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Longitudinal Changes of Human Milk Nutrient Content in the First 6 Months of Lactation

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Abstract Human milk is considered to be the optimal source of nutrition during the first six months of a child's life. Since the milk is the only source of nutrition for the first six months of an infant's life, understanding its composition remains a matter of public health concern. Further, few studies have determined the breast milk composition of lactating mothers in African countries. The aim of this research was to assess longitudinal changes of human milk nutrient content in the first 6 months of lactation**.** A longitudinal descriptive study with repeated measures was adopted by the study. Breast milk nutrient composition was assessed among lactating mothers at the 1st and the 5th month of lactation. The milk energy, lactose, protein, lipids, vitamin A, calcium, magnesium, zinc and iron was assessed at the two time points of lactation (first and fifth month). A total of 104 mature breast human milk samples were collected and analysed for energy and the selected nutrients at the two stages of lactation. Significant differences in the mean nutrient content of proteins (p value = 0.029), vitamin A (p value = 0.004) and iron (p value = 0.015) was observed between the first and the fifth month of lactation. A downward trend in the mean nutrient content for protein was observed while an upward trend was observed for both vitamin A (retinol) and iron between the 1st and 5th month of lactation. Human milk nutrient content varies longitudinally in the first six months of lactation. Studies investigating the causes of the variations are critical in improving the quality of human breast milk and ultimately the growth and development of a child.

Keywords: *human milk, lactating mother, infant, nutrient composition*

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1. Introduction

Exclusive breastfeeding for the first 6 months of life, with continued breastfeeding for 2 years of life or longer alongside appropriate complementary feeding, is recognized as the normative standard for infant feeding [1,2]. This recommendation is supported by extensive evidence which has shown that breast milk is exceptionally suited to human infants due to its nutritional and other bioactive components required for optimal growth and development of a child [1]. The human milk is considered ideal food for the infant as it provides all the nutrients required by the infant for survival and optimal growth [3]. Substantial evidence has indicated that breastmilk contains nutrients and bioactive components that enhances infant gastrointestinal tract health, immune system, physical and cognitive development [4,5,6,7]. Studies have further provided new evidence suggesting that human milk may have a role in programming of late metabolic diseases, particularly protecting against obesity and type 2 diabetes [8]. Breastfeeding has also been shown to confer numerous health benefits to the mother. These benefits include; rapid return of the mother to her pre-pregnancy weight, delayed fertility and have a reduced risk of developing type 2 diabetes [7,9]. Mothers are therefore encouraged to exclusively breastfeed for the first six months and thereafter introduce appropriate complementary foods with continued breastfeeding for up to two years of age and beyond [10]. Since breast milk is the only source of nutrition for the first six months of an infant life, understanding its composition remains a matter of public health concern. Studies have documented that human milk could vary based on many factors [1,11]. Notably, few studies have determined the breast milk composition of lactating mothers in African countries. The aim of this research was therefore to determine the longitudinal changes of breast milk nutrient content among lactating women (0-6 months) in Nyeri, County, Kenya.

2. Materials and Methods

2.1. Study Design and Participants

The study was a longitudinal study which was carried out among lactating women (0-6 months) in Nyeri County, Kenya. The participating women provided samples of

their breast milk at the first and fifth months after delivery. In total, 104 mature milk samples were collected from the lactating mothers at each of the two time points.

2.2. Inclusion and Exclusion Criteria

The study only included mothers who consented to participate in the study, were above 18 years, and had a full-term delivery (≥ 37 weeks in gestational age). The study excluded lactating mothers who had any chronic disease condition.

2.3. Breast Milk Sample Collection

Breast milk samples were collected in the morning (between 0800-1030). Samples were stored at -20°C using portable freezers to allow for transportation to the laboratory. Once in the laboratory, the samples were stored at - 80°C until the final nutrient content analysis was done. The milk samples were collected into 100 mL clean plastic vials. The vials were covered with aluminum foil to protect the milk from light. To allow for comparable samples, mothers were requested to hand express the milk from their breast and the hind milk collected. The process was repeated again within a span of two weeks to reduce day to day variations.

2.4. Breast Milk Sample Analysis

2.4.1. Lactose Content Determination

Analysis and quantification of the mother milk lactose was achieved using chromatographic technology. High Pressure Liquid Chromatography (HPLC) (Shimadzu LC-20A) equipped with a refractive index detector (Shimadzu RID-10A) was used. The mobile phase consisted of acetonitrile: water (75:25) at a flow rate of 0.8 ml/min on NH2P column of (250mm x 4.6 mm x 5ul). Quantification was done using standard solutions of lactose at the concentration range of 0.5-1% g/100ml.

2.4.2. Protein Content Determination

Protein was determined using the Kjeldahl method. In human milk, about 20-25% of nitrogen is non-protein nitrogen (NPN). For this reason, the total protein nitrogen was obtained by subtracting the NPN from the total nitrogen [1,12]. The non-protein nitrogen compounds include creatinine, urea, uric acid and nucleotides [1]. In the analysis, Trichloroacetic acid (TCA) solution was used to precipitate the protein and remove the non-protein nitrogen. Total protein nitrogen was converted to total (true) protein by a factor of 6.38 [13].

2.4.3. Lipid Content Determination

Lipid fraction was extracted and determined from the breast milk as recommended by the Association of Official Analytical Chemists International (AOAC) Official Method 989.05 [14] with slight modifications.

2.4.4 Energy Content Determination

Total energy in the breast milk was calculated from proximate composition of macronutrients (carbohydrates, proteins and fats) through application of energy conversion factors. The study adopted the UN's FAO published energy conversion factor for milk and milk products. The factors are; 3.87, 4.27 and 8.79 for carbohydrates, proteins and fats respectively [13]. The macronutrient (carbohydrates, proteins and fats) were measured in grams per 100 mL of milk.

2.4.5. Retinol Content Determination

Retinol was analyzed by HPLC using a modification of the method by Zahar and Smith [15]. A Shimadzu 20 A series liquid chromatograph equipped with a (250mmx 4.6mm x5 ul) stainless steel ODS reversed- phase column was used to quantify α retinol as measures of vitamins A. The mobile phase was 95:5 methanol: water, for separation at a flow rate of 1 ml/min and injection volume was 20ul. The retinol was monitored at 325 nm wavelength on a UV-VIS detector (Shimadzu SPD 20 A). External standards were compared to sample extracts for determination of the vitamin concentrations.

2.4.6. Minerals Content Determination

Determination of minerals was done by dry ashing and atomic absorption spectrophotometer (AAS), according to AOAC [16]. In this study, the minerals that were determined were calcium, magnesium, zinc and iron. Acid washed mineral free containers were used to store the samples. For calcium determination, lanthanum chloride as a releasing agent was used. Suitable standard solutions were prepared, their absorbance's measured and calibration curves prepared. Concentrations of the minerals in the samples was determined by reading absorbance's against calibration curves prepared from standard stock solutions of each mineral. The mineral detection wavelengths were; 422.7 nm, 285.2 nm, 248.3 nm and 213.9 nm for calcium, magnesium, iron and zinc respectively.

2.5. Statistical Analysis

The statistical analysis was performed using Statistical Package for Social Sciences (S.P.S.S) version 24 for windows. Data are presented as means ± SD (standard deviation). Normality test were conducted using the Shapiro-Wilk test. Where data was normally distributed Student t-test was used to check for mean differences based on the stage of lactation. For skewed data, Wilcoxon Signed Rank test was used. Frequency and percentages were used to for categorical variables to describe the study population. A p -value less than 0.05 was considered statistically significant.

2.6. Ethical Considerations

The study received ethical approval from Kenyatta University Ethics Review Committee (KUERC). Further, the study was approved by the Kenya National Council for Science, Technology and Innovation (NACOSTI). All the participants provided their written consent to participate in the study.

3. Results

3.1. Characteristics of the Study Participants

The mean age of the participants was 28.83 (± 6.47 SD) years. Majority (76.0%) of the mothers had a vaginal delivery and most (90.4%) of them were multiparous. Majority of the mothers indicated that they had initiated breastfeeding within 1 hour after delivery. Notably, slightly more than half (55.8%) of the participants had male infants. All the infants were exclusively breastfed. Table 1 presents the characteristics of the participants.

Table 1. Characteristics of the study participants

Characteristic (N=104)	(n)	(%)
Age category (years)		
≤24	31	29.8
25-34	51	49.0
35-44	22	21.2
Mean age: 28.83 (± 6.47 SD)		
Marital status		
Married	86	82.7
Unmarried	18	17.3
Mode of delivery		
Caesarean section	25	24.0
Vaginal delivery	79	76.0
Infant sex		
Male	58	55.8
Female	46	44.2
Mothers parity		
Primiparous	10	9.6
Multiparous	94	90.4

3.2. Breast Milk Nutrient Composition of the Lactating Mothers

Where necessary, the human milk specific gravity of 1.031 g/mL was used for unit conversion [12]. The lactating mother's milk energy and nutrient profile is detailed in Table 2. The mean values at the two stages of lactation are also presented. Downward trends for energy and all the other nutrients was observed except for vitamin A and iron which had an upward trend. The study further observed that average milk concentration at the two stages of lactation for proteins, vitamin A and iron was significantly different (p value < 0.05). The mean concentration of energy and the other selected nutrients were not significantly different at the first and fifth month postpartum among the mothers.

Table 2. Energy and nutrient profile of the lactating women milk

		N=104		
		Month of lactation		
Nutrient	Units	Month 1	Month 5	P value
Energy	Kcal	66.36 ± 21.45	63.20 ± 19.87	0.284
Carbohydrate (Lactose)	g/dL	6.94 ± 1.40	7.08 ± 1.48	0.413
Proteins	g/dL	0.96 ± 0.31	0.85 ± 0.25	0.029*
Fats	g/dL	3.88 ± 2.33	3.50 ± 2.13	0.231
Vitamin A (retinol)	µg/dL	22.48 ± 23.51	31.61 ± 21.97	0.004*
Calcium	mg/dL	28.64 ± 17.87	27.39 ± 18.09	0.626
Magnesium	mg/L	19.16 ± 17.79	15.92 ± 13.04	0.128
Zinc	mg/L	0.63 ± 0.51	0.51 ± 0.45	0.090
Iron	mg/L	0.39 ± 0.40	0.47 ± 0.32	0.015*

p value; paired student t-test/Wilcoxon Signed Rank test.

The Figure 1 below presents the lactating women breastmilk trend between 1st and 5th month of lactation postpartum.

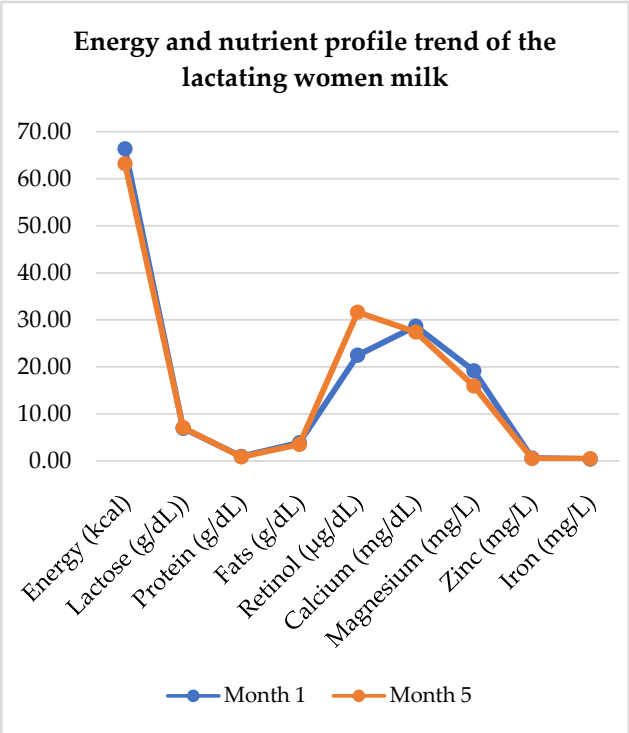

Figure 1. Lactating women breastmilk trend between 1st and 5th month of lactation postpartum

4. Discussion

4.1. Macronutrients and Energy Composition

Human milk has higher concentration of lactose than any other species reflecting higher nutritional requirements for human beings [17]. As reported by previous studies, this study confirmed that breast milk macro nutrients content has a wide variation [18,19]. According to Ballard et al. [1] the lactose content of mature breast milk is estimated to range from 6.7 to 7.8 g/dL in the first year of lactation.

The mean lactose concentration observed by this study (month one-6.9 g/dL and month five-7.1 g/dL) was in agreement with the estimated ranges. The results of this study were also comparable to those reported by other studies. A study conducted among Korean mothers and another conducted among Chinese mothers reported a mean lactose concentration of 7.1 g/dL [18,20]. The concentration of human milk lactose is said to be the least variable and has been reported to vary from 6.3-8.1 g/100mL [21]. In agreement with the current research, the concentration of lactose concentration in this study did not differ significantly during the two stages of lactation (1st month- 6.94 g/100mL, 5th month- 7.08 g/100mL) that were focused by this study.

Milk proteins provide nutritional, immunological and hormonal support to the growing infants. The protein content of mature milk observed in this study (month one-1.0 g/dL and month five-0.9 g/dL) was comparable to that of Chinese mothers (0.9 g/100mL) and Australian mothers

(1.0 g/100mL) [20,22]. However, results from a study conducted among Korean mothers showed a higher protein level (1.4 g/100mL) compared to this study [18]. The difference could be due to the non-inclusion of the non-protein nitrogenous substances in the current study which account for approximately 25% of the total nitrogen. These non-protein nitrogenous substances include urea, uric acid, amino acids, creatine, creatinine and nucleotides [1]. This study also observed significant difference in the protein levels at the 1st and 5th month of lactation. This denotes that the stage of lactation is a predictor of human breast milk protein content. Similar decrease in protein content as lactation progresses have been observed by other studies [23,24].

The mean fat content in the breast milk of the current study participants (3.9 g/100mL in 1st month and 3.5 g/100ml in 5th month post-delivery) was comparable to findings of other studies conducted in New Zealand (3.8 g/100mL), China (3.4 g/100mL), Japan (3.6 g/100mL), Poland (3.5 g/100mL), Korea (3.0 g/100mL) France (3.5 g/100mL) and the United States (3.2 g/100mL) [18,20,25-29]. Some of the minimal divergence between the findings may relate to differences inherent to the method of milk collection, time of collection, dietary habits and storage conditions. It is reported that fat is the most variable macronutrient in the human milk. Factors such as time of collection (morning or evening) and type of milk (fore or hind milk), frequency of feeding and maternal diet could cause such variability [30,31].

Energy content of the milk observed in the present study was 66.4 kcal/100mL in the first month and 63.2kcal/100mL in the fifth month postpartum. The energy content reported in the current study was consistent with values reported by Bzikowska et al. [26] who reported an energy value of 65.9 kcal/100mL at first month and 61.2 kcal/100mL at fifth month postpartum. Zielinska et al. [32] reported an energy value of 69.5 kcal/100mL at first month of lactation. Another study by Czosnykowska-Łukacka et al. [33] reported an average energy of 65.8 kcal/100mL among mothers with infant 1-12 months which agreed with the present study. Contrary to these studies, a relatively lower mean energy (56.7kcal/100mL) was observed by Abranches et al. [34] in their study. The difference could be explained by the variations in the milk macronutrient contents. The participants in that study had a low milk fat content. Milk fat content is the major contributor of the overall energy content of milk [35,36]. Notably, there was no significant difference on the mean energy at first and fifth month postpartum. This indicates that energy content of breast milk from 1-5 month post-delivery may not vary based on the stage of lactation.

4.2. Micronutrients Composition

The mean concentration of vitamin A observed in the present study was 22.5 µg/100mL and 31.6 µg/100mL at first and fifth month respectively. There was a significant difference in the means at the two stages of lactation. This indicates that stage of lactation is an important determinant of breast milk vitamin A content. The mean concentrations from the present study were slightly lower or higher than those reported in other studies. A study conducted among mothers in South Korea reported breast milk retinol levels of 36.4 µg/100mL at the fifth month of lactation which was consistent with this study [37]. Two other studies documented a slightly higher concentration of 59.8 µg/100mL and 57.1 µg/100mL [38,39]. Considerable higher levels (81.5 µg/100mL) in comparison with this study were reported among Turkish lactating mothers [40]. On the other end, a study conducted by Hailu et al. [41] reported a lower mean of 12.9 µg/100mL. The differences could be due to differences in dietary intakes and nutrition status among the populations or due to methodological factors, such as time of milk collection, stage of lactation and sample storage conditions.

The mothers' milk calcium mean was 28.6 mg/100mL and 27.4 mg/100mL at first and fifth month respectively. These means are comparable with that reported by other studies from different populations. For example, Butts et al. [25] reported a mean of 27.5 mg/100mL, 29.1 mg/100mL and 30.9 mg/100mL in three different ethnic groups of New Zealand. Another study by Vítolo, et al. [42] reported a mean calcium concentration of 28.0 mg/100mL. Further, Kim et al. [37] reported a breast milk calcium concentration of 29.8 mg/100mL at first month of lactation and 27.1 mg/100mL at 5th month of lactation.

The lactating mothers' breast milk magnesium mean was 1.9 mg/100mL and 1.5 mg/100mL at the two stages of lactation. Other studies have reported relatively similar or slightly different values. Butts et al. [25] in their study reported a mean of 3.08 mg/100mL, Mastroeni et al. [43] reported a mean concentration of 2.9 mg/100mL while Daniels et al.[38] reported a mean of 3.0 mg/100mL in the mature milk of the participating mothers. Additionally, a study conducted among South Korea mothers documented a concentration of 2.9 mg/100mL and 3.0 mg/100mL at first and fifth month respectively [37]. The differences could be inherent to the analytic methods used or maternal factors influence on the milk composition. In the present study there were no statistically significant differences in the means observed in this study based on the mother's stage of lactation.

A downward trend was observed in the mothers' breast milk zinc concentration (first month- 0.6 mg/100mL^{-1} and fifth month- 0.5 mg/100mL^{-1}). Similar trends have been observed in other studies where statistically significant differences were noted based on the stages of lactation [44]. A systematic review by Yang et al. [20] supports this observation and indicates that zinc concentration in breast milk decreases rapidly as the stage of lactation progresses. Comparable, slightly lower or higher zinc content level was observed in mature breast milk of lactating mothers from Latvia (0.1 mg/100mL^{-1}), Iran (0.1 mg/100mL), Indonesia (0.1 mg/100mL) and Tehran (0.3 mg/100mL) [3,38,45,46]. The differences could be due to the analytic methodologies used, sampling time (morning-afternoon-night or foremilk-hind milk), dietary habits and other environmental factors. Notably, zinc is essential for normal functioning of the body and its deficiency can lead to retardation in child growth and development. However, when taken in excess the metal can be harmful to the body [47,48].

The iron content of breast milk is often characterized as low. However, the iron amount provided by human breast

milk is adequate to prevent iron deficiency anaemia for at least the first 6 months of a child's life. Longitudinal analysis of milk composition in this study showed an iron concentration of 0.39 mg/L and 0.47 mg/L during first and fifth month postpartum. Other previous studies have reported similar or close values for the iron concentration of breast milk, ranging from 0.2 to 0.7 mg/L [38,49-52]. The differences reported by these studies may be due to differences in sampling procedures, analytical procedures, inter-individual variability as well as stage of lactation. Furthermore, a difference was found in the concentration of the breast milk iron content at first and fifth month of lactation. A significant increase in the concentration was observed. This finding is however inconsistent with a previous study in which breast-milk concentrations of iron decreased significantly between 1st and 3rd month of lactation [50]. The difference could be due to maternal iron status of the participating mothers. A recent study has reported that maternal iron status during pregnancy may affect the quantity of iron during lactation [53].

5. Conclusion

This study confirms that human milk nutrient composition is variable and varies longitudinally. Due to the nutrient content variability observed in this study, establishing the factors that causes human milk variability during lactation could importantly help in improving human milk quality and the eventual growth and development of a child.

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Conflicts of Interest

The authors declare no conflict of interest.

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