RESEARCH PAPER

Macronutrients in Breastmilk of Mothers of Preterm Infants

SHIWANI MAHAJAN, DEEPAK CHAWLA, *JASBINDER KAUR AND SUKSHAM JAIN

*From Departments of Pediatrics and *Biochemistry, Government Medical College and Hospital, Chandigarh, India.*

Correspondence to: Dr Deepak Chawla, Department of Pediatrics, Government Medical College and Hospital, Chandigarh, India. drdeepakchawla@gmail.com

Received: August 26, 2016; Initial Review: November 15, 2016; Accepted: May 16, 2017

Objectives: To evaluate the variability in macronutrient and energy content of breastmilk of Indian women delivering at ≤34 weeks of gestation. **Methods**: In this cross-sectional study, samples of breastmilk expressed manually for feeding of preterm neonates were collected from 106 mothers at 3±1 (*n*=26), 7±2 (*n*=34), 14±2 (*n*=24), 21±3 (*n*=12) and 28±3 (*n*=10) days after birth. Protein, fat and carbohydrate content were estimated and total energy content was calculated. **Results:** Protein content in the human milk declined from 4.1±2.1 g/dL on the 3rd postpartum day to 2.2±0.6 g/dL by the 28th day postpartum. Lactose (from 2.2±0.7 g/dL to 3.0±0.9 g/dL), fat (1.9±1.8 g/dL to 3.4±2.1 g/dL) and energy (42.3±18.8 Kcal/dL to 51.9±21.5 Kcal/dL) contents increased from day 3 to day 28. **Conclusions:** Preterm human milk has high temporal and inter-individual variation in the macronutrient composition and without fortification is unlikely to meet the nutritional requirement of preterm neonates.

Keywords: *Lactose, Lipids, Proteins.*

Published online: June 04, 2017. PII:S097475591600068

Breast milk decreases the risk of late-onset sepsis and necrotizing enterocolitis in preterm neonates and is therefore preferred over formula milk [1]. For adequate growth, enteral nutrition must be able to provide 110-135 Kcal/kg/day of energy and 3.5-4.5 g/kg/day of proteins [2]. Breastmilk secreted by mothers of preterm neonates is deficient on both these counts and therefore needs fortification [3]. However, neonates fed human milk even after fortification grow slower than the neonates fed formula milk [4]. One probable reason of this is variation in composition of human milk. Previous studies, mainly from developed countries, demonstrated that the breastmilk composition changed as lactation progressed [3,5]. However, fortification strategies assume a uniform value of nutrient composition of breastmilk [6]. Evaluation of the nutritional content of human breastmilk is important to determine its sufficiency as the source of nutrients for preterm neonate. Data is sparse about temporal changes and inter-individual variation in breastmilk composition in Indian mothers delivering prematurely. Objective of this study was to evaluate variability in macronutrient composition of human breastmilk of Indian mothers who deliver prematurely.

METHODS

This cross-sectional study was carried out at the Departments of Pediatrics and Biochemistry of a tertiary care hospital in Northern India from July, 2014 to September, 2014. Study protocol was approved by the Institutional Research and Ethics Committee and written informed consent was obtained from lactating women who participated in the study. Mothers of preterm neonates born in the hospital were enrolled if they fulfilled the following criteria: gestation at delivery ≤34 completed weeks, normal blood hemoglobin and glucose levels, normal renal and liver function tests and milk supply sufficient to meet the the volume needed by the baby. Mothers who had chronic illness like hypertension, diabetes mellitus or thyroid dysfunction were excluded. All women obtained comprehensive instructions about breastfeeding techniques and milk collection procedures including pumping. Samples of milk for the study were taken from either or both breasts by manual expression on 3±1, 7±2, 14±2, 21±3 and 28±3 days after birth. To minimize any effect of diurnal variation, sample collection was done in the morning hours between 9:00 AM and 11:00 AM 10 mL of milk was collected for analysis. Total protein estimation was done by the Modified Lowry method [7,8]. Fat content was measured using a commercial enzymatic kit. In this method, the fat is measured as triglycerides [8]. Lactose content of the milk sample was measured by Nelson Somogyi method [8]. The coefficient of variation of these three methods was less than 5%. Energy estimate was made by using Atwater energy multiplication factors: carbohydrates (4 Kcal/g), protein (4 Kcal/g) and fat (9 Kcal/g) [9].

Data was entered in a spreadsheet and analyzed with statistical software R. Variability within each epoch of milk collection was evaluated by calculating percent variability [10]. Percent variability was calculated using following formula: Percent variability = (x - mean) × 100/Mean.

A previous study had reported 15±13% variability in protein and fat content of non-pooled own mother's milk [10]. To detect 20±13% variability with alpha error of 0.05 and power of 80% we needed to enroll 107 mothers. Significance of temporal change in macronutrient contents was assessed using Kruskal-Wallis rank sum test.

RESULTS

Milk samples were collected from a total of 106 mothers. Of these, 15 delivered at <30 weeks of gestation, 56 at 30-32 weeks and 35 at 33-34 weeks of gestation. Milk samples were collected from 26 women on postpartum day 3±1, from 34 women on postpartum day 7±2, from 24 women on postpartum day 14±2, from 12 women on postpartum day 21±3, and from 10 women on postpartum day 28±3. Mothers delivering prematurely were of the mean (SD) age of 25±4.2 years. The most common factors responsible for delivery at ≤34weeks of gestation were preterm rupture of membranes (41%) and eclampsia (15%). About one-third women delivered by a C-section and the median gestational age at delivery was 32 weeks (IQR 31-33).

Over the first 4 weeks postpartum, there was a significant decline (*P*=0.006) in the protein content from 4.1±2.1g/dL on the 3rd day to 2.2±0.6 g/dL by the 28th day. The fat content of colostrum (3rd day) was lower than the fat content of transitional milk (7th day) and mature milk (14th, 21st, 28th day, *P*=0.03). The lactose content also increased from 2.2 g/dL in the first week to 3.0 g/dL by the fourth week. The energy content of milk escalated progressively from 42.3 kcal/dL in the first week to 52 kcal/dL by the fourth week (*P*=0.01). High degree of variability was observed in macronutrient composition even when milk expressed at each time-point was assessed (***Table* I**).

To determine whether the nutritional requirements of the preterm neonate were being adequately met, the recommended and actual values for protein and energy intake was calculated for each neonate assumed to be receiving either 150mL/kg/day or 180mL/kg/day of breastmilk. It was observed that in 102 (96.2%) neonates the minimum prescribed energy intake of 110 Kcal/kg was not met even with 180 mL/kg of breastmilk. With intake of 180 mL/kg of breast milk, the minimum prescribed protein intake of at least 3.5 g/kg was not met in 67 (63.2%) neonates.

DISCUSSION

This study reiterates significant inter-individual and temporal variation in the macronutrient content of human breastmilk and inability of preterm milk to meet macronutrient requirement of preterm neonates. The preterm milk is rich in protein initially and with increase in postpartum age there is a decline in protein content and an increase in the amount of lactose, fat and energy. Although multicomponent fortification of milk can improve the macronutrient profile, wide inter-individual variation reported in the present study highlights the inappropriateness of use of fixed-composition commercially available milk fortifiers. Large variability in the protein and fat content of human milk has also been observed in a systematic review of the studies which have reported macronutrient composition of human milk [3]. Although studies from developing countries were excluded from this review to exclude mothers with suboptimal nutrition, protein content reported is lower than the present study at all the postnatal age time-points. The increase in fat content with postnatal age and stabilization of the content by second postnatal week are comparable with the present study. Lactose content observed in the present study is lower than levels reported by many other studies [8,11,12]. However, lactose content of the milk decreases with degree of prematurity and similar low levels have been reported previously by Narang, *et al*. [13] in the milk obtained during first week after birth. In addition, it is possible that some of milk samples in the present study consisted predominantly of hind-milk which is relatively rich in fat and contains less of proteins and lactose.

TABLE I COMPOSITION OF BREAST MILK OF PRETERM MOTHERS

Age	*Protein (g/dL)*		*Fat (g/dL)*		*Lactose (g/dL)*		*Energy (kcal/dL)*	
Post-partum	*Mean (SD)*	*Percent Variability*	*Mean (SD)*	*Percent Variability*	*Mean (SD)*	*Percent Variability*	*Mean (SD)*	*Percent Variability*
Day 3±1 (*n*=26)	4.1 (2.1)	43.7	1.9 (1.8)	24.4	2.2 (0.7)	68.2	42.3 (18.8)	29.7
Day 7±2 (*n*=34)	2.8 (0.7)	20.7	2.6 (1.4)	29.9	2.4 (0.9)	39.9	44 (12.4)	21.0
Day 14±2 (*n*=24)	2.6 (1.0)	24.7	3.2 (1.1)	14.5	2.8 (0.5)	28.0	50.5 (11.9)	19.3
Day 21±3 (*n*=12)	2.3 (0.6)	21.8	3.3 (0.8)	19.4	3.0 (0.3)	43.2	50.7 (8.2)	25.8
Day 28±3 (*n*=10)	2.2 (0.6)	47.5	3.4 (2.1)	79.9	3.0 (0.9)	53.0	51.9 (21.5)	33.8

WHAT THIS STUDY ADDS?

- Preterm breastmilk shows marked temporal and inter-individual variation in protein and energy content and is not able to meet nutritional requirements of preterm neonates.

Decrease in protein and inadequate fat content of breastmilk can compromise the physical and brain growth of preterm neonates. Small studies from developed countries have demonstrated the feasibility of targeted and individualized fortification [6,14]. This consists of adding medium-chain triglycerides and/or protein hydrolysates to meet the prescribed range of protein and energy intake. However, influence of individualized fortification on long-term growth and neuro-developmental outcomes has not been investigated.

The milk analyzed in this study was collected by a standardized procedure designed to minimize variations and sample collection was restricted to 2 hours in the morning to prevent any diurnal variation. Although, a 24-hour pooled sample would have minimized the effect of diurnal variation in the fat content, this approach would have deprived the infant of mother's milk for a day. Another limitation of the study is the cross-sectional samples of breastmilk instead of longitudinal follow-up of all the enrolled mothers. The modified Lowry method used for measurement of proteins is susceptible to interference by other compounds like carbohydrates, calcium, magnesium and other chemicals used as buffers in the laboratory.

In conclusion, this study suggests that the macronutrient composition of breastmilk is highly variable and may not be able to meet the energy and protein demands of growing preterm neonates. In view of large inter-individual variation observed, further studies are needed to evaluate the role of routine measurement of content of macronutrients and individualized fortification of the breastmilk.

Acknowledgement: ICMR-STS-2014 program for providing a student fellowship to complete the project.
Contributors: SM, DC, JK: planned the study and wrote study protocol; SM: collected the data; DC: analyzed the data; JK: laboratory measurements were supervised; DC,SJ. data was interpreted; SM: wrote first draft of the manuscript. All authors approved the final draft.
Funding: None; *Competing interest*: None stated.

REFERENCES

1. Section on Breastfeeding. Breastfeeding and the Use of Human Milk. Pediatrics. 2012;129:e827-41.
2. Agostoni C, Buonocore G, Carnielli VP, De Curtis M, Darmaun D, Decsi T, *et al*. Enteral nutrient supply for preterm infants: Commentary from the European Society of Paediatric Gastroenterology, Hepatology and Nutrition Committee on Nutrition. J Pediatr Gastroenterol Nutr. 2010;50:85-91.
3. Gidrewicz DA, Fenton TR. A systematic review and meta-analysis of the nutrient content of preterm and term breast milk. BMC Pediatr. 2014;14:216.
4. Rozé J-C, Darmaun D, Boquien C-Y, Flamant C, Picaud J-C, Savagner C, *et al*. The apparent breastfeeding paradox in very preterm infants: relationship between breast feeding, early weight gain and neurodevelopment based on results from two cohorts, EPIPAGE and LIFT. BMJ Open. 2012;2:e000834.
5. Kreissl A, Zwiauer V, Repa A, Binder C, Thanhaeuser M, Jilma B, *et al*. Human Milk Analyser shows that the lactation period affects protein levels in preterm breastmilk. Acta Paediatr. 2016;105:635-40.
6. Reali A, Greco F, Fanaro S, Atzei A, Puddu M, Moi M, *et al*. Fortification of maternal milk for very low birth weight (VLBW) pre-term neonates. Early Hum Dev. 2010;86:33-6.
7. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193:265-75.
8. Paul VK, Singh M, Srivastava LM, Arora NK, Deorari AK. Macronutrient and energy content of breast milk of mothers delivering prematurely. Indian J Pediatr. 1997;64:379-82.
9. Nichols BL. Atwater and USDA nutrition research and service: a prologue of the past century. J Nutr. 1994;124:1718S-27S.
10. de Halleux V, Rigo J. Variability in human milk composition: benefit of individualized fortification in very-low-birth-weight infants. Am J Clin Nutr. 2013;98:529S-35S.
11. Faerk J, Skafte L, Petersen S, Peitersen B, Michaelsen KF. Macronutrients in milk from mothers delivering preterm. Adv Exp Med Biol. 2001;501:409-13.
12. Bauer J, Gerss J. Longitudinal analysis of macronutrients and minerals in human milk produced by mothers of preterm infants. Clin Nutr Edinb Scotl. 2011;30:215-20.
13. Narang APS, Bains HS, Kansal S, Singh D. Serial composition of human milk in preterm and term mothers. Indian J Clin Biochem. 2006;21:89-94.
14. Radmacher PG, Lewis SL, Adamkin DH. Individualizing fortification of human milk using real time human milk analysis. J Neonatal Perinatal Med. 2013;6:319-23.