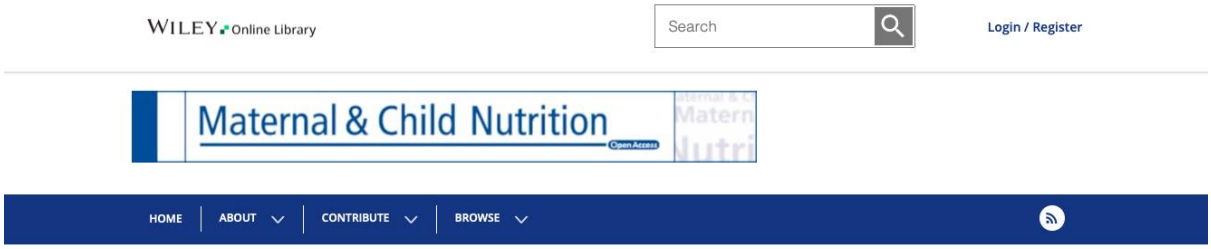
APPENDIX N: *SOUTH AFRICAN JOURNAL OF CLINICAL NUTRITION* AUTHOR GUIDELINES

www.sajcn.co.za

Please see Author Guidelines for submission to the South African Journal of Clinical Nutrition

© University of Pretoria

APPENDIX O: *MATERNAL & CHILD NUTRITION* AUTHOR GUIDELINES



https://onlinelibrary.wiley.com/journal/17408709

Please see Author Guidelines for submission to Maternal & Child Nutrition:

© University of Pretoria

APPENDIX P: LETTER FROM LANGUAGE EDITOR

Krige Lane
Irene
Centurion

30 October 2023

Confirmation of Editing of Thesis

I hereby confirm that I, Dr Michelle Janse van Rensburg, have edited the thesis entitled "GROWTH, FEEDING PRACTICES, AND HAEMOGLOBIN LEVELS IN 6- TO 12-MONTH-OLD INFANTS EXPOSED AND UNEXPOSED TO MATERNAL HIV STATUS IN A PERI-URBAN AREA IN GAUTENG PROVINCE, SOUTH AFRICA", which is being submitted for the degree Doctor of Philosophy (PhD) in Nutrition by **Phumudzo Tshiambara**.

I have considered spelling, grammar, punctuation, formatting, numbering and any other aspects relating to the editing of this document. The content of the thesis and the accuracy thereof remain the responsibility of the PhD candidate and her supervisors.

(Note: Dr Cheryl Tosh edited the article manuscripts contained in Chapters 3, 4, and 5 of this document according to the relevant academic journal guidelines.)

Dr Michelle Janse van Rensburg (PhD)

Email: michellejvr.ot.copc@gmail.com
Cell: 0834065648

© University of Pretoria