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Maternal Phenotype, Infant Size and Breast Milk Composition in Women Living With HIV

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ABSTRACT

The impact of maternal factors on the size of HIV-exposed-uninfected (HEU) infants and breast milk composition is poorly understood. Anthropometry, bio-electrical impedance, haemoglobin and HIV viral load data of women living with HIV (WLWH) and without HIV (WLWOH) were compared and related to their infants' anthropometric Z-scores and breast milk macronutrients 6 weeks and 6 months postnatally. At both time points, WLWH (6-week: $n = 83$; 6-month: $n = 63$) had lower reactance (measure of body cell mass) (6-week: $p = 0.016$; 6-month: $p < 0.001$), phase angle (PhA) (measure of cell health) (6-week: $p = 0.001$; 6-month: $p = 0.002$) and haemoglobin (6-week: $p = 0.002$; 6-month: $p = 0.004$) than WLWOH (6-week: $n = 90$; 6-month: $n = 73$). HEU infants had lower weight-for-age Z-scores (WAZ) (6-week: $p = 0.010$; 6-month: $p = 0.005$). Breast milk composition did not differ between groups. At 6 weeks, HEU infants had lower head circumference-for-age Z-scores (HCAZ) ($p = 0.014$). Bivariate regression demonstrated maternal HIV predicted lower infant WAZ (ß = −0.442; $p = 0.011$) and HCAZ (ß = −0.445; $p = 0.014$). Maternal body mass index (BMI) and mid-upper arm circumference were positively associated with breast milk protein content (ß = 0.018; $p = 0.014$ and ß = 0.025; $p = 0.002$, respectively). At 6 months (bivariate regression) maternal HIV predicted lower infant WAZ (ß = −0.609; $p = 0.005$) and length-for-age Z-scores (ß = −0.741; $p = 0.018$). Higher maternal BMI and PhA were associated with higher infant WAZ (ß = 0.622; $p = 0.015$ and ß = 0.055; $p = 0.017$, respectively). On multivariable analysis, maternal HIV remained a predictor of lower WAZ (ß = −0.568; $p = 0.024$). In conclusion, maternal HIV infection and phenotype predict the size of infants and breast milk composition up to 6 months postnatally.

1 | Introduction

Antiretroviral therapy (ART) for pregnant women diagnosed with HIV has resulted in the birth of many infants who are HIV-exposed but uninfected (HEU) (Slogrove et al. 2020). Despite not contracting HIV, infants appear to grow sub-optimally, with the risk factors remaining poorly understood (Ramokolo et al. 2022). In South Africa, these issues have considerable public health implications, where 3.5 million (23.8%) children aged 0–14 years were reported as HEU in 2020 (Slogrove et al. 2020).

Factors influencing infant growth include duration of gestation, infant feeding practices, infant illnesses, maternal nutritional

Summary

- Maternal HIV status predicted a lower infant weight-for-age Z-score at 6 weeks and 6 months, which necessitates close monitoring of HIV-exposed-uninfected (HEU) infants in the first 6 months of life.
- Maternal body mass index and phase angle, an indicator of cell membrane health, were lower in WLWH and these two measures were positively associated with infant weight-for-age Z-scores at 6 months, confirming the association of maternal health with infant growth and supporting the need to perform prepregnancy screening and intervention.
- Maternal body mass index and mid-upper arm circumference of WLWH and WLWOH related positively to breast milk protein content at 6 weeks. These maternal measurements are easily performed and could be utilised as a screening tool when breastfed infants show suboptimal growth.

status, socioeconomic conditions, education, social networks and access to healthcare (Scherbaum and Srour 2016; von Salmuth et al. 2021; Wells 2010). Additionally, maternal body composition as part of the maternal phenotype (any observable traits, resulting from genetic and environmental factors, e.g., body mass index [BMI]) has been reported to influence foetal programming (Kwon and Kim 2017), the stimulus that affects foetal organ development, metabolic and immunological responses. The impact of these factors on infant growth specifically in the context of maternal HIV infection remains poorly understood.

Anthropometric measurements and bioelectrical impedance analysis (BIA) are used to determine maternal body composition. BIA was used in the first 4 months postpartum, to differentiate fat-free mass and fat mass, as part of maternal body composition. Compared to nonpregnant women, population-specific equations are not needed in the postpartum period and the existing age-, race- and sex-adjusted equations are suitable (Garr Barry et al. 2022).

Breast milk macronutrient composition is genetically determined and influenced by parity, maternal body composition and nutritional status (Bzikowska-Jura et al. 2018). A meta-analysis established an association between maternal BMI and breast milk fat content, but few studies focused on the impact of maternal HIV infection (Daniel et al. 2021). South Africa recommends breastfeeding regardless of HIV status, aligning with the World Health Organization (WHO) guidelines (West et al. 2019). Whether the maternal body composition of women living with HIV (WLWH) influences breast milk composition remains unknown.

This substudy is embedded in the Siyakhula prospective cohort study in South Africa that explored the influence of *in utero* HIV exposure on foetal and infant growth, including breast milk composition (White et al. 2021). This substudy explored associations between maternal phenotype, infant size and breast milk composition and compared these factors between WLWH and women living without HIV (WLWOH).

2 | Materials and Methods

2.1 | Study Design

Cross-sectional analyses were performed on WLWH and WLWOH based on maternal phenotype characteristics, their breast milk composition and their infants' anthropometric indices at 6 weeks and 6 months postnatally.

2.2 | Recruitment and Data Collection

Inclusion criteria for the Siyakhula study were adult (≥ 18 years) pregnant (18–22 weeks gestation) women with singleton pregnancies and known HIV status. ART was provided according to the South African national guidelines to all WLWH, most commonly a fixed-dose combination of tenofovir, emtricitabine and efavirenz as the first-line regimen at the time of study. Participants were recruited from antenatal clinics by dedicated research nurses in the southwest Tshwane District in the Gauteng Province of South Africa, between January 2018 and January 2021, with a focus on low-risk healthy pregnant women, as per local antenatal care guidelines. Data collection was conducted from June 2018 to December 2021 at Kalafong Hospital. Sampling for the Siyakhula study was performed in a stratified convenient way to ensure similar numbers of WLWH and WLWOH (final recruitment numbers: WLWH $n = 153$; WLWOH $n = 162$). The mother–infant pairs for this substudy were followed up to 6 months *postpartum*. Exclusion criteria included inability to obtain informed consent, maternal hypertension, diabetes, tuberculosis or other serious pre-existing medical disorders, multiple pregnancies and foetal chromosomal or structural abnormalities. Preterm infants were included. The number of mother–infant pairs who attended the postpartum visits for the Siyakhula study is shown in Figure 1. The numbers reflect the expected postnatal attrition, due to known high population mobility around the time of pregnancy and delivery. Women often return to their hometowns after giving birth to benefit from family support.

2.3 | Ethics Statement

Written informed consent was obtained from women by research nurses who could speak various local languages. Participant names are only reflected in the research files and for the sake of anonymity and confidentiality, only study numbers were used in electronic data sheets and breast milk samples. The Research Ethics Committee of the Faculty of Health Sciences, University of Pretoria (reference: 480/2021) and the Research Committee of Kalafong Provincial Tertiary Hospital approved the study. Written informed consent was obtained from all study participants.

2.4 | Data Collection

2.4.1 | Maternal Variables

A structured questionnaire was used to collect maternal variables such as sociodemographic information, including age, obstetric history, marital status, employment and education status.

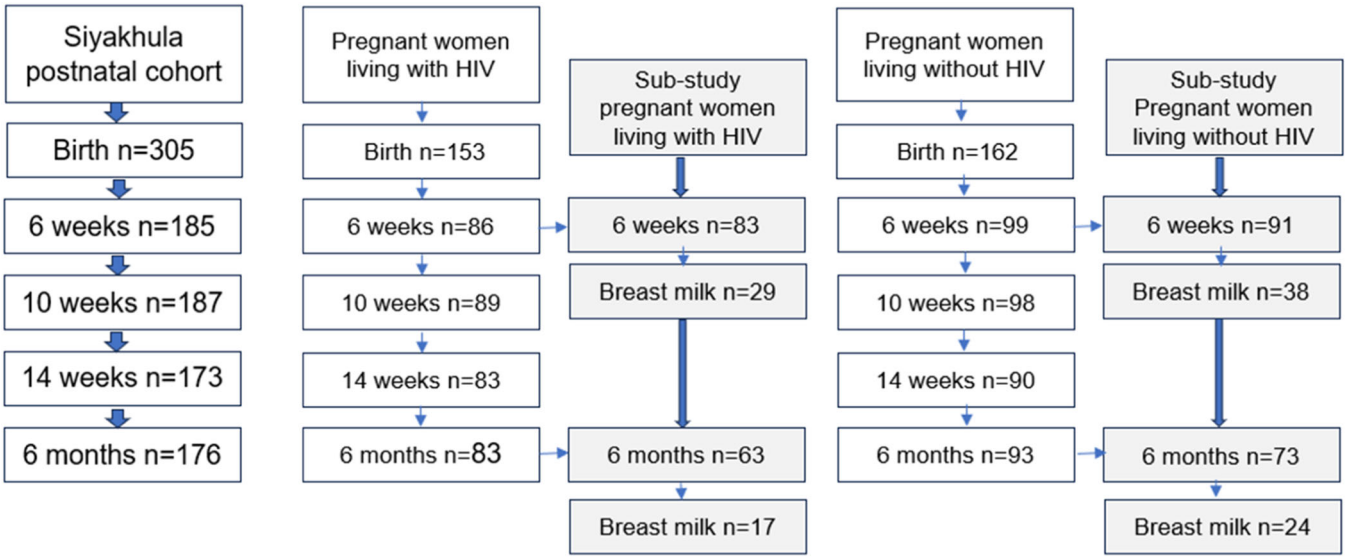

FIGURE 1 | Number of women living with and without HIV and their infants included in the Siyakhula study and this substudy (shaded in grey), per postnatal visit.

Maternal body composition measurements were collected by the researcher and one trained research assistant, with participants barefoot and included anthropometric measurements (height, weight and mid-upper-arm circumference [MUAC]) and BIA parameters. Maternal height was measured to the nearest 0.1 cm with a metal portable stadiometer (Seca 310, Hamburg, Germany), as per standard operating procedures (Dezateux et al. 2016). Weight was measured with the participant lightly clothed with the 8-point segmental multifrequency medical Body Composition Analyser (Seca 514, Hamburg, Germany), and BMI (kg/m^2) was calculated by the medical Body Composition Analyser using the weight and manually entered height. MUAC (cm) was measured twice on the left arm to the nearest 0.1 cm with a flexible measuring tape ([Empisal, Johannesburg, South Africa) following standardised procedures (Lee and Nieman 2012). BIA relies on the electrical conductance and resistance properties of body tissues. The impedance (measured in ohm;[Ω]) refers to a measure of resistance (R) and reactance (Xc) to a nonharmful multifrequency electrical current, passing through the body. Fat-free mass, which includes muscles and organs, has a high water and electrolyte content and produces a low resistance. It includes all the determinants of the resting metabolic rate, so the higher the fat-free mass the higher the resting metabolic rate. Fat mass and fat-free mass are predicted with anthropometric and biographic data and skeletal muscle mass through additional algorithms. Xc reflects the cell-membrane capacitance and body cell mass, and the ratio of R to Xc is expressed as the phase angle (PhA). The PhA is used to determine cell membrane health. A higher PhA and Xc indicate increased body cell mass (Khalil, Mohktar, and Ibrahim 2014).

The BIA parameters were determined in the morning after a 2-h fast and included R (Ω), Xc (Ω), PhA (°), fat mass (kg), fat-free mass (kg) and skeletal muscle mass (kg).

The HIV viral load (copies/mL) result, determined by the National Health Laboratory Services, was recorded for WLWH, and the maternal haemoglobin (g/dL) levels were collected using the HemoCue Hb 201$^+$ System Analyser (Ängelholm, Sweden), which is calibrated according to the International Council for Standardisation in Haematology method (calibration-(https://www.hemocue.com/en/products/hematology/hemocue-hb-201plus-system/).

2.4.2 | Infant Variables

A dedicated research nurse took the infants' anthropometric measurements. Weight was measured to the nearest 0.01 kg using a baby scale (Seca 354, Hamburg, Germany). The length was recorded to the nearest 0.1 cm with an infantometer (Seca 410, Hamburg, Germany) and the head circumference (HC) was measured to the nearest 0.1 cm, using a flexible measuring tape, following standardised procedures (Lee and Nieman 2012).

2.4.3 | Breast Milk Variables

Milk samples were collected between 9h00 and 11h00 and stored in a MIRIS-dedicated film-coated container, to prevent fat adhesion to the container sides. Mothers were instructed to feed their infants without restraint while hand-expressing 10 mL of breast milk. This approach aimed to create a more representative sample, reflecting the average composition at the specific time of day (Ballard and Morrow 2013; Miller et al. 2013; Pham et al. 2020; Samuel et al. 2020). The samples were frozen within a few hours at −20°C and analysed within 3 months of storage.

2.4.4 | Data Management and Storage

The Siyakhula study data were entered into Research Electronic Data Capture (REDCap) v 9.3.5., a secure web-based application for data capture. BIA parameters were directly captured by the Seca medical Body Composition analyser in an Excel spreadsheet. Maternal height (m) and MUAC (cm) as well as infant anthropometric data were manually captured, transferred to Excel and then to Stata (v 13.1) for statistical analysis. Data

were inspected for outliers, which were corrected or in the case of implausible values, were excluded from the analysis. Missing data exceeding 5% per variable were imputed using the median, as the data was missing completely at random.

Maternal height (m), BMI (kg/m^2), MUAC (cm) and BIA parameters (R [Ω], Xc [Ω] and PhA [°]) were used as measured, but skeletal muscle mass (kg), fat-free mass (kg) and fat mass (kg) were converted to percentages (kg skeletal muscle mass/fat-free mass/fat mass) ÷ weight (kg) × 100) of body weight.

The WHO Child Growth Standards (2006) were used to convert infant size measurements to age- and sex-standardised Z-scores for weight-for-age (WAZ), length-for-age (LAZ), HC-for-age (HCAZ) and BMI-for-age (BMIZ) (World Health Organization 2006). Z-score calculations were based on the corrected age. The birth gestational age was determined as the difference between the actual date of birth and the expected date of birth according to the early ultrasound.

Breast milk macronutrient composition was analysed using the MIRIS Human Milk Analyser (Uppsala, Sweden). The analysis included protein (g/100 mL), carbohydrates (g/100 mL), fat (g/100 mL) and energy content in kcal/100 mL (conversion kJ = kcal × 4.184). The analyser uses a combination of established mid-infrared transmission spectroscopy principles and a patented innovation (Kotz 2018) to measure macronutrients in breast milk against a known reference library (Miller et al. 2013). Duplicate results were recorded and found to be within 0.1 g/100 mL of each other.

2.4.5 | Statistical Analysis

Differences between the groups were tested with Pearson's χ^2 test or Fisher's exact test for categorical data, and an independent t test for normally distributed continuous variables. The median and interquartile range (IQR) were compared for nonnormally distributed variables using the Mann–Whitney U test. Bivariate- and multivariate linear regression analyses were used to examine relationships between maternal factors, infant anthropometric indices and breast milk macronutrient and energy content at 6 weeks and 6 months. Statistical analysis assumed a two-sided 5% significance level ($p < 0.05$).

3 | Results

3.1 | Study Sample

There were 83 WLWH and 91 WLWOH participants at 6 weeks, and 63 WLWH and 73 WLWOH at 6 months. Some participants attended the 6-week visit but not the 6-month visit and vice versa. The number of participants who attended the 6-week and 6-month visits was: $n = 56$ (WLWH) and $n = 62$ (WLWOH). In Table 1, the participants were grouped, without duplication and stratified according to HIV status (WLWH $n = 83 + 63 - 56 = 90$; WLWOH $n = 91 + 73 - 62 = 102$).

The number of breast milk samples was as follows: $n = 29$ and $n = 17$ in WLWH and $n = 38$ and $n = 24$ in WLWOH at 6 weeks and 6 months, respectively. Not all attending women were breastfeeding and additionally, there was a delay in the delivery of collection bottles from Sweden.

3.2 | Sample Characteristics

Maternal characteristics of the participants are presented in Table 1.

Compared to WLWOH, WLWH were significantly older ($p < 0.001$), had higher gravidity ($p = 0.043$) and parity ($p = 0.005$) and had less education equal to or higher than Grade 12 ($p = 0.041$). Results that were not significantly different, included fewer WLWH having access to electricity (91.6%; $n = 76$ vs. 94.6%; $n = 87$, $p = 0.40$).

No cigarette and illicit drug use was reported and only 2.0% ($n = 2$) of WLWOH reported using alcohol during pregnancy. Comparing home language, fewer WLWH spoke one of the South African languages (Northern Sotho, Southern Sotho, Zulu, Tswana, Ndebele, Tsonga, Venda, Xhosa, English, Afrikaans) (92.1%; $n = 82$ vs. 96.9%; $n = 95$), while foreign languages (Swazi, Shona, Chichewa) were spoken by seven (7.9%) WLWH compared to three (3.1%) WLWOH.

TABLE 1 | Maternal characteristics in the substudy of the WLWH and WLWOH.

Maternal characteristics			WLWH ($N = 90$)	WLWOH ($N = 102$)	p value
Age		Median [IQR]	38 [34;39]	31 [26;36]	**< 0.001**[a]
Obstetric history	Gravidity	Median [IQR]	3 [2;4]	3 [2;3]	**0.043**[a]
	Parity	Median [IQR]	2 [1;2]	1 [1;2]	**0.005**[a]
Marital status	Single, divorced, widowed	n (%)	65 (73.9)	74 (74.7)	0.890[b]
Employment	Any	n (%)	52 (58.4)	49 (51.0)	0.313[b]
Education	Grade 12 or above	n (%)	45 (52.3)	59 (60.8)	**0.041**[b]

Note: Bold values: Significant difference ($p < 0.05$). Missing values: Marital status: WLWH: $n = 2$; WLWOH: $n = 3$. Employment: WLWH: $n = 1$; WLWOH: $n = 6$. Education: WLWH: $n = 4$; WLWOH: $n = 5$.
Abbreviations: IQR, interquartile range; WLWH, women living with HIV; WLWOH, women living without HIV.
[a]Mann–Whitney U test.
[b]χ^2 tests.

3.3 | Comparative Analysis

The comparisons of maternal phenotype characteristics, stratified by HIV status, for 6 weeks and 6 months, are shown in Table 2 and the anthropometric Z-scores of the infants at 6 weeks and 6 months are shown in Table 3.

WLWH included at 6 weeks were significantly taller than WLWOH ($p = 0.044$) but they had a significantly lower BMI ($p = 0.007$), Xc ($p = 0.016$), PhA ($p = 0.001$) and %fat mass ($p = 0.016$). Nonetheless, the mean BMI of both groups exceeded 25 kg/m^2 at both time points. At 6 months, WLWH still had a lower Xc ($p < 0.001$) and PhA ($p = 0.002$). At both visits, WLWH had significantly lower serum haemoglobin levels ($p = 0.002$ and $p = 0.004$, respectively). At 6 weeks 63/83 (75.9%) of WLWH and 39/91 (42.9%) WLWOH were anaemic (< 12 mg/dL), ($p < 0.001$). At 6 months 43/63 (68.3%) of WLWH and 34/73 (46.6%) of WLWOH were anaemic ($p < 0.001$). At 6 weeks, 85.3% ($n = 64$) and at 6 months, 76.2% ($n = 48$) of WLWH had a suppressed HIV viral load (< 50 copies/mL). About one-fifth of the WLWH at 6 weeks: 20.5% ($n = 17$) were receiving ART other than the fixed-dose combination of tenofovir, emtricitabine and efavirenz, but none were on a dolutegravir-based regimen.

At 6 weeks, the HEU infants were smaller in terms of WAZ ($p = 0.010$) and HCAZ ($p = 0.014$), while at 6 months the WAZ ($p = 0.005$) and LAZ ($p = 0.018$) were significantly lower. The weight range for HEU infants was 2.1–6.6 kg, and that for HUU infants was 3.0–6.8 kg at 6 weeks, while at 6 months weights ranged between 5.4 and 11 kg for HEU infants and 5.5 and 11.5 kg for HUU infants.

Breast milk macronutrient and energy composition for WLWH and WLWOH are shown in Table 4. At 6 weeks and 6 months the breast milk macronutrient and energy composition did not differ significantly between the two groups. Only three HEU infants (3.3%) were fed infant formula milk, compared to no HUU infants at 6 weeks. At 6 months, eight of the HUU infants (11%) and three

TABLE 2 | Maternal phenotype characteristics in the WLWH and WLWOH at both postnatal visits at 6 weeks and 6 months.

	6-week visit			6-month visit		
Maternal phenotype	**WLWH ($n = 83$) Mean (SD)**	**WLWOH ($n = 91$) Mean (SD)**	***p* value**	**WLWH ($n = 63$) Mean (SD)**	**WLWOH ($n = 73$) Mean (SD)**	***p* value**
Anthropometry						
Height (m)	1.59 (0.07)	1.57 (0.05)	**0.044**[a]	1.60 (0.06)	1.60 (0.05)	0.063[b]
BMI (kg/m^2)	25.4 (3.9)	27.0 (3.8)	**0.007**[b]	25.9 (4.6)	27.3 (4.7)	0.100[b]
MUAC (cm)	28.7 (3.6)	29.0 (3.2)	0.561[b]	29.3 (3.6)	30.1 (3.5)	0.203[b]
BIA (raw values)						
R (Ω)	685.4 (76.6)	687.5. (71.5)	0.850[b]	690.1 (67.1)	708.1 (63.5)	0.112[b]
Xc (Ω)	57.9 (8.3)	60.9 (7.6)	**0.016**[b]	62.2 (7.5)	66.6 (6.6)	**< 0.001**[b,c]
PhA (°)	4.8 (0.4)	5.1 (0.5)	**0.001**[b]	5.2 (0.4)	5.4 (0.4)	**0.002**[b]
BIA-derived values						
%fat-free mass	65.4 (8.5)	63.3 (6.9)	0.087[b]	63.7 (10.3)	61.6 (9.9)	0.223[a,c]
%fat mass	34.9 (6.4)	37.2 (5.9)	**0.016**[b]	37.8 (8.7)	38.3 (8.7)	0.723[a,c]
%skeletal muscle mass	30.3 (4.1)	30.0 (4.0)	0.548[b]	30.1 (5.0)	29.8 (4.9)	0.698[a,c]
Haemoglobin (g/dL)	11.2 (1.6)	12.0 (1.6)	**0.002**[b]	11.1 (1.8)	11.9 (1.6)	**0.004**[b]

Note: Bold values: Significant difference ($p < 0.05$).
Imputed data:

- MUAC: WLWH $n = 4$; WLWOH $n = 3$, 6 weeks and WLWH $n = 3$; WLWOH $n = 1$, 6 months.
- R: WLWH $n = 17$; WLWOH $n = 15$, 6 weeks and WLWH $n = 15$; WLWOH $n = 21$, 6 months.
- Xc: WLWH $n = 17$; WLWOH $n = 15$, 6 weeks and WLWH $n = 15$; WLWOH $n = 21$, 6 months.
- PhA: WLWH $n = 17$; WLWOH $n = 15$, 6 weeks and WLWH $n = 15$; WLWOH $n = 21$, 6 months.
- %fat-free mass: WLWH $n = 20$; WLWOH $n = 16$, 6 weeks and WLWH $n = 21$; WLWOH $n = 26$, 6 months.
- %fat mass: WLWH $n = 20$; WLWOH $n = 16$, 6 weeks and WLWH $n = 21$; WLWOH $n = 26$, 6 months.
- %skeletal muscle mass: WLWH $n = 20$; WLWOH $n = 16$, 6 weeks and WLWH $n = 21$; WLWOH $n = 26$, 6 months.
- Haemoglobin: WLWH $n = 5$; WLWOH $n = 3$, 6 weeks and WLWH $n = 1$; WLWOH $n = 1$, 6 months.

Abbreviations: BIA, bioelectrical impedance analysis; BMI, body-mass-index; MUAC, mid-upper arm circumference; PhA, phase angle; R, resistance; SD, standard deviation; WLWH, women living with HIV; WLWOH, women living without HIV; Xc, reactance.
[a]Mann–Whitney U test.
[b]Independent *t* test.
[c]Nonnormally distributed variables were reported as mean and SD for comparison.

TABLE 3 | Anthropometrical Z-scores of HEU and HIV-unexposed-uninfected (HUU) infants at both postpartum visits of 6 weeks and 6 months.

	6-week-visit			6-month-visit		
Infant factors	**HEU ($N = 83$) Mean (SD)**	**HUU ($N = 91$) Mean (SD)**	***p* value**	**HEU (N = 63) Mean (SD)**	**HUU ($N = 73$) Mean (SD)**	***p* value**
WAZ*	−0.40 (1.20)	0.02 (1.06)	**0.010**[a]	−0.33 (1.12)	0.28 (1.33)	**0.005**[b]
LAZ*	−0.42 (1.49)	−0.11 (1.32)	0.081[b]	−0.27 (1.71)	0.47 (1.87)	**0.018**[a]
HCAZ*	0.45 (1.23)	0.88 (1.13)	**0.014**[b]	0.56 (1.43)	0.70 (4.50)	0.831[a]
BMIZ	−0.71 (4.08)	0.12 (1.26)	0.068[a,c]	−0.20 (1.17)	0.05 (1.11)	0.202[a]

Note: Bold values: Significant difference ($p < 0.05$). *Data not evenly distributed but mean and SD reported for comparison.
Abbreviations: BMIZ, body-mass index-for-age z-score; HCAZ, head circumference-for-age z-score; HEU, HIV-exposed-uninfected; HUU, HIV-unexposed-uninfected; LAZ, length-for-age z-score; SD, standard deviation; WAZ, weight-for-age z-score. Z-scores were calculated using the corrected age.
Statistical analysis:
[a]Mann–Whitney U test.
[b]Independent *t* test.
[c]Nonnormally distributed variables were reported as mean and SD for comparison.

TABLE 4 | Breast milk macronutrient and energy composition per 100 mL in WLWH and WLWOH at both postnatal visits of 6 weeks and 6 months.

	6-week visit			6-month visit		
Breast milk components	**WLWH ($n = 29$) Mean (SD)**	**WLWOH ($n = 38$) Mean (SD)**	***p* value**	**WLWH ($n = 17$) Mean (SD)**	**WLWOH ($n = 24$) Mean (SD)**	***p* value**
Protein (g)[a]	1.0 (0.2)	0.9 (0.2)	0.338[b]	0.8 (0.3)	0.8 (0.3)	0.658[b]
Carbohydrates (g)[c]	7.5 (0.4)	7.6 (0.4)	0.216[d]	7.5 (0.5)	7.5 (0.7)	0.906[d]
Fat (g)	3.1 (1.2)	3.3 (1.6)	0.524[d]	3.1 (1.1)	3.9 (1.9)	0.116[d]
Energy (kcal)	267 (47.7)	276.2.0 (65.7)	0.507[d]	261.6 (45.6)	293.8 (72.4)	0.121[b]

Note: Nonnormally distributed variables were reported as mean and SD for comparison: carbohydrates (6 weeks and 6 months), fat (6 weeks and 6 months) and energy (6 weeks).
Abbreviations: g, gram; kJ, kilojoule (MIRIS reports kcal: kilocalories (1 kcal = 4.185 kJ); SD, standard deviation; WLWH, women living with HIV; WLWOH, women living without HIV.
[a]Protein (g): Crude protein (including nonprotein nitrogen) and true protein (includes only protein), are measured by the MIRIS. True protein is reported in the results.
[b]Independent *t* test.
[c]Carbohydrates include 70% lactose and 30% oligosaccharides.
[d]Mann–Whitney U test.

of the HEU infants (4.8%) were formula-fed. Mixed feeding (breast milk and formula) was recorded for one HUU infant (1.1%) and 12 HEU infants (14.5%) at 6 weeks, while at 6 months, 17 HUU infants (23.3%) and 24 HEU infants (38.1%) were mixed feeding.

3.4 | Regression Analysis

At 6 weeks, bivariate regression analysis revealed that maternal HIV infection was associated with significantly lower infant WAZ ($n = 174$; ß = −0.442; $p = 0.011$) and HCAZ ($n = 173$; ß = −0.445; $p = 0.014$), while maternal employment was positively related to infant LAZ ($\beta = 0.483$; $p = 0.013$). Maternal haemoglobin levels were not associated with any anthropometric indices at either 6 weeks or 6 months. Conversely, maternal BMI and MUAC were positively associated with breast milk protein content ($n = 67$; ß = 0.018; $p = 0.014$ and $n = 67$; ß = 0.025; $p = 0.002$, respectively). Increased maternal %fat mass predicted an increase in infant BMIZ ($n = 138$; ß = 9.487; $p = 0.040$).

At 6 months, similar results were noted, whereby maternal HIV infection predicted lower infant WAZ ($n = 136$; ß = −0.609; $p = 0.005$) and LAZ ($n = 136$; ß = −0.741; $p = 0.018$) in bivariate analysis. Positive associations were also found between maternal PhA and infant WAZ ($n = 98$; ß = 0.622; $p = 0.015$) and between maternal BMI and infant WAZ ($n = 136$; ß = 0.055; $p = 0.017$). Bivariate analysis revealed no significant associations between breastfeeding (exclusive breastfeeding) and infant WAZ ($n = 130$; $\beta = -0.398$; $p = 0.743$), LAZ ($n = 130$; $\beta = 0.647$; $p = 0.709$), HCAZ ($n = 130$; $\beta = -1.66$, $p = 0.222$) or BMIZ ($n = 130$; $\beta = -1.018$, $p = 0.358$). Multivariate regression analysis, adjusted for maternal BMI and PhA, revealed maternal HIV infection to be a predictor of reduced infant WAZ at 6 months ($n = 98$; ß = −0.568; $p = 0.024$).

4 | Discussion

This study showed that maternal HIV infection was associated with significantly lower infant WAZ and HCAZ scores at 6 weeks and lower infant LAZ and WAZ at 6 months, without any effect on breast milk composition.

Recent international and South African studies highlight lower anthropometric indices found in HEU infants during early life

compared to their HEU counterparts (Aizire et al. 2020; Fowler et al. 2022; Leary et al. 2006; Mabaya et al. 2021). The precise determinants underlying the growth and development of HEU children remain incompletely understood hampering the development and implementation of effective interventions (Ruck and Smolen 2022).

Although not HIV-infected, HEU infants are at increased risk of mortality, infectious morbidity and growth faltering, signalling the future burden of noncommunicable diseases, even for subsequent generations (Evans, Jones, and Prendergast 2016). The first 1000 days of life, a critical developmental window, are influenced by environmental and socioeconomic factors, including unemployment, food insecurity, limited access to healthcare and pathogen exposure, all of which impact long-term nutritional and neurodevelopmental outcomes (Evans, Jones, and Prendergast 2016). Notably, before ART, studies revealed mixed findings regarding the growth trajectories of breastfed HEU infants, some showing no significant difference in the WAZ of breastfed HEU infants, while others showed lower WAZ and LAZ in HEU newborns exposed to ART (Powis et al. 2016).

Feeding modality also impacts infant growth, with formula feeding historically associated with increased risks of morbidity and mortality (Bork et al. 2014; Clavano 1982; Lamberti et al. 2011). Despite the risk of vertical HIV transmission, innate immune factors and immunoglobulins are transferred from the mother to her infant through breast milk, positively influencing infant health (Evans, Jones, and Prendergast 2016). However, challenges persist, as evidenced by a cohort of women on ART in Botswana, where despite catch-up growth in low birth weight infants by 6 months, the heightened risk of infectious morbidity persisted (Powis et al. 2016).

While recent studies have shown no adverse growth outcomes in infants exposed to *in utero* ART, various maternal factors were found to independently predict infant anthropometric indices (Ramokolo et al. 2022). Maternal education, food security and employment status are among the factors that significantly determine infant growth (Gillette, Lohman, and Neppl 2017; Ramokolo et al. 2022; von Salmuth et al. 2021; Widen et al. 2019). Lower infant weight and length indices are associated with WLWH on ART, highlighting the need for targeted interventions to mitigate these effects. WLWH in this current study had a lower education level than their WLWOH counterparts, but no relationships were observed between education and infant size. Significant associations were found between maternal employment and infant LAZ at 6 weeks, which could be a proxy for better financial means and diet quality, but in low and middle-income countries, maternal employment was also associated with a lower probability of preparing food and breastfeeding (Oddo and Ickes 2018). The dietary intake of women, which has the potential to influence the maternal gut microbiome with transfer thereof to the infants, was not assessed (Lundgren et al. 2018). In this study differences in maternal age, parity and haemoglobin levels were found. Low haemoglobin affects birth outcomes, but its effect on infant growth is unclear. No relationships were found on further analysis.

Most WLWH were initiated on ART at or before the first antenatal care visit and were virally suppressed. The South African first-line antiretroviral regimen has changed to include dolutegravir since the research was conducted, but none of the participants in this substudy were on dolutegravir. The effects of different ART regimens on maternal body composition need further research (Esber et al. 2022).

The first BIA was performed at 6 weeks when the excess fluid accumulated during pregnancy was negligible. Postpartum weight loss primarily consists of extracellular water, intracellular water, total body water and fat-free mass (Cho et al. 2011). In this study, maternal BMI and PhA were positively associated with infant WAZ at 6 months and WLWH had notably lower Xc and PhA at 6 weeks and 6 months, indicating a lower body cell mass, which affects metabolism as it comprises all metabolically active tissue (Aldobali and Pal 2021). Additionally, lower PhAs indicate suboptimal cell membrane health (Norman et al. 2012). Body cell mass integrity determines health, including muscle function and immune response, and is insensitive to major body fluid shifts and other nonnutritional factors (Fiaccadori et al. 2014). Chronic viral infections, such as HIV and the resulting immune response to it, alter the body's cellular milieu, possibly explaining the altered cell membrane function. Maternal nutritional status could additionally alter the body's response to viral infections due to the accompanying immune insufficiency (Sumbria et al. 2021).

Our findings suggest that maternal health is positively associated with infant size. Notably, these factors have not been well studied in the context of HIV (Wells 2018). Growing evidence suggests that sustained improvements in early life and adult prepregnancy nutrition may improve offspring birth outcomes and infant health. The link between birth weight and maternal prepregnancy diet suggests a need for nutritional intervention policies aimed at optimising intergenerational health outcomes (Thayer, Rutherford, and Kuzawa 2020). The added burden of maternal HIV infection and ART could further necessitate interventions in the prepregnancy phase of a woman's life to optimise infant outcomes.

Feeding practices are known to influence infant growth (Gillette, Lohman and Neppl 2017; von Salmuth et al. 2021; Widen et al. 2019). In this study, the comparative analysis demonstrated that most infants were breastfed exclusively for 6 months aligning with the WHO recommendations (World Health Organization 2023). More HUU infants were formula-fed (11% vs. 4.8%) and fewer mixed-fed (23.3% vs. 38.1%) by 6 months, possibly because the mothers were working and separated from their infants for long periods or due to cultural practices, including the early introduction of solids (Vitalis et al. 2021). The prevalence of breastfeeding is also associated with school, work obligations and the availability of formula milk (Vitalis et al. 2021). We found that breast milk macronutrient composition was not affected by the maternal HIV infection status. Macronutrient composition in mature breast milk recorded in our study fell within the ranges reported in the literature (fat: literature: 1.7–3.6 vs. 3.1–3.9 g/100 mL in this study) (Prentice et al. 2016), but the breast milk was lower in carbohydrate and protein content than cited in the literature: (carbohydrates: 8.2–8.6 [in literature] vs. 7.5–7.6 g/100 mL [in current study] and proteins: 1–1.2 [in literature] vs. 0.8–1 g/100 mL [in current study]) (Prentice et al. 2016). Breast milk composition is influenced by feeding frequency, infant age, time of

day, maternal age, method of expression, handling, storage, the total volume of milk removed at the previous feed and the interval between feedings (Ballard and Morrow 2013; Pham et al. 2020; Samuel et al. 2020). Most of this information was not available in our study, but milk collection was standardised, by expressing at a specific time during the morning (Miller et al. 2013).

In this study, BMI and MUAC were positively associated with breast milk protein content. These maternal parameters can be measured at the primary healthcare level, making it a readily available screening tool when considering supplementation for women of breastfed infants. Interventions such as maternal protein supplementation (Wati et al. 2023) could improve breast milk protein content, subsequently improving the protein intake of the infant, but more research is needed.

The study's strengths include its prospective design, the inclusion of an HIV-unexposed control group and the use of standardised measurement protocols. However, study limitations included missing data, small sample sizes and potential selection bias due to participant attrition. The WLWOH control group differed demographically (age, parity, gravidity and education level) from the WLWH. These demographics did not relate to infant size or breast milk macronutrient composition, yet it is possible that our study was not powered to detect such relationships. Additionally, breast milk micronutrient composition was not analysed, which could influence infant growth. Although the feeding modality was recorded and analysed, a detailed breastfeeding schedule was not collected. Additionally, the majority of infants were on breast milk up to 6 months and, therefore, a detailed dietary intake was not assessed. Further research could validate these findings and inform targeted interventions aimed at optimising maternal nutrition and health outcomes.

5 | Conclusion

In this study, HEU infants were smaller than their HUU counterparts up to 6 months of age. Differences in sociodemographic and maternal phenotype between WLWH and WLWOH included level of education, haemoglobin levels, Xc and PhA. Maternal phenotype was associated with infant size and breast milk protein content, and maternal HIV infection predicted decreased infant size for up to age 6 months. Research with larger sample sizes and participants on different antiretroviral regimens, especially with the updated first-line regimen, which now includes dolutegravir, is warranted to define the role of maternal phenotype in infant growth trajectories in the context of HIV and, in so doing, to assist targeted strategies that will provide long-term benefits for both mother and child.

Author Contributions

Marlene Gilfillan: conceptualisation, data collection, data curation, writing of the draft article. **Friedeburg A. M. Wenhold:** conceptualisation, data curation, editing of the draft article for publication. **Helen Mulol:** data curation, statistical analysis, editing of the draft article for publication. **Ute D. Feucht:** Principal investigator of the Siyakhula study, conceptualisation, data curation, editing of the draft article for publication, funding for Siyakhula. All authors read and approved the manuscript.

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Disclosure

The authors confirm that this manuscript is an honest, accurate and transparent account of the study being reported. The reporting of this work is compliant with the STROBE guidelines. The lead author affirms that no important aspects of the study have been omitted.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data presented in this study are available upon request from the primary investigator and the data repository details are omitted for the sake of anonymity.

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