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Breast milk fatty acid profiles in relation to infant growth during the the first four months of life

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Abstract:
Background: *Breast milk of healthy and well nourished, lactating women is a unique and ideal source of nutritional factors such as hormones, cytokines, chemokines and growth factors that ensures adequate growth and development of infants. Among the main components of breast milk, fat is an important energy source and a regulatory factor. Fatty acid composition determines the quality of milk fat. The aim of the study was to determine the association between breast milk polyunsaturated fatty acid profiles and infant growth.*
Materials and Methods: *A prospective cohort study of 114 lactating women was conducted in Gweru, Zimbabwe. The women's 114 babies were also recruited. The mother-infant pairs were followed up from 6 weeks to 16 weeks post-delivery to determine breast milk fatty acid composition as well as infant growth.*
Results: *A significant positive association was observed between breast milk d*ocosahexaenoic acid (*DHA) and infant length at 16 weeks (r (95% CI): 0.05(0.09-0.01; p= 0.018) as well as with weight from 6-16 weeks respectively (r (95% CI): 0.29(0.5-0.08; p=0.007); 0.29(0.49-0.09; p= 0.005). Breast milk e*icosapentaenoic acid (*EPA) levels were significantly positively associated with infant length throughout lactation from 6 weeks: (r (95% CI): 0.06(0.09-0.25; p= 0.001) to 16 weeks 0.04(0.07-0.01; p= 0009). In contrast, there was a significant negative association between breast milk a*rachidonic acid (*AA) and infant length from 6 weeks to 16 weeks (r (95% CI): -0.21(-0.30-0.11; p< 0.001; -0.2(-0.29-0.11; p<0.001) respectively.*
Conclusion: *Human milk fatty acid composition influences infant growth during lactation.*
Keyword: *Fatty acids, Breast milk, Infant growth*

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I. Introduction

Breast milk is the ideal food for neonates and infants, which constitutes a variety of nutrients, growth factors, immune components, and energy[1]. Human milk has a fat content of about 4%, triacylglycerols account for 98% of human milk lipids, and their properties are largely influenced by their fatty acid composition [2]. Breast milk contains more than 200 fatty acids. The majority are saturated fatty acids (48.2%) or monounsaturated (39.8%) followed by polyunsaturated fatty acids (PUFA: 10.8%) [3]. The most important long chain LC-PUFAs in nutrition are Eicosapentaenoic acid (EPA: C 20:5 n-3), Arachidonic acid (AA: C 20:4 n-6) and Docosahexaenoic Acid (DHA: C 22:6 n-3). LC-PUFAs are produced endogenously from the precursors; alpha-linolenic acid (ALA) and linoleic acid (LA) through a series of desaturation and chain elongation steps present in the omega-3 and omega-6 pathways. ALA is eventually converted into DHA, while LA becomes AA after successive reactions in plants[4]. LC-PUFAs are essential for the functional and structural development of an infant. They play a critical role in infants with regards to cognitive, motor, visual and brain development [5]. Additional benefits include modulating the immune response and reduction of blood pressure.

II. Materials and Methods

Study Design: A prospective study of 114 breast feeding women together with their infants (114) was conducted.
Study Location: Participants were recruited from Gweru district polyclinics in Zimbabwe at 6 weeks post-delivery after obtaining written informed consent from each participant.
Sample size: 114 mothers and their infants
Sample size calculation: Schumann's sample size calculation formula was used to determine the minimum sample size (n) as n=3CV2×(Zα+Zβ)2d2, where coefficient of variation (CV)=50% is the expected inter-

individual variability in fatty acid composition, Zβ=0.84, the standard value for a normal distribution at power (β)=80%, Zα=1.96 the critical value for a standard normal distribution at level of significance (α)=5%, d=20%, the difference in fatty acid exposure considered clinically significant , n:=2CV2×(Zα+Zβ)2d2=2×0.52×(1.96+0.84)20.202= 114.

Inclusion criteria:

1. Infants whose mothers had given written informed consent
2. 6 weeks of age,
3. Born singleton
4. Breastfed exclusively for 6 months
5. Visiting the same clinic with their mothers for post antenatal care.

Exclusion Criteria:

1. Mothers with severe obstetric complications
2. Psychiatric disorders.

Procedure Methodology:

Matured breast milk samples (10 m) were collected by manual expression into labelled sterile conical tubes from all the participants at 6 weeks postpartum for determination of LC-PUFAs (EPA, DHA and AA). Milk samples were stored at −80°C pending analysis. The mother-infant pairs were followed up from enrolment to 16 weeks post-delivery to determine the association between fatty acid composition and infant growth. Ethical approval was granted by the Medical Research Council of Zimbabwe (MRCZ/A/2466)

Maternal anthropometric measurements including mid upper arm circumference (MUAC), height and weight were recorded at enrolment. The MUAC was measured from the non-dominant arm. The midpoint between the shoulder and the elbow was determined and the MUAC was then measured with a non-stretchable tape with the limb relaxed and hanging down. The height measurements were taken using a stadiometer (Seca 213, Scorpia Medicare, India). Weight was measured using a bathroom scale (Walmart 573314462, San Leandro, USA). For the infants; birth date, gender, gestational age and anthropometry namely weight (kg), length (cm) and head circumference (cm) recorded at birth were abstracted from the birth records. Thereafter, weight and length measurements were recorded at each visit; 6 weeks and 16 weeks postpartum with an average of two readings was taken. The infant weights were measured while they were putting on light clothing with a digital scale (Seca 3741321004, Hamburg, Germany). The length was measured using a measuring tape.

Whole milk samples were first homogenised for 30 seconds using a vortex mixer (Heidolph Vortex Shaker REAX 1. 220 V. 30 W Germany). Fatty acid extraction was conducted using a method by Kelishadi et., al (2012)[6] .The esterified sample extract (1 µl) was injected into the gas chromatography machine (GC-7890A, Agilent Technologies, USA) coupled with 5975C VL MSD with Triple- axis detector equipped with a splitless capillary intel system. Fatty acids of interest (DHA 22:6n-3, AA 20:4n-6, EPA 20: 5n-3) were identified by comparing the retention times of sample FAME with a standard FAME mixture (SUPELCO 37 Component, FAME mix, Sigma Aldrich, USA).

Statistical Analysis

Statistical analysis was performed using STATA version 14.2 software (Stata Corporation, College Station, Texas, USA). Data was summarised by proportions (%), mean ± standard deviation for normally distributed data and median interquartile range (IQR) for non-normal data. The Pearson's Chi square test was used to assess differences in categorical variables. The independent t-test was used for analysis of normally distributed continuous variables. The association between breast milk fatty acid profile and infant growth parameters was assessed using multiple regression analysis.

III. Results

Table 1 shows demographic and clinical characteristics of lactating mothers. Altogether, 114 participants were enrolled into the study. The mean age of mothers was 29.6±5.2 years. The majority of HIV infected mothers were married (n=42, 36.8%), whilst the secondary level of education was more prevalent (n=64, 56.1%). Furthermore, a greater percentage of the mothers were self-employed (n=55, 48,2%). The monthly income of the mothers was Median (IQR); 81(69-98).

Table 1: Demographic and clinical characteristics of lactating mothers

Characteristics		**Lactating Mothers N=114**
Age (years)	mean (SD)	29.6 (5.2)
Marital status (n %)	Married	42 (36.8)

	Divorced	22 (19.3)
	Single	36 (31.6)
	Widowed	14 (10.5)
Education (n %)	Primary	16 (14.0)
	Secondary	64 (56.1)
	Tertiary	34 (28.1)
Employment (n %)	Employed	35 (30.7)
	S-employed	55 (48.2)
	Unemployed	24 (21.1)
Monthly Income US$	Median(IQR)	81(69-98)
Gravidity	Mean (SD)	2.6 (1.3)
Parity	Mean (SD)	2.5 (1)
MUAC (cm)	Mean (SD)	28.4 (3.5)
Height (cm)	Mean (SD)	165.3 (5.6)
Weight (kg)	Mean (SD)	67.2 (11.6)

Key: *S-employed* : Self –employed; *IQR*: Interquartile range; *SD*: Standard deviation; *US$*: United States dollar

Table 2 shows baseline infant characteristics. A total of 114 infant were included in the study. The mean gestational age was 37.1± 1.2 weeks. Only 16.7% of the infants were born preterm and females constituted 52.6 % of the infant population .

Table 2: Baseline Infant Characteristics

Infant Characteristics		**Infants**
		n=114
Gender n %	Female	60 (52.6)
Birth Weight (kg)	mean (SD)	2.7 (1.5)
Birth weight (<2.5kg)	n %	5 (4.38)
Birth length (cm)	mean (SD)	47.7 (1.6)
Head circumference, cm	mean (SD)	33.5 (1.6)
Gestational age (weeks)	mean (SD)	37.1 (1.2)
Preterm birth <37 weeks	n %	19 (16.7)

Key: *SD*: Standard deviation

Breast Milk Fatty Acid Profile and Infant Growth

Table 3 shows the association between the 6-weeks breast milk fatty acid profile and infant growth parameters. A significant positive association was observed between breast milk DHA and infant length at 16 weeks (r (95% CI): 0.05(0.09-0.01; p= 0.018) as well as with weight from 6-16 weeks respectively (r (95% CI): 0.29(0.5-0.08; p=0.007); 0.29(0.49-0.09; p= 0.005). In addition, breast milk EPA levels were significantly positively associated with infant length throughout lactation from 6 weeks: (r (95% CI): 0.06(0.09-0.25; p= 0.001) to 16 weeks 0.04(0.07-0.01; p= 0009). In contrast, there was a significant negative association between breast milk AA and infant length from 6 weeks to 16 weeks (r (95% CI): -0.21(-0.30-0.11; p< 0.001; -0.2(-0.29-0.11; p<0.001) respectively. However, no association was observed between breast milk fatty profile and infant head circumference during lactation .

Table 3: Association between 6-weeks breast milk fatty acid profile and infant growth

Growth Index	**Age (weeks)**	**DHA 6 Weeks r (95% CI)**		**P**	**EPA 6 Weeks r (95% CI)**	**P**	**AA 6 Weeks r (95% CI)**	**P**
Length (cm)	6 16	0.03(0.07-0.01) 0.05(0.09-0.01)		0.177 **0.018**	0.06(0.09-0.25) 0.04(0.07-0.01)	**0.001** **0.009**	-0.21(-0.30-0.11) -0.2(-0.29-0.11)	**<0.001** **<0.001**
Weight (kg)	6 16	0.29(0.5-0.08) 0.29(0.49-0.09)		**0.007** **0.005**	0.01(0.15-0.18) 0.02(0.14-0.18)	0.896 0.783	-0.27(-0.73-0.18) -0.35(-0.79-0.08)	0.232 0.110
Head circumference (cm)	6 16	0.3(-0.07-0.04) 0.02(1.84-6.98)		0.551 0.549	0(-0.04-0.04) 0.02(-0.06-0.03)	0.932 0.421	0.06(-0.05-0.17) 0(0.12-0.11)	0.291 0.956

Key: *DHA*: docosahexaenoic acid; *EPA*: eicosapentaenoic acid; *AA*: arachidonic acid; *r*: correlation coefficient; *95% CI*: 95% confidence interval; A p-value<0.05 was considered statistically significant. Multiple regression analysis was used to determine the association between breast milk fatty acids and infant growth parameters

IV. Discussion

An important finding from this study was the significant positive association observed between early (6 weeks) breast milk omega-3 fatty acids; EPA, DHA and infant length/weight during lactation. However, negative association of early breast milk AA concentrations with infant length was observed over the course of lactation. This is consistent with findings of Much et al., (2013), who reported consistent negative associations between total omega-6 LCPUFAs and AA with body weight, ponderal index, BMI and lean body mass up to 4 months postpartum [7]. In addition, omega-6 fatty acids were significantly negatively associated with skin-fold thickness and body fat in that study. Therefore, results from the current study suggest that breast milk AA levels serve as important regulators of growth in early postnatal life particularly reducing BMI in infants. Further studies are required to confirm this aspect of infant growth. In contrast, a study conducted in Iran revealed an inverse interaction between maternal dietary fat intake and breast milk omega-3 fatty acids with infant weight at 4 and 12 months of age [8].

In a study done in India, a significant positive correlation was observed between EPA+DHA intake during pregnancy and birth weight of infants[9]. In an observational study from Brazil, breast milk omega-3 fatty acids were positively associated with growth in preterm infants[10]. Rocquelin et al., (2003) reported a significant association between breast milk fatty acids and infant weight in Congo but not in Burkina Faso infants[11]. The discrepancies observed could be related to the different dietary habits among the studied countries[12]. It is postulated that geographical locations do impact the breast milk composition of fatty acids in human milk and consequently differences in n-3 and n-6 fatty acids between Spanish and Finnish breast milk samples have been reported before[13].

Results obtained from this current study suggest that omega-3 fatty acids; DHA, EPA during lactation and early life, possess significant benefits for infant growth and adipose tissue development.

V. Conclusion

Breast milk omega-3 fatty acids; EPA, DHA were positively associated with infant length/weight during lactation. However, a negative association was observed between omega- 6 fatty acids AA and infant growth up to 16 weeks postpartum.

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