Associations of plasma total phospholipid fatty acid patterns with feeding practices, growth, and psychomotor development in 6-month-old South African infants

Linda P. Siziba¹ | Jeannine Baumgartner¹ | Cristian Ricci¹ | Adriaan Jacobs¹ | Marinel Rothman¹ | Tonderayi M. Matsungo¹ | Namukolo Covic² | Mieke Faber^{1,3} | Cornelius M. Smuts¹

¹Centre of Excellence for Nutrition, North-West University, Potchefstroom, South Africa

²Poverty, Health and Nutrition Division, International Food Policy Research Institute, Washington, DC, USA

³Non-Communicable Disease Research Unit, South African Medical Research Council, Cape Town, South Africa

Correspondence

Linda P. Siziba, Centre of Excellence for Nutrition, Faculty of Health Sciences, North-West University, 11 Hoffman Street, Potchefstroom Campus, Potchefstroom, 2520 South Africa.
Email: sizibalinda@gmail.com

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Abstract

The objective of this study was to assess plasma fatty acid (FA) patterns of 6-month-old South African infants and to determine their association with feeding practices, growth, and psychomotor development. Plasma total phospholipid FA composition (% of total FAs) of 6-month-old infants ($n = 353$) from a peri-urban township was analysed, and principal component and factor analysis were performed to identify plasma FA patterns. Feeding practices, anthropometric measurements, and psychomotor development scores were determined. Four major plasma phospholipid FA patterns were identified: A *plant-based C18 FA*, a *high n-6 long-chain polyunsaturated fatty acids (LCPUFA)*, a *C16:1 and long-chain saturated fatty acid (SFA)*, and a *high n-3 and low n-6 LCPUFA* pattern. Formula feeding was associated with higher, whereas breastfeeding was associated with lower scores for the *plant-based C18 FA* and *C16:1 and long-chain SFA* patterns. On the other hand, breastfeeding, the consumption of cow's milk, and the consumption of semisolid foods were associated with higher scores, whereas formula feeding was associated with lower scores for the *high n-6 LCPUFA* pattern. Breastfeeding and the consumption of semisolids were also associated with higher *high n-3 and low n-6 LCPUFA* pattern scores. The *C16:1 and long-chain SFA* and *high n-3 and low n-6 LCPUFA* patterns were positively associated with psychomotor development scores. In 6-month-old South African infants, we identified distinct plasma FA patterns that presumably represent the FA quality of their diet and that are associated with psychomotor development. Our results suggest that breast milk is an important source of n-6 LCPUFAs and formula-fed infants may be at risk of inadequate LCPUFA intake.

KEYWORDS

breast milk, FA patterns, feeding practices, infant nutrition, LCPUFA status, psychomotor development

1 | INTRODUCTION

Infancy is a critical period of rapid neurodevelopment that requires adequate and proper nutrition. Thus, poor nutrition during the first 1,000 days of a child's life may lead to cognitive and behavioural

deficits later in life (Prado & Dewey, 2014). Long-chain polyunsaturated fatty acids (LCPUFAs) are important structural components of membrane phospholipids in the central nervous system (CNS), the retina, and other tissues (Crawford & Broadhurst, 2012). The LCPUFAs arachidonic acid (AA; C20:4n-6) and docosahexaenoic acid (DHA;

C22:6n-3) are particularly needed for the development and functioning of the CNS.

During the third trimester of pregnancy, DHA and AA are transferred to the foetus via the placenta. Their rapid accretion in the CNS continues during the early stages of infancy after birth (Carver, Benford, Han, & Cantor, 2001). Although DHA and AA can be synthesised *de novo* from their parent essential fatty acids (EFAs) alpha linolenic (ALA; C18:3n-3) and linoleic acid (LA; C18:2n-6), respectively, this conversion process is very limited in infants (Uauy & Dangour, 2009). The European Food Safety Authority considers 100 mg of DHA/day and 140 mg of ARA/day as adequate intakes of LCPUFA for the majority of infants from birth up until the age of 6 months (Agostoni et al., 2013). The World Health Organization (World Health Organization [WHO], & United Nations Children's Fund [UNICEF], 2003) recommends exclusive breastfeeding (EBF) for the first 6 months of life and timely introduction of nutritionally adequate and safe complementary foods thereafter, with continued breastfeeding for up to 2 years and beyond. Breast milk is the main source of LCPUFAs in breastfed infants, and the relative fatty acid (FA) composition of breast milk is associated with maternal plasma FA status and can be influenced by maternal diet (S. M. Innis, 2014).

In South Africa, inappropriate feeding practices, such as mixed feeding and early introduction of complementary foods, were found to be the rule rather than the exception (Faber, Laubscher, & Berti, 2016). With a very high prevalence of human immunodeficiency virus (HIV) in South Africa, inappropriate infant feeding practices in the HIV-positive population are often driven by a desire to protect the infant from HIV. However, there is a higher risk of HIV transmission associated with these feeding practices (Zulliger, Abrams, & Myer, 2013).

Despite a high (83%) breastfeeding initiation rate (Shisana et al., 2013), the percentage of South African children reported to be exclusively breastfed in 2016 decreased with age from 44% in infants aged 0 to 1 month, to 24% in infants aged 4 to 5 months (National Department of Health (NDoH), Statistics South Africa (Stats SA), South African Medical Research Council (SAMRC), & ICF, 2017). Moreover, infants from most low- and middle-income countries are typically introduced to plant-based complementary foods that are insufficient to meet their dietary needs as growing infants (S. Forsyth, Gautier, & Salem, 2016). As a result, infants are introduced to complementary foods that may result in a reduction of dietary LCPUFAs as reflected in their blood FA profiles. This reduction may also be due to a simultaneous reduction in breast milk intake in combination with intake of LCPUFA-poor weaning foods (Rise et al., 2013).

Over a fifth (22%) of the children under 5 years in South Africa are stunted in growth, and stunting is the most prominent form of malnutrition in the country (Hall et al., 2016). Research has shown that inadequate EFA intake could be inversely associated with linear growth of children aged between six and 18 months (Phuka et al., 2008). Thus, a continued supply of dietary DHA and AA during this critical period of rapid growth and development is an important nutritional objective. There are limited data on the LCPUFA profiles of infants in developing countries. Despite inappropriate feeding practices in South Africa, the LCPUFA status of infants who are breastfed and those who receive complementary foods remains unknown; yet infants should be assured

Key messages

- Six-month-old breastfed South African infants living in a peri-urban setting have adequate EFA status.
- Plasma total phospholipid FA patterns presumably represent the FA quality of an infant's diet and are associated with psychomotor development.
- South African infants fed infant formula milk are at risk of inadequate DHA and AA intake, whereas breastfed infants are ensured of n-6 LCPUFA intake.
- Lactating mothers need to consume adequate dietary n-3 LCPUFAs to ensure that breastfed infants are guaranteed of a sufficient supply of n-3 LCPUFAs through breast milk.

of an adequate intake of LCPUFAs for optimal neurodevelopment, growth, and other positive health outcomes (Colombo et al., 2013).

Previously, FAs have been reported and expressed individually as a relative measure of the percentage of total FAs. However, as these FAs are mutually dependent, a change in the proportion of one FA may influence the proportions of many other FAs (Imamura et al., 2012; Voortman et al., 2017). Therefore, synergistic or additive effects may be missed when analysing individual FAs. Principal component analysis (PCA) is an approach that can be used to overcome this limitation because it takes into account these interrelations and generates novel patterns in complex data (Voortman et al., 2017). Thus, in the current study, PCA and factor analysis were used to identify specific plasma phospholipid FA patterns in 6-month-old peri-urban South African infants. Therefore, the aim of the present study was to determine the associations of plasma phospholipid FA patterns with feeding practices, growth, and psychomotor development in 6-month-old South African infants.

2 | METHODS

2.1 | Participants and study site

This cross-sectional study was performed using baseline data of the Tswaka trial, which was a randomised, controlled trial designed to investigate the effects of two small-quantity lipid-based nutrient supplements on linear growth in infants aged six to 12 months (Smuts et al., 2018). The Tswaka trial was conducted during the period of September 2013 and January 2015 in the Jouberton area of the greater Matlosana Municipality in the North West Province, South Africa. The trial was carried out on 6-month-old infants and their mothers, who were recruited from five different clinics in the study area.

Infants were enrolled in the study at the age of 6 months. Infants were excluded if they had never previously been breastfed; had experienced severe known congenital abnormalities; were severely anaemic (haemoglobin <7 g/dl) or severely wasted (weight-for-length Z-score < -2 SD); had other diseases that were referred for

hospitalisation by the clinic staff; had known allergies and/or intolerances to peanuts and/or soy, cow's milk protein, and/or fish; were receiving special nutritional supplements as part of feeding programmes; or were not born as singletons.

2.2 | Data collection and measurements

At enrolment, the infants' health cards were used to confirm their age. A 4-ml venous blood sample was drawn into ethylenediaminetetraacetic acid-coated vacutainers from the antecubital arm area or dorsal area of the hand at the age of 6 months. Venous blood samples were centrifuged for 10 min at 2,000 g and 100- μ l plasma aliquoted and stored at 4°C on site. These samples were transported daily from the study site to the main laboratory at the Potchefstroom campus of the North-West University (NWU) for storage at -80°C until analysis.

If a blood sample could not be obtained from the antecubital arm area or dorsal area of the hand, a blood sample was taken by means of a finger prick only for the haemoglobin (Hb) measurement. The Hb concentrations were determined using the HemoCue system (HemoCue 201+; HemoCue® AB). Anaemia was defined as Hb <11 g/dl. Weight and recumbent length were measured according to WHO (1995) standardised techniques. Infants without clothing were weighed by a research nurse to the nearest 0.01 kg using a digital baby scale (Seca model 354, GmbH & Co. KG., Hamburg, Germany, maximum 20 kg). Recumbent length was measured to the nearest 0.1 cm (Seca 416 infantometer; Seca GmbH & Co. KG., Hamburg, Germany). The WHO (2006) growth standards were used to calculate age and gender-specific Z-scores for weight and length.

Breast milk (fore-milk) samples (5 ml) were collected at baseline between 8:00 a.m. and 12:00 noon from mothers who were still breastfeeding at the time of sample collection and who were willing to provide a sample. Samples were collected in sterile polypropylene tubes by manual expression of the breast that was not used at the last feed. Breast milk samples were aliquoted and stored at -80°C until analysis.

2.3 | Feeding practices

Information on their breastfeeding and complementary feeding practices was collected from participants. A structured questionnaire was developed, based on the WHO (2010) guidelines for assessing infant and young child feeding practices, and was used to collect this information. Some of the indicators included for this study were breastfeeding practices at the time of the study, EBF up to the age of 6 months and whether the infant had been introduced to infant feeding formula. Descriptive qualitative information on the usual consumption of foods by the infants over the past 7 days was collected by the use of a set of unquantified food frequency questions as explained in detail by Rothman et al. (2018). These questions have also been used in other similar studies (Faber et al., 2016; C. M. Smuts et al., 2005).

2.4 | Psychomotor development

The Kilifi Developmental Inventory (KDI; Abubakar, Holding, Van Baar, Newton, & van de Vijver, 2008), which was developed and evaluated

in Africa using materials and activities that both parents and infants can relate to, and a parent rating scale developed for South Africa, were used to assess psychomotor development, as previously described (Osei et al., 2017). Although the tools were developed by a qualified psychologist, for the purpose of this study, we included only those activities applicable to 6-month-old infants. Prior to the commencement of the trial, the KDI was translated into the local language by a team member who was proficient in speaking and writing the language. The KDI was specifically used to assess locomotor development and fine motor skills by means of a parental report and direct observation by fieldworkers.

In this study, the KDI was used to determine different developmental scores for locomotor skills and eye-hand coordination. Locomotor skills were assessed by the use of different activities, which included movement in space, static, and dynamic balance, as well as motor coordination. Although the infant's ability to manipulate objects and engage in activities requiring fine motor coordination was the basis of eye-hand coordination assessments. All activities were scored separately and ranked according to the infant's ability to perform the specific task, as explained in detail by Rothman et al. (2018) and Osei et al. (2017). In addition, a questionnaire on parent rating of motor development was used for parental assessments. This questionnaire allowed each caregiver to rate the infants' gross motor developmental milestones.

2.5 | FA analysis

2.5.1 | FA extraction from plasma samples

Lipids were extracted from plasma samples with chloroform: methanol (2:1, vol:vol) by using a modification of the method of Folch, Lees, and Sloane-Stanley (1957). Lipid extracts were concentrated using nitrogen gas, and thin-layer chromatography (silica gel 60 plates without fluorescent indicator, 10 × 20 cm; Merck) was used to separate neutral lipids from the phospholipids. Phospholipids were eluted using petroleum ether: diethyl ether (peroxide free): acetic acid (90:30:1, vol:vol) and a pinch of ultraviolet (UV) fluorescent indicator 2,5-Bis(5-tert-butyl-benzoxazol-2-yl) thiophene. The lipid fraction containing the phospholipids was visualised under long-wave UV light, removed from the thin-layer chromatography plate and trans-methylated with methanol: sulphuric acid (95:5, vol: vol) at 70°C for 2 hr. This led to the formation of fatty acid methyl esters (FAMES). The FAMES were extracted using hexane and water. The organic layer was aspirated, evaporated, redissolved in hexane, and analysed by gas chromatography-electron ionisation mass spectrometry. All solvents used during the extraction procedure contained 0.01% butylated hydroxytoluene.

2.5.2 | FA extraction from breast milk samples

FAs from breast milk samples were derivatised to their respective FAMES and determined using a modification of the methods described by Liu, Mühlhäusler, and Gibson (2014) and Folch et al. (1957). Aliquots of breast milk samples were subjected to direct *trans*-methylation by incubation with methanol: sulphuric acid (95:5; vol:

vol) at 70°C for 3 hr. Samples were cooled to room temperature, after which the FAMES were extracted with methanol, hexane and water, followed by centrifugation at 1,200×g for 5 min. The top phase containing FAMES was collected by aspiration and evaporated to dryness under nitrogen gas. Samples were redissolved in chloroform: methanol:saline (86:14:1, vol:vol:vol) and subjected to thin-layer chromatography (silica gel 60 plates without fluorescent indicator, 10 × 20 cm; Merck) using petroleum ether:diethyl ether (peroxide free):acetic acid (90:30:1, vol:vol:vol) and a pinch of UV fluorescent indicator 2,5-Bis(5-*tert*-butyl-benzoxazol-2-yl) thiophene as the mobile phase. The separated FAME fraction was visualised under long-wave UV light and removed from the thin-layer chromatography plate. An extraction with methanol: sulphuric acid (95:5, vol: vol), hexane, and water followed, after which the top hexane phase containing the FAMES, was aspirated and evaporated to dryness under nitrogen gas. The FAMES were redissolved in a small volume of hexane and analysed by gas chromatography–electron ionisation mass spectrometry. All solvents used during the extraction procedure contained 0.01% butylated hydroxytoluene.

2.5.3 | Analysis by gas chromatography mass spectrometry

Samples were analysed on an Agilent Technologies 7000 GC/MS Triple Quad system comprising an Agilent 7890A gas chromatograph equipped with an Agilent G7001B triple quad mass spectrometer (Agilent Technologies). The gas chromatography separation of FAMES was carried out on an HP-88 capillary column (100 m × 0.25 mm × 0.20 µm; Agilent Technologies) by using helium as the carrier gas at a flow rate of 2.2 ml/min. Initial inlet temperature was held at 70°C for 0.02 min, after which it was ramped to 270°C at 500°C/min. The mass spectrometer source was maintained at a temperature of 230°C. A sample volume of 1 µl was injected, and split ratios of 80:1 for plasma samples and 10:1 for breast milk samples were used. The oven temperature was maintained at 50°C for 1 min, then ramped to 170°C at 30°C/min, then from 170°C to 215°C at 2°C/min, after which it was ramped to 230°C at 4°C/min. The temperature was then held isothermally at 230°C for 7 min. The total analysis time was 38.25 min. Mass spectrometry with 70 eV electron ionisation was carried out in multiple-reaction monitoring mode, with at least two transitions per compound. The FAMES were quantified using MassHunter Quantitative Analysis software (Version B.05.02, Agilent Technologies). FAME peaks were identified and calibrated against a standard reference mixture of 33 FAMES (Nu-Check-Prep) and two single FAME standards (Larodan Fine Chemicals AB). Relative percentages of FAs were calculated by expressing the concentration of a given FAME as a percentage of the total concentration of all FAMES identified in the sample (Baumgartner et al., 2012).

2.6 | Statistical methods

Data were checked for normal distribution using the Kolmogorov–Smirnov test and visual inspection of histogram plots. Categorical variables were reported as frequencies and percentages. Skewed variables (individual FAs) were log transformed. Data were described

using arithmetic means and standard deviation if normally distributed; the geometric means were used elsewhere. A PCA and factor analysis of the correlation matrix of Blom's normal ranks were (PCA and factor analysis were both used) used to determine the patterns of association between plasma FA (Blom, 1958). The Kaiser criterion (eigenvalues > 1) and the scree plot visual inspection were used to define the factors to be retained. Based on these procedures, the first four principal components or FA patterns were retained for subsequent analyses. To name factors 1, 2, and 3, variable loadings with an absolute value higher than 0.5 were considered, whereas variable loadings with an absolute value higher than 0.2 were considered to name factor 4. Finally, associations of plasma phospholipid FA pattern scores with feeding practices, growth, and psychomotor development were evaluated using a generalised linear model adjusted for sex, age, and Hb. The models determining the associations with psychomotor development were adjusted for sex, age, Hb, and LAZ, as Hb concentrations and LAZ were found to be significant predictors of psychomotor development (Rothman et al., 2018). All statistical tests were two-tailed, and type-I error rate was set to 5%. Statistical analysis was performed using SAS® (version 9.4; SAS Institute, Inc, Cary, NC).

2.7 | Ethical considerations

The Tswaka trial was registered at <http://clinicaltrials.gov> as NCT01845610. The study was approved by the ethics committees of NWU (NWU-00001-11-A1) and the South African Medical Research Council (EC-01-03/2012), adhered to the principles of the Declaration of Helsinki and followed good clinical practice guidelines. The North West Provincial Department of Health and Social Development also reviewed the trial protocol, and the trial was further registered with the Directorate for Policy, Planning and Research. Informed consent was obtained from the parents/legal guardians of the infants before any study-specific procedures were performed.

3 | RESULTS

3.1 | Baseline characteristics and FA status of infants aged 6 months and breast milk FA composition

Of the 750 infants enrolled in the Tswaka trial, plasma samples for total phospholipid FA analysis were available from 353 infants. Table 1 shows the characteristics of these infants. This subsample comprised 53% ($n = 187$) male and 47% ($n = 166$) female infants, with a mean age of 6.2 ± 0.3 months. There were no differences in these characteristics between infants of the Tswaka trial with and without FA data available (data not shown).

Table 2 shows the plasma total phospholipid FA composition of all infants and separately in nonbreastfed and breastfed infants at the time of the survey, as well as the FA composition in available breast milk samples. The geometric mean (95% CI) plasma DHA and AA contents were 4.1 (4.0, 4.3) and 11.5 (11.2–11.8)%, respectively. The plasma total n-6 PUFA content was 6 times higher than plasma total n-3 PUFA. The infants in this study had an essential PUFA status index

TABLE 1 Characteristics of 6-month-old infants with plasma total phospholipid fatty acid data available

Characteristic		Plasma FAs available (n = 353)		Mean ± SD
		n	%	
Males		187	53.0	
Females		166	47.0	
LAZ				-1.4 ± 1.1
Stunting	Stunted (LAZ < -2 SD)	104	29.5	
	Not stunted (LAZ ≥ -2 SD)	249	70.5	
WLZ				0.5 ± 1.1
Wasting	Wasted (WLZ < -2 SD)	1	0.3	
	Not wasted	352	99.7	
Overweight	Overweight (> +2 WLZ)	13	3.7	
	Not overweight	340	96.3	
WAZ				-0.6 ± 1.2
Underweight	Underweight (WAZ < -2 SD)	36	10.2	
	Not underweight	317	89.8	
Hb (g/dl)				11.3 ± 1.1
Hb status	Anaemic (Hb < 11 g/dl)	126	35.7	
	Not anaemic	227	64.3	

Note. BAZ, BMI-for-age Z-scores; FA, fatty acids; Hb, haemoglobin; LAZ, length-for-age Z-score; WAZ, weight-for-age Z-score; WLZ, weight-for-length Z-score.

and a plasma omega-3 index of 3.8 (3.7, 3.8) and 4.4 (4.3, 4.6) %, respectively.

Two thirds (66%, $n = 236$) of infants included in this study were still breastfeeding at the time of the study. However, of only 164 breastfed infants (47%) with FA data available, mothers provided a breast milk sample. There were significant differences between the plasma FA composition of infants who were still breastfeeding and those who were no longer receiving breast milk ($p < 0.05$; Table 2). Breastfed infants had significantly higher plasma DHA and AA but lower plasma ALA and LA compared with non-breastfed infants.

The breast milk samples ($n = 380$) collected from the mothers whose infants were participating in the Tswaka study contained $47.4 \pm 5.3\%$ saturated FAs, $27.5 \pm 3.6\%$ monounsaturated FAs (MUFAs), and $24.1 \pm 4.8\%$ PUFAs. The ALA and LA contents were 0.7 (0.6, 0.7) and 20.6 (20.1, 21.0) %, respectively. The DHA and AA contents were 0.2 (0.2, 0.3) % and 0.8 (0.7, 0.8) %, respectively.

3.2 | Plasma total phospholipid FA patterns

Four latent factors were retained according to the Kaiser criterion (50% cumulative variance of all 29 FAs; Table 3). Factor loadings >0.5 and < -0.5 were used to define each FA pattern (0.2 cut off was used to define factor 4). Each factor was named according to the positive and negative loadings observed with the individual FAs.

3.3 | Associations between baseline characteristics and plasma total phospholipid FA patterns

Associations of baseline characteristics with the FA patterns are presented in Table 4. None of the patterns were associated with growth measurements. Anaemic infants had higher factor scores for the *high n-6 LCPUFA* pattern compared with the nonanaemic infants.

3.4 | Associations between infant feeding practices and total phospholipid FA patterns

Associations of feeding practices with FA patterns are summarised in Table 5. More than two thirds (67%, $n = 236$) of the infants were still breastfeeding at the time of the study (6 months). Almost half (47%, $n = 167$) of the infants had been introduced to formula milk, and most (93%, $n = 329$) of the infants had already been introduced to semi-solids at the time of the study. Only 8% ($n = 27$) of the infants were still exclusively breastfed at 6 months.

Formula feeding was associated with higher, whereas breastfeeding was associated with lower scores for the *plant-based C18 FA* and *C16:1 and long-chain SFA* patterns. On the other hand, breastfeeding, the consumption of cow's milk and the consumption of semisolid foods were associated with higher scores, whereas formula feeding was associated with lower scores for the *high n-6 LCPUFA* pattern. Breastfeeding and the consumption of semisolids were also associated with higher scores of the *high n-3 and low n-6 LCPUFA* pattern.

3.5 | Associations between phospholipid FA patterns and infant psychomotor scores at 6 months

The results of a general linear model to examine the relationship between plasma phospholipid FA patterns and psychomotor scores are shown in Table 6. The mean (95% CI) scores for the different psychomotor developmental categories were as follows: eye-hand coordination subscale: 20.4 (20.1, 20.7), locomotor skills subscale: 16.4 (16.1, 16.6), and parent rating: 20.1 (19.8, 20.4). The maximum possible scores for the combined psychomotor score and eye-hand coordination subscale are 53 and 27, respectively. The *C16:1 and long-chain SFA* pattern was positively associated with locomotor skills, whereas

TABLE 2 Breast milk and infant plasma total phospholipid fatty acid composition of 6-month-old South African infants (% total fatty acids)

Fatty acid		Infant plasma (n = 353)	Plasma non-BF infants (n = 117)	Plasma BF infants (n = 236)	Breast milk (n = 380)
SFA		44.5 (44.4, 44.7) ^b	44.6 (44.3, 44.8)	47.5 (44.4, 44.7)	47.4 ± 5.26 ^c
C14:0	Myristic acid	0.41 (0.4, 0.4)	0.4 (0.4, 0.4)	0.9 (0.4, 0.5)	11.4 (11.1, 11.7)
C16:0	Palmitic acid	25.7 (25.6, 25.9)	26.6 (26.6, 26.8)	25.3 (25.5, 25.5)	19.2 (19.1, 19.4)
C18:0	Stearic acid	15.5 (15.4, 15.6)	15.0 (14.4, 14.9)	16.0 (15.8, 16.1)	6.2 (6.0, 6.3)
C20:0	Arachidic acid	0.5 (0.5, 0.5)	0.5 (0.4, 0.5)	0.5 (0.5, 0.5)	0.1 (0.1, 0.1)
C22:0	Behenic	1.1 (1.1, 1.2)	1.1 (1.1, 1.2)	1.1 (1.1, 1.2)	0.2 (0.2, 0.2)
C24:0	Lignoceric	1.1 (1.1, 1.2)	1.1 (1.1, 1.2)	1.2 (1.1, 1.2)	0.1 (0.1, 0.1)
MUFA		11.5 (11.3, 11.7)	13.2 (12.9, 13.6) ^a	10.7 (10.5, 10.9)	27.5 ± 3.8
C16:1n-7	Palmitoleic acid	0.3 (0.3, 0.3)	0.3 (0.3, 0.4)	0.3 (0.3, 0.3)	2.9 (2.8, 3.1)
C18:1n-9 T	Trans-Vaccenate	0.1 (0.1, 0.1)	0.1 (0.1, 0.1)	0.1 (0.1, 0.2)	0.3 (0.2, 0.3)
C18:1n-9	Elaidate	0.1 (0.1, 0.1)	0.1 (0.1, 0.1)	0.1 (0.1, 0.1)	0.2 (0.2, 0.2)
C18:1n-9	Oleic acid	6.7 (6.5, 7.0)	8.6 (8.3, 8.9)	5.9 (5.8, 6.1)	22.5 (22.2, 22.7)
C20:1n-9	Eicosenoic acid	0.2 (0.2, 0.2)	0.2 (0.2, 0.3)	0.2 (0.2, 0.2)	0.3 (0.2, 0.3)
C24:1n-9	Nervonic acid	2.8 (2.7, 2.8)	2.7 (2.5, 2.8)	2.8 (2.8, 2.9)	0.1 (0.1, 0.1)
n-9 fatty acids					
C20:3n-9	Mead acid	0.00 (0.00, 0.00)	0.01 (0.01, 0.01) ^a	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)
PUFA		42.9 (42.7, 43.1)	41.2 (40.9, 43.9) ^a	43.7 (43.5, 43.9)	24.1 ± 4.8
n-3 fatty acids					
C18:3n-3	ALA	0.12 (0.11, 0.13)	0.24 (0.22, 0.26) ^a	0.09 (0.08, 0.09)	0.65 (0.63, 0.68)
C20:5n-3	EPA	0.27 (0.25, 0.28)	0.33 (0.31, 0.36) ^a	0.24 (0.22, 0.25)	0.06 (0.06, 0.68)
C22:5n-3	DPA	0.86 (0.84, 0.88)	0.81 (0.78, 0.85) ^a	0.88 (0.85, 0.91)	0.14 (0.14, 0.15)
C22:6n-3	DHA	4.11 (3.98, 4.26)	3.12 (2.97, 3.27) ^a	4.73 (4.57, 4.90)	0.24 (0.23, 0.25)
n-6 fatty acids					
C18:2n-6	LA	21.4 (21.1, 21.7)	23.4 (22.9, 23.9) ^a	20.4 (20.1, 20.8)	20.6 (20.1, 21.0)
C20:4n-6	AA	11.5 (11.2, 11.8)	8.85 (8.61, 9.10) ^a	13.1 (12.8, 13.3)	0.76 (0.74, 0.77)
C20:3n-6	DGLA	2.31 (2.26, 2.37)	2.30 (2.21, 2.40)	2.31 (2.25, 2.38)	0.55 (0.54, 0.57)
C22:4n-6	Adrenic	0.66 (0.65, 0.68)	0.57 (0.55, 0.60) ^a	0.71 (0.69, 0.73)	0.19 (0.19, 0.19)
C22:5n-6	Osbond	0.72 (0.70, 0.74)	0.66 (0.63, 0.70) ^a	0.75 (0.72, 0.78)	0.08 (0.08, 0.08)
Total fatty acids					
Σn-3 ^d	n3 PUFA	5.56 (5.43, 5.70)	4.70 (4.53, 4.88) ^a	6.04 (5.88, 6.20)	1.18 (1.15, 1.22)
ΣLC n-3 ^e	n3 LCPUFA	5.39 (5.25, 5.54)	4.43 (4.26, 4.61) ^a	5.94 (5.78, 6.11)	0.50 (0.48, 0.51)
Σn-6 ^f	n6 PUFA	37.7 (37.5, 37.9)	33.8 (36.5, 37.2) ^a	38.1 (37.9, 38.4)	22.8 (22.3, 23.3)
ΣLC n-6 ^g	n6 LCPUFA	15.8 (15.5, 16.2)	12.9 (12.8, 13.3) ^a	17.5 (17.2, 17.8)	2.00 (1.97, 2.04)
Fatty acid ratios					
Total (n6/n3)	n6: n3 PUFA	6.78 (6.61, 6.96)	7.83 (7.51, 8.16) ^a	6.31 (6.13, 6.50)	19.3 (18.6, 19.9)
Total LC (n6/n3)	n6: n3 LCPUFA	2.93 (2.87, 3.00)	2.93 (2.81, 3.04)	2.94 (2.86, 3.02)	4.03 (3.90, 4.16)
Mead/AA	Mead: AA	0.00 (0.00, 0.00)	0.00 (0.00–0.00) ^a	0.00 (0.00, 0.00)	0.00 (0.00, 0.00)
LA/ALA	LA: ALA	176.5 (166.6, 186.9)	97.4 (91.3, 103.8) ^a	237.3 (226.8, 248.3)	31.4 (30.1, 32.8)
DHA/Osbond	DHA: Osbond	5.73 (5.47, 6.02)	4.70 (4.34, 5.09) ^a	6.33 (5.99, 6.70)	2.97 (2.84, 3.09)
	EPA: DHA	0.06 (0.06, 0.07)	0.24 (0.22, 0.26) ^a	0.05 (0.05, 0.05)	0.25 (0.24, 0.26)
	Essential PUFA status index ^h	3.75 (3.67, 3.84)	3.11 (3.02, 3.22) ^a	4.11 (4.02, 4.20)	0.88 (0.86, 0.91)
	Omega 3 index ⁱ	4.43 (4.29, 4.57)	3.49 (3.33, 3.65) ^a	4.99 (4.83, 5.15)	0.31 (0.30, 0.32)

Note. AA, arachidonic acid; ALA, α-linoleic acid; BF, breastfeeding; DGLA, dihomog-γ-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentanoic acid; EPA, eicosapentaenoic acid; FA, fatty acid; LA, linoleic Acid; LCPUFA, long-chain polyunsaturated fatty acids; PUFA, polyunsaturated Fatty Acids.

^aSignificant differences between BF and non-BF infants' plasma total phospholipid FA composition ($p < 0.05$), assessed using general linear models adjusted for age and sex.

^bFAs were log transformed, values presented as Geometric Mean (95% confidence limits), all such values.

^cMean ± SD all such values.

^dSum of all n-3 FAs.

^eSum of all n-3 long-chain fatty acids (20–22 carbons).

^fSum of all n-6 fatty acids.

^gSum of all n-6 long-chain fatty acids (20–22 carbons).

^hRatio between all essential PUFAs (the sum of all n-3 and n-6 FAs) and all nonessential unsaturated fatty acids (the sum of all n-7 and n-9 FAs).

ⁱSum of EPA and DHA.

TABLE 3 Factor loadings of individual plasma total phospholipid fatty acid patterns

Fatty acid		Factor 1 <i>Plant-based C18 FA</i>	Factor 2 <i>High n-6 LCPUFA</i>	Factor 3 <i>C16:1 and long chain SFAs</i>	Factor 4 <i>High n-3 and low n-6 LCPUFA</i>
C14:0	Myristic acid	-0.08512	0.31146	-0.07953	0.38901
C16:0	Palmitic acid	0.54233	0.12284	0.05214	0.07235
C16:1	Palmitoleic acid	0.02023	0.65337	0.50995	0.16399
C18:0	Stearic acid	-0.41741	0.18208	-0.60562	-0.05663
C18:1n-7c	Vaccenic	-0.25638	0.24497	0.40845	0.0189
C18:1n-9T	<i>Trans</i> -Vaccenate	-0.29611	0.38145	0.0973	0.66839
C18:1n-9	Elaidate	-0.20819	0.4299	0.08623	0.69548
C18:1n-9c	Oleic acid	0.70669	0.19602	0.44031	0.10626
C18:2n-6	LA	0.68539	-0.36829	-0.39032	-0.12903
C18:3n-3	ALA	0.84873	-0.12337	0.18656	-0.00985
C18:3n-6	GLA	0.64956	0.40874	0.16755	-0.21754
C18:4n-3	Stearidonic acid	0.31164	-0.02208	-0.18744	0.29924
C20:0	Arachidic acid	-0.28515	-0.64462	0.29343	-0.03793
C20:1n-9	Eicosenoic acid	0.67964	-0.1959	0.08684	-0.10552
C20:2n-6	Docosadienoic acid	-0.26369	0.20097	-0.38591	-0.20464
C20:3n-9	Mead acid	0.52956	0.56111	0.29702	-0.09632
C20:3n-3	Eicosatrienoic acid	0.61321	-0.08161	-0.05916	0.23473
C20:3n-6	DGLA acid	-0.01261	0.54763	0.23014	-0.36373
C20:4n-6	AA	-0.83172	0.21304	-0.05638	-0.0299
C20:5n-3	EPA	0.40659	-0.01193	0.28877	0.17838
C22:0	Behenic	-0.28517	-0.71415	0.48086	-0.01943
C22:1n-9	Erucic acid	-0.01195	-0.19268	0.25176	-0.00831
C22:3n-3	Docosatrienoic acid	0.21827	-0.00046	-0.04247	0.33888
C22:4n-6	Adrenic acid	-0.48409	0.56814	0.20476	-0.3436
C22:5n-3	DPA	-0.25676	0.28583	0.28335	-0.15873
C22:5n-6	Osbond acid	-0.27148	0.60172	0.24431	-0.33654
C22:6n-3	DHA	-0.68521	-0.11894	-0.05851	0.24021
C24:0	Lignoceric acid	-0.34656	-0.62771	0.50642	0.02366
C24:1n-9	Nervonic acid	-0.39641	-0.50198	0.59426	0.02275
Eigen value		6.26	4.45	2.83	1.99
Total variance (%)		22	15	10	7

Note. AA, arachidonic acid; ALA, α -linoleic acid; DGLA, dihomo- γ -linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentanoic acid; EPA, eicosapentaenoic acid; FA, fatty acid; GLA, γ -linolenic acid; LA, linoleic acid; LCPUFA, long-chain polyunsaturated fatty acids; SFA, saturated fatty acids.

Factor loadings >0.5 contribute to defining factors 1, 2, and 3 and are in bold italics. Factor loadings >0.2 contribute to defining factor 4 and are in bold italics

the *high n-3 and low n-6 LCPUFA* pattern was associated with both locomotor skills and eye-hand coordination.

4 | DISCUSSION

In this study, we identified four major plasma total phospholipid FA patterns that were associated with feeding practices and psychomotor development in 6-month-old South African infants from a periurban township: A *plant-based C18 FA* pattern, a *high n-6 LCPUFA* pattern, a *C16:1 and long-chain SFA* pattern, and a *high n-3 and low n-6 LCPUFA* pattern. The *C16:1 and long-chain SFA* and *high n-3 and low n-6 LCPUFA* patterns were positively associated with psychomotor development scores of infants. No associations were found with growth.

The infants in this present study had an adequate EFA and LCPUFA status overall, based on several FA indices in structural

phospholipids that have been used as proxies for assessing EFA and LCPUFA status. However, infants who were no longer breastfed at the time of the study had a significantly lower LCPUFA status. Historically, mead acid levels above 0.21%, the ratio of mead to AA >0.02% (Siguel, 1998), the ratio of DHA to osbond, and the essential PUFA status index (Hornstra, 2000) have been used to determine essential FA deficiency. The presence of mead acid indicates a possible shortage of essential PUFAs, whereas a higher essential PUFA status index indicates a better essential PUFA status. In this regard, infants in the present study had mead levels below 0.21% and a higher essential PUFA status index, which indicate an adequate EFA and LCPUFA status overall.

Infants who were still receiving breast milk, consumed semisolid foods or cow's milk, but not infant formula milk at the time of the survey had higher factor scores for the *high n-6 LCPUFA* pattern. This FA pattern was characterised by high plasma contents of the LA

TABLE 4 Associations between infant characteristics at 6 months and plasma total phospholipid fatty acid patterns

Characteristics	Factor 1		Factor 2		Factor 3		Factor 4			
	N	Plant-based C18 FA aLS (95% CI)	P value ^b	High n-6 LCPUFA aLS (95% CI)	P value ^b	C16:1 and long-chain SFA aLS (95% CI)	P value ^b	High n-3 and low n-6 LCPUFA aLS (95% CI)	P value ^b	
Gender	Female	187	-0.03 (-0.18, 0.12)	0.604	-0.12 (-0.27, 0.03)	0.034	0.08 (-0.07, 0.24)	0.154	0.04 (-0.11, 0.19)	0.486
	Male	166	-0.03 (-0.12, 0.17)		0.10 (-0.04, 0.25)		-0.07 (-0.21, 0.07)		-0.03 (-0.18, 0.11)	
LAZ			0.07	0.204	-0.004	0.938	-0.045	0.437	0.031	0.596
Stunting	Stunted (LAZ < -2 SD)	104	-0.12 (-0.32, 0.07)	0.127	0.09 (-0.11, 0.28)	0.266	0.15 (-0.05, 0.34)	0.082	-0.09 (-0.28, 0.10)	0.268
	Not stunted	249	0.05 (-0.07, 0.17)		-0.04 (-0.17, 0.08)		-0.06 (-0.18, 0.07)		0.04 (-0.08, 0.17)	
WLZ			-0.036	0.199	-0.037	0.145	-0.042	0.338	0.006	0.540
Overweight	Overweight (> + 2 WLZ)	13	-0.06 (-0.61, 0.48)	0.817	-0.33 (-0.88, 0.21)	0.220	0.31 (-0.23, 0.86)	0.251	-0.43 (-0.98, 0.11)	0.114
	Not overweight	340	-0.0 (-0.10, 0.11)		0.01 (-0.09, 0.12)		-0.01 (-0.23, 0.86)		0.12 (-0.09, 0.12)	
WAZ			0.011	0.864	-0.037	0.560	-0.072	0.257	0.024	0.699
Underweight	Underweight (WAZ < -2 SD)	36	0.09 (-0.24, 0.42)	0.551	-0.07 (-0.41, 0.26)	0.671	0.14 (-0.19, 0.47)	0.399	-0.13 (-0.46, 0.20)	0.404
	Not underweight	317	-0.01 (-0.12, 0.10)		0.00 (-0.11, 0.11)		-0.01 (-0.12, 0.10)		0.02 (-0.09, 0.13)	
Hb (g/dL)			0.221	<0.001	-0.209	<0.001	0.09	0.120	0.003	0.959
Hb status	Anaemic (Hb < 11 g/dl)	126	-0.29 (-0.47, -0.12)	<0.001	0.20 (0.02, 0.37)	0.007	-0.09 (-0.27, 0.09)	0.220	0.01 (-0.16, 0.19)	0.844
	Not anaemic	227	0.16 (0.04, 0.29)		-0.10 (-0.24, 0.02)		0.05 (-0.08, 0.18)		-0.01 (-0.14, 0.12)	

Note. FA, fatty acids; Hb, haemoglobin; LAZ, length-for-age Z-score; LCPUFA, long-chain polyunsaturated fatty acids; SFA, saturated fatty acids; WAZ, weight-for-age z-score; WLZ, weight-for-length Z-score.

^aLeast squares (LS) means (95% confidence limits [CL]) of factor scores all such values.

ΔP value is the differences between the factor scores in each category, determined by general linear model with factor scores as outcome variables and characteristics as fixed factor, adjusted for sex, age and Hb (growth parameters).

TABLE 5 Associations between feeding practices and plasma total phospholipid fatty acid patterns

Feeding practice	N	Factor 1		Factor 2		Factor 3		Factor 4	
		Plant-based C18 FA	P value ^c	High n-6 LCPUFA	P value ²	C16:1 and long-chain SFA	P value ^c	High n-3 and low n-6 LCPUFA	P value ^c
BF	Yes	-0.54 (-0.63, -0.47) ^b	<0.001	0.11 (-0.02, 0.24)	0.003	-0.18 (-0.30, -0.05)	<0.001	0.10 (-0.03, 0.23)	0.009
	No	1.11 (0.98, 1.21)		-0.22 (-0.40, -0.04)		0.36 (0.18, 0.54)		-0.20 (-0.38, -0.02)	
EBF	EBF up to 6 mo	-0.68 (-1.05, -0.31)	<0.001	-0.22 (-0.59, 0.16)	0.254	0.06 (-0.32, 0.44)	0.770	-0.24 (-0.62, 0.14)	0.187
	Stopped EBF	0.05 (-0.05, 0.16)		0.01 (-0.10, 0.12)		-0.00 (-0.11, 0.11)		0.02 (-0.09, 0.13)	
FM	Yes	0.62 (0.50, 0.74)	<0.001	-0.25 (-0.40, -0.10)	<0.001	0.13 (-0.02, 0.29)	0.014	-0.02 (-0.17, 0.13)	0.556
	No	-0.60 (-0.71, -0.48)		0.22 (0.08, 0.37)		-0.13 (-0.27, 0.02)		0.03 (-0.12, 0.17)	
Cow's milk	≥ 4 days/week	-0.01 (-0.12, 0.10)	0.995	0.56 (0.15, 0.96)	0.006	0.24 (-0.13, 0.08)	0.215	0.03 (-0.38, 0.43)	0.919
	Seldom/never	-0.01 (-0.42, 0.40)		-0.04 (-0.15, 0.07)		-0.03 (-0.17, 0.66)		0.00 (-0.10, 0.11)	
Semi-solid foods ^a	Yes	-0.00 (-0.11, 0.11)	0.847	0.02 (-0.08, 0.13)	0.017	0.02 (-0.09, 0.13)	0.376	0.03 (-0.07, 0.14)	0.033
	No	-0.05 (-0.48, 0.39)		-0.52 (-0.96, -0.09)		-0.19 (-0.63, 0.25)		-0.46 (-0.89, -0.02)	
Fats	Yes	-0.02 (-0.16, 0.12)	0.888	0.06 (-0.09, 0.20)	0.224	0.01 (-0.13, 0.16)	0.734	-0.05 (-0.20, 0.08)	0.186
	No	-0.01 (-0.17, 0.16)		-0.08 (-0.24, 0.09)		-0.02 (-0.18, 0.14)		0.09 (-0.07, 0.24)	

Note. BF, breastfeeding; EBF, exclusive breastfeeding; FM, formula milk; FA, fatty acid; LCPUFA, long-chain polyunsaturated fatty acids; SFA, saturated fatty acids.

^aFoods first introduced were commercial infant cereal; jarred infant foods; maize meal porridge; other (sorghum or oats porridge, mashed vegetables).

^bLeast squares (LS) means (95% confidence limits) all such values.

^cP value is for the difference between the means of the factor scores in the different categories, determined using general linear model with factor score as outcome variable and feeding practice as fixed factor, adjusted for sex and age.

TABLE 6 Associations between Hb, LAZ, and plasma phospholipid fatty acid patterns and psychomotor development at 6 months

Parameter	Psychomotor development					
	Eye-hand co-ordination		Locomotor skills		Parent rating	
	β estimates	P value	β estimates	P value	β estimates	P value
Hb	-0.332	0.062	-0.050	0.734	-0.102	0.493
LAZ	0.562	0.002	0.409	0.006	0.570	0.000
Age	1.671	0.028	1.576	0.012	1.442	0.024
Gender	0.030	0.939	-0.001	0.997	0.216	0.510
Plant-based C18 FA pattern scores	-0.208	0.287	-0.154	0.337	0.180	0.270
High n-6 LCPUFA pattern scores	-0.011	0.956	-0.005	0.977	-0.088	0.590
C16:1 and long-chain SFA pattern scores	0.076	0.690	0.316	0.046	0.205	0.200
high n-3 and low n-6 LCPUFA pattern scores	0.467	0.014	0.356	0.024	0.179	0.260

Note. FA, Fatty acids; Hb, haemoglobin; LAZ, length-for-age Z-score; LCPUFA, long-chain polyunsaturated fatty acids; SFA, saturated fatty acids.

General linear model adjusted for age, sex, Hb, and LAZ.

Hb concentration was a significant predictor, $p < 0.05$ (Rothman et al., 2018).

LAZ is a significant covariate, $p < 0.05$ (Rothman et al., 2018).

metabolite dihomo- γ -linolenic acid (DGLA; C20: 3n-6), the very long-chain n-6 PUFAs adrenic (C22:4n-6), and osbond acid (C22:5n-6). Similar to AA, adrenic acid accumulates rapidly after birth during the period of the brain growth spurt in infants (Hadley, Ryan, Forsyth et al., 2016).

Furthermore, the DHA composition of breastmilk in this study was lower than the reported mean concentration of $0.32 \pm 0.22\%$, and AA was higher than the average mean concentration $0.47 \pm 0.13\%$ reported by Brenna et al. (2007). Therefore, the observation that this continued breastfeeding-associated FA pattern was characterised by n-6 LCPUFAs but not by n-3 PUFAs, as well as the levels of breastmilk DHA suggest that maternal intake of n-3 LCPUFAs, that is, from fatty oily fish, is very low in this population. Thus, as a balanced supply of the LCPUFAs AA and DHA is important for adequate accumulation of AA and DHA in the growing brain during infancy (Novak, Dyer, & Innis, 2008), breastfeeding women are advised to consume at least 200 mg of dietary DHA to provide breast milk with a DHA content of at least 0.3%. This will ensure that a breastfed infant is guaranteed to receive a daily supply of 100 mg DHA/day, which is enough to meet their metabolic needs (Koletzko, 2016).

In addition, continued breastfeeding and the consumption of semisolid foods were both associated with higher scores for the *high n-3 and low n-6 LCPUFA* pattern. This FA pattern comprised trans-FAs and n-3 LCPUFAs with factor loadings above 0.2, respectively, and negative n-6 LCPUFA factor loadings. Although we used a higher factor loading cut-off to define the other patterns, we used a lower cut-off specifically to define this pattern, because this was the only pattern with positive loadings for DHA and other n-3 LCPUFAs. Also, it is plausible that some of the infants who were breastfed or who had already started eating semisolids were receiving a considerable amount of n-3 LCPUFAs through breast milk or semi-solid foods, respectively.

Omega-3 LCPUFAs play a crucial role in the growth and development of infants, with special implications in the CNS and visual function (González & Báez, 2017). Thus, the positive association between the *high n-3 and low n-6 LCPUFA* pattern and psychomotor

development may be attributed to the role of DHA in brain development. This association could be attributable to the differences in how optimal brain function manifests in different developmental functions. It is for this reason that an adequate intake of n-3 LCPUFAs should be ensured from pregnancy, in early infancy, and during childhood (Meldrum & Simmer, 2016). Therefore, supplementation with n-3 LCPUFAs should be considered when dietary intake is inadequate.

In South Africa, all infant feeding formulas contain the EFAs LA and ALA, but the addition of DHA as an ingredient in infant formula is optional (Codex Alimentarius Commission, 2007). Despite the known importance and need for AA during infancy, the addition of AA to infant formulas has not yet been regulated. Thus, similarly with other studies (J. Forsyth & Willatts, 2002; Makrides, Neumann, Byard, Simmer, & Gibson, 1994), the factor scores for the *plant-based C18 FA* pattern were higher in infants who were receiving formula milk, while being lower in infants who were still exclusively or nonexclusively breastfed. The FA composition of infant formula varies according to lipid sources used, which is based on a mixture of vegetable oils, potentially characterising this *plant-based C18 FA* pattern (Delplanque, Gibson, Koletzko, Lapillonne, & Strandvik, 2015). These results suggest that formula-fed infants may not synthesise enough LCPUFAs to allow adequate accretion in the brain at a rate that is comparable with and corresponds to breastfed infants who receive LCPUFAs through breast milk. Therefore, because preformed LCPUFAs are necessary and because of the acknowledged importance of the LCPUFAs DHA and AA during infancy, LCPUFA-enriched formulas are now common worldwide (Hadley, Ryan, Forsyth, Gautier, & Salem, 2016).

On the other hand, the formula feeding-associated *plant-based C18 FA* pattern was positively associated with Hb concentrations. A possible explanation for this could be that the infants in this study were receiving mostly iron-fortified infant formula milk. In contrast, factor scores for the continued breastfeeding-associated pattern of *high n-6 LCPUFA* were higher in anaemic than nonanaemic infants. This is in agreement with data from Europe (Eussen, Alles, Uijterschout, Brus, & Van Der Horst-graat, 2015), China (Luo et al., 2014), and South Africa (Faber, 2007), showing that the prevalence of anaemia is lower in infants who receive infant formula. Breast milk contains

highly bioavailable iron but in low concentrations. Therefore, the infants' iron status during the first 6 months is dependent on the infants' iron stores at birth, which in turn are dependent on maternal iron status during pregnancy. Thus, the WHO (2009) recommends that infants with healthy birth weight must be supplied with iron at the age of 6 months by the introduction of iron-rich complementary foods. It is plausible that although fortified infant formula milk may not provide the much needed LCPUFAs, it may be a good source of iron in these children.

Moreover, formula feeding was positively, whereas continued breastfeeding was negatively associated with the *C16:1 and long-chain SFA* pattern. This pattern was characterised by high loadings of palmitoleic acid (*C16:1n-7*), which is one of the two predominant MUFAs found in infant formula milk. However, the potential purpose and impact of MUFA supply to the immune system and other functional outcomes have not yet been explored in infants and their nutritional relevance remains unknown (Delplanque et al., 2015). Although found in very low levels in formula milk, nervonic acid is a major very long-chain FA important for myelination, brain growth, and development (Innis, Sprecher, Hachey, Edmond, & Anderson, 1999). It may also be synthesised endogenously in newborns and could therefore have contributed to the positive association observed between the *C16:1 and long-chain SFA* pattern and locomotor skills in psychomotor development. However, its relevance in dietary supply still remains speculative (Delplanque et al., 2015).

In the present study, there were no associations with growth, although research suggests that EFAs are important for optimal linear growth during early infancy (Adu-Afarwuah et al., 2007) and in older children (Jumbe et al., 2016). It is unclear why associations between growth and the plasma FA patterns were not observed in this study, but data from human studies attempting to establish a relationship between FA intake and linear growth resulted in mixed conclusions (Jumbe et al., 2016).

A limitation of this study is the low success rate of 64% (480/750) in drawing blood at baseline. Furthermore, of the successfully drawn blood samples, adequate amounts for FA analysis were only available from 353 infants (47%), owing to the fact that iron status analyses had the highest priority. This led to reductions in sample size for the analyses done in this study. Data used in this current study were collected in one area of the North-West province in South Africa. Thus, our results cannot be generalised to infants in all areas of South Africa. Also, the cross-sectional and observational nature of this study restricts the possibility of drawing conclusions on causation, particularly between FA status and psychomotor development. However, one of the strengths of this study is the use of the PCA and factor analysis to determine FA patterns. The latent factors retained were characterised by high factor loadings of different FAs, hence the strict criterion that was used to define the patterns. The application of PCA is an innovative approach that can be used in future studies to determine distinct patterns taking into account the complexity of individual FA data (Imamura et al., 2012). Therefore, in light of the present study, a combination of FAs may potentially be important in determining the quality of the diet of infants in future studies.

In conclusion, this study identified four distinct plasma FA patterns in 6-month-old South African infants. Our findings suggest that

infant formula-fed infants are at risk of inadequate DHA and AA intake, whereas breastfed infants are likely ensured an adequate supply of n-6 LCPUFAs. However, it is plausible that lactating women do not consume adequate dietary n-3 LCPUFAs, which might lead to an insufficient supply of n-3 LCPUFAs through breast milk. In addition, mothers and caregivers should be advised to give their infants at least two portions of fatty fish a week in order to enhance their intake of EPA and DHA (Smuts & Wolmarans, 2013) during the complementary feeding period. These findings reinforce the importance of the WHO recommendation for EBF up to the age of 6 months, timely introduction of LCPUFA-rich complementary foods and continued breastfeeding for up to 2 years and beyond.

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CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest. CMS received travel support from Unilever, D.S.M., and Sight and Life; TMM received a speaking honorarium from D.S.M; LPS received travel support from D.S.M and Sight and Life.

CONTRIBUTIONS

CMS, JB, MF, and NC conceptualised and designed the study; CMS was the principal investigator of the Tswaka study and had overall responsibility for data collection. MF was the coprincipal investigator of the Tswaka study, contributed to the study design, and provided guidance on analysis of feeding practices. MR and TMM were the study coordinators of the Tswaka study and helped to execute the study. JB, MR, TMM, and LPS executed the study and collected data. AJ developed the method for breast milk analysis and performed biochemical analysis; CMS provided guidance on the development of the method and biochemical analysis and LPS quantified FAs. LPS, CR, and JB performed statistical analyses; CMS, JB, and CR provided guidance on statistical analysis and interpretation. LPS wrote the first draft of the manuscript and all authors read, revised, and edited the manuscript.

ORCID

Linda P. Siziba <https://orcid.org/0000-0002-4773-252X>

Cristian Ricci <https://orcid.org/0000-0003-4113-8682>

Mieke Faber <https://orcid.org/0000-0002-8878-254X>

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