The Effects of Energy and Macronutrient Content of Human Milk on the Growth of Preterm Infants: A Prospective Cohort Pilot Study

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ABSTRACT

Objective: To assess the effect of energy and macronutrient content of expressed breast milk on the growth of preterm infants.

Design: An observational prospective cohort pilot study.

Setting: At the NICUs following the three main hospitals in the Kingdom of Bahrain, Salmaniya Medical Complex (SMC), Bahrain Defence Force Royal Medical services (BDF), and King Hamad University Hospital (KHUH).

Methods: Expressed breast milk samples from 15 mothers of preterm infants were analyzed for protein, total lipids, and total carbohydrates using Bradford, Folch, and phenol-sulfuric acid methods respectively. The energy was calculated using the Atwater general factor system. Anthropometric measurements of 19 preterm infants were taken and growth rates were calculated.

Results: The mean energy content of the samples was 72.77 ± 12.86 kcal/dL (21.83 ± 3.86 kcal/ounce) and mean macronutrient content was as follows: Protein (2.11 ± 0.35 g/dL), total lipids (3.63 ± 1.49 g/dL) and total carbohydrates (7.90 ± 1.29 g/dL). The protein to energy ratio was lower than the recommended ESPGHAN ratio. The energy content was positively correlated to total lipids. Total lipids were negatively correlated to gestational age and weight at birth. The protein content was significantly higher in milk samples from mothers who were vaginally delivered. The mean daily growth rates were found to be lower than the recommended growth rates. ESPGHAN recommended levels of protein and total carbohydrates combined with low total lipids levels yielded a better daily weight gain.

Conclusion: Most of the studied subjects failed to reach the recommended daily growth and head circumference increment rates. The growth rates were found to be affected by the macronutrients composition of breast milk.

INTRODUCTION

Preterm infants born less than 32 weeks of gestation or infants with very low birth weight (VLBW) less than 1500 g are vulnerable to many difficulties during their stay in the neonatal intensive care unit (NICU). Necrotizing enterocolitis (NEC), sepsis, and restricted growth and neurodevelopment are some of the short-term challenges facing preterm infants. In addition, they have an increased risk of developing hypertension, heart diseases, type 2 diabetes, and osteoporosis in their later life[1-3].

All infants, including preterm infants, are recommended to be fed on human breast milk (HBM) as the optimal nutrition for newborns[4-6]. Human breast milk composition is highly variable between mothers and influenced by many factors such as gestational age, postnatal age, and mother's dietary intake. Furthermore, it varies in the same lactation course depending on the time of the day and the stage of lactation[7,8].

Rapidly growing preterm infants have higher energy and nutrient requirements compared to term infants. Their growth and development have to reflect the growth of a healthy fetus of matched age[6]. Ehrenkranz et al. suggested a growth velocity (GV) rate of 15 – 20 g/kg/day and a head circumference (HC) and length increment of 0.9 cm/week as they found that these values are consistent with the intrauterine growth[2]. Unfortunately, HBM may not be sufficient to meet their nutritional needs[5].

It is recommended to fortify HBM to meet preterm and VLBW infants' nutritional needs[4-6]. It was found that preterm infants receiving fortified HBM had a higher growth rate than infants fed on HBM alone[9]. Based

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on the assumption that HBM contains 20 kcal/ounce, fixed doses of fortifiers are used for all infants. Not accounting for variation in HBM composition may lead to energy and nutrient deficiencies that influence the growth of the preterm infant[7]. The European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) guidelines for preterm infants are used as reference values for comparing HBM composition and for target fortification[6].

This study is the first to be done in the Kingdom of Bahrain and in the region, which provides baseline data for the human breast milk composition of mothers delivering preterm infants in the Kingdom. In addition, it included the three main NICUs located at the three main hospitals in the Kingdom of Bahrain. The study aims to assess the effect of energy and macronutrient content of expressed breast milk on the growth of preterm infants.

METHODS

Study Design: This study is an observational prospective cohort pilot study, were healthy hospitalized preterm infants with birth gestational age less than 32 weeks or birth weight less than 1500 g, consuming their mothers expressed breast milk by enteral nutrition either using a Nasogastric tube (NGT), Orogastric tube (OGT) or feeding bottle, were included in the study. Preterm infants with feeding intolerance, NEC and gastrointestinal issues or anomalies, fed donor milk, or exclusively preterm formula were excluded from the study. The study was held at the NICUs following the three main hospitals in the Kingdom of Bahrain, Salmaniya Medical Complex (SMC), Bahrain Defence Force Royal Medical services (BDF), and King Hamad University Hospital (KHUH) from July 2018 to January 2020. The study permissions were obtained from the Technical Support Team (RTST) Committee at the Ministry of Health (AUHR/457/2018) followed by a second approval from the Secondary Health Care Research Sub Committee (SHCRC) at SMC, Research Ethics Committee at BDF (BDF/R&REC/2019-331) and Research and Ethics Committee at KHUH (Ref.KHUH/Research/No.286/2019). Informed consent was signed by infants' guardians before samples and data collection.

Data Sources/ Measurement: Demographic data were collected from preterm infants' records were preterm sex, birth date, method of delivery, gestational age at birth, anthropometric measurements at birth (weight, length, and head circumference), place of delivery, and nationality. Anthropometric measurements, corrected age, volume and type of milk formula per day, and intravenous infusion intake volume were also obtained from hospital records.

Anthropometric Measurements: The daily weight of preterm infants was taken using electronic balance available at the NICU units to the nearest 0.001 kg. Weekly head circumference was measured using a non-stretchable measuring tape to the nearest 0.1 cm.
The daily growth rate (GV) was calculated in g/kg/day using the Exponential model suggested by Patel et al.[10]:

$$GV = [1000 \times \ln(\frac{W_n}{W_1})] / (D_n - D_1)$$

Where W_1 = weight at the beginning of the time interval, W_n = weight at the end of the time interval, D_1 = first day at the time interval, D_n = end day of the time interval.

The head circumference (HC) growth rate was calculated as follows [2]:

$$HC(cm/week) = \frac{sum\ of\ weekly\ increment\ of\ HC}{number\ of\ weeks}$$

Breast Milk Collection and Storage: Mothers of healthy preterm infants were asked to collect milk samples one day per week for two weeks when infants reached full enteral feed of 120 ml/kg/day and after two weeks of birth in which the milk is more stable in its composition and avoiding colostrum milk[7]. Each day, two samples were collected in breast milk storage bags (5 ml each) at different times of the day (morning and evening) from expressed milk used for their infant feeding. The samples were frozen at the NICU, then transferred to the University of Bahrain, and kept frozen at -100°C until analysis.

Breast Milk Analysis: Milk samples were thawed at 37°C water bath and vortexed for 30 seconds before analysis. The protein content of human milk samples was determined by the Bradford method modified by Atwood & Hartmann and Mitoulas et al. regarding the sample dilution (1:30) and the reading absorbance (620 nm)[11-13]. Protein values were then adjusted by subtracting non-protein nitrogen specific values used by Boyce et al. to give more representative values of bioavailable protein which are 17.6% for lactation weeks 1 - 4 and 24% for lactation weeks above 4[14-16]. The protein content of one milk sample was determined by the Kjeldahl method to be used as a standard stock solution[13,17]. The Folch method was used to determine total lipids concentration[18], and total carbohydrates were measured with the phenol-sulfuric acid method suggested by Dubois et al. after the sample diluted to 1:1000 with deionized water[19]. All macronutrients results were expressed in g/dL and g/100 kcal. While energy (kcal/dL) was calculated using the Atwater general factor system in which proteins and carbohydrates give 4 kcal/g each and fat gives 9 kcal/g[20]. The energy content in kcal/ounce was calculated by multiplying the energy (kcal/dL) by 0.3 dL/ounce[21].

Statistical Methods: All statistical analyses were performed using Excel 2013 (Microsoft Corporation) and SPSS Statistics version 22.0 (IBM Corp., Armonk, NY, USA) programs. Descriptive statistics were presented as frequencies, means ± standard deviations (SD), and ranges (minimum and maximum). Kendall`s tau-b correlation coefficient (r) was used to investigate the correlation between energy and macronutrients, also the correlation between gestational age at birth and birth weight with energy and macronutrient content. Independent samples T-test was used to investigate if there was a relationship between sex, the number of preterm per mother (Singleton or Twins), method of delivery, energy, and macronutrient content. Univariate analysis of variance was used after adjusting for the following covariates: gender, the number of preterms, gestational age at birth, method of delivery, place of delivery, birth weight, fortification days and formula days, and using Bonferroni adjustment for the confidence interval. Repeated measures test was used to plot the effect of energy and macronutrients and different follow up from the first day of full feed to 15 days on preterm infants' weight accounting for possible confounders. p-values less than 0.05 were considered statistically significant.

RESULTS

Baseline Characteristics of Study Subjects: Fifteen mothers of 19 preterm infants were recruited in this study. Table 1 shows the baseline characteristics of the study subjects (n = 19). Preterm infants recovered their birth weight when they reached full feed of 120 ml/kg/day and the intravenous infusion was discontinued. Full feed was reached within two weeks from birth except for four subjects with birth weight less than 1000 g needed 3 to 4 weeks to reach this point.

Expressed Breast Milk Energy and Macronutrient Content: Table 2 shows the energy and macronutrient content of the expressed breast milk samples collected from the mothers of preterm infants. The mean energy content was higher than the assumed value of 20 kcal/ounce. Compered to recommended ESPGHAN values[6], the mean protein content in g/100 kcal was lower than the recommended value for

Table 1: Baseline characteristics of preterm infants

Characteristics	**All preterm infants (n = 19)**
Sex:	
Male, n (%)	11 (57.9)
Female, n (%)	8 (42.1)
No. f preterm per mother:	
Singleton, n (%)	8 (42.1)
Twin, n (%)	11 (57.9)
Method of delivery:	
NVD/SVD, n (%)	5 (26.3)
LSCS, n (%)	14 (73.7)
Place of delivery:	
SMC, n (%)	7 (36.8)
BDF, n (%)	8 (42.1)
KHUH, n (%)	4 (21.1)
Nationality:	
Bahraini, n (%)	15 (78.9)
Non-Bahraini, n (%)	4 (21.1)
Birth weight:	
< 1000 g, n (%)	7 (36.8)
≥ 1000 g, n (%)	12 (63.2)
Gestational age at birth (weeks), mean ± SD (min-max)	27.82 ± 2.44 (23.5 - 31)
Weight at birth (g), mean ± SD (min-max)	1105.30 ± 356.49 (520 - 1610)
Length at birth (cm), mean ± SD (min-max)	36.00 ± 5.72 (25 - 44)
Head circumference at birth (cm), mean ± SD (min-max)	25.90 ± 3.11 (19.5 - 29.5)
Gestational age at Full feed (weeks), mean ± SD (min-max)	30.46 ± 1.87 (25.6 - 33.4)
Weight at full feed (g), mean ± SD (min-max)	1109.50 ± 303.45 (590 - 1610)

BDF: Bahrain Defence Force Royal Medical services; KHUH: King Hamad University Hospital; LSCS: Lower Segment Caesarean Section; max.: maximum; min: minimum; n: number of subjects; NVD: Normal Vaginal Delivery; SD: Standard deviation; SMC: Salmaniya Medical Complex; SVD: Spontaneous Vaginal Delivery.

Table 2: Energy and macronutrient content of expressed breast milk samples

	mean ± SD (g/dL)	**min – max (g/dL)**	**mean ± SD (g/100 kcal)**	**Recommended ESPGHAN values (g/100 kcal)[6]**
Energy	72.77 ± 12.86 (21.83 ± 3.86 kcal/ounce)	55.90 – 108.12 (16.77 – 32.44 kcal/ounce)		
Protein	2.11 ± 0.35	1.61 – 3.05	2.97 ± 0.67	For <1000 g bodyweight: 3.6 – 4.1 For ≥ 1000 g bodyweight: 3.2 – 3.6
Total lipids	3.63 ± 1.49	1.97 – 7.43	4.86 ± 1.19	4.4 – 6.0
Total carbohydrates	7.90 ± 1.29	5.30 – 10.70	11.08 ± 2.22	10.5 – 12

ESPGHAN: European Society for Pediatric Gastroenterology, Hepatology and Nutrition; max.: maximum; min: minimum; SD: Standard deviation

preterm infants weighing less than or more than 1000 g. On the other hand, both total lipids and carbohydrates were in the recommended range.

Total lipids were found to be positively correlated to energy and negatively correlated to gestational age at birth and birth weight. while total carbohydrate content was positively correlated to gestational age (Table 3).

Protein content was significantly different according to the method of delivery, in which it was higher in mothers who had Normal Vaginal Delivery (NVD) or Spontaneous Vaginal Delivery (SVD) than mothers who undergone Lower Segment Caesarean Section (LSCS) (Table 4).

No significant associations were found after investigating the effect of sex and number of preterm per mother on energy and macronutrient content of breast milk.

Association Between Preterm Infants Growth and Breast Milk Content: The growth rates of preterm infants from the start of full feed until 30 days or discharge are represented in (Table 5). Compared to the recommended growth rates suggested by Ehrenkranz et al.[2], both mean daily growth rate (GV) and mean HC rate were lower than these values. No significant effects were found comparing the energy and macronutrient content with growth velocity groups.

Table 3: Kendall`s tau-b Correlation between energy, gestational age at birth and birth weight with energy and macronutrient content of milk

	Energy (kcal/dL)		Gestational age at birth (weeks+days)		Birth weight (g)	
	r	p-value	r	p-value	r	p-value
Energy (kcal/dL)			-0.085	0.621	-0.219	0.194
Protein (g/dL)	-0.054	0.745	0.000	1.000	0.131	0.440
Total lipids(g/dL)	0.611	0.000**	-0.453	0.009**	-0.451	0.008**
Total carbohydrates (g/dL)	0.204	0.216	0.440	0.011*	0.190	0.261

*p-value is statistically significant at < 0.05
**p-value is statistically significant at < 0.01
r = Kendall`s tau-b Correlation coefficient

Table 4: The effect of method of delivery on energy and macronutrient content of milk

	Method of delivery mean ± SD		
	LSCS	NVD/SVD	p-value
Energy (kcal/dL)	75.19 ± 14.08	68.18 ± 8.06	0.389
Protein (g/dL)	2.04 ± 0.24	2.24 ± 0.60	0.001**
Total lipids (g/dL)	3.85 ± 1.69	3.29 ± 0.72	0.168
Total carbohydrates (g/dL)	8.08 ± 1.17	7.36 ± 1.73	0.227

*p-value is statistically significant at < 0.05
**p-value is statistically significant at < 0.01
LSCS: Lower Segment Caesarean Section; NVD: Normal Vaginal Delivery; SD: Standard deviation; SVD: Spontaneous Vaginal Delivery

Table 5: The growth rates of preterm infants during the study

	n	mean ± SD	min-max	LGV n	NGV n	Recommended values [2]
Daily growth rate (GV) (g/kg/day)	19	13.45 ± 4.13	6.47 – 20.00	12	7	15 - 20
HC (cm/week)	17	0.50 ± 0.22	0.00 – 1.00	15	2	0.9

GV: Growth velocity; HC: Head circumference; LGV: low growth velocity (< 15 g/kg/day); max.: maximum; min: minimum; n: number of subjects; NGV: normal growth velocity (≥ 15 g/kg/day); SD: Standard deviation

The effect of energy and macronutrients of the milk on different follow up from the first day started full feed until day 15 on preterm infants weight is represented in (Figure 1). Using repeated measures test and adjusting for possible covariates that might affect the measures such as sex, the number of preterm per mother, gestational age at full feed, method of delivery, place of delivery, weight at full feed, days received fortified breast milk or preterm formula milk.

In Figure 1 (A), milk samples were divided into milk samples with low energy (< 20 kcal/ounce) and normal energy (≥ 20 kcal/ounce) according to the assumed energy value of breast milk. According to ESPGHAN recommended macronutrient values (g/100 kcal)[6], milk samples were categorized as milk that provides low or normal levels of a macronutrient in Figure 1 (B, C, D). Infants who received milk with normal energy levels exhibit normal steady daily increments in their weight, while infants who received milk with low energy levels had a fluctuating increment in their daily weight. Since there is an overlap between low and normal energy groups, there was no significant difference between them.

Infants who received milk with normal protein and total carbohydrates levels combined with low total lipid levels exhibited better, larger and significant weight increment in their daily weight.

DISCUSSION

Although Human breast milk is considered the best nutrition for rapidly growing preterm infants, some preterm infants may not achieve their optimal growth if fed with expressed HBM alone due to differences in energy and macronutrient content of the milk[5,6].

The mean energy content of milk samples was 21.83 ± 3.86 kcal/ounce, which was higher than the assumed 20 kcal/ounce used. These high values of energy could have been reduced if the lactose content of the milk was used instead of total carbohydrates. It is preferable to determine lactose concentration because it constitutes 70-83% of the total carbohydrates in human milk and can be digested and absorbed by the preterm infant's digestive system[14].

The protein to energy ratio is important for achieving healthy growth since this ratio affects tissue synthesis and thus energy is required for effective protein utilization[22]. This may be one of the reasons for the hindered daily growth rate and HC growth of preterm infants in this study because of low protein to energy ratio compared to recommended ESPGHAN value, even that most infants received fortified expressed breast milk or formula milk in some days. This means that using standard fortification may fail to provide some preterm infants with their nutrients needs[23,24]. A study by Costa-Orvay et al. found that both protein and energy improves weight and fat-free mass gain if added in appropriate proportions[25]. Another study found that the energy content of the milk affects the weight gain of preterm infants, while protein content affects head circumference and length[26].

Unlike Bauer & Gerss and Hsu et al., no inverse or significant relationship was found between gestational age and birth weight of preterm infants with protein content, which could be attributed to the

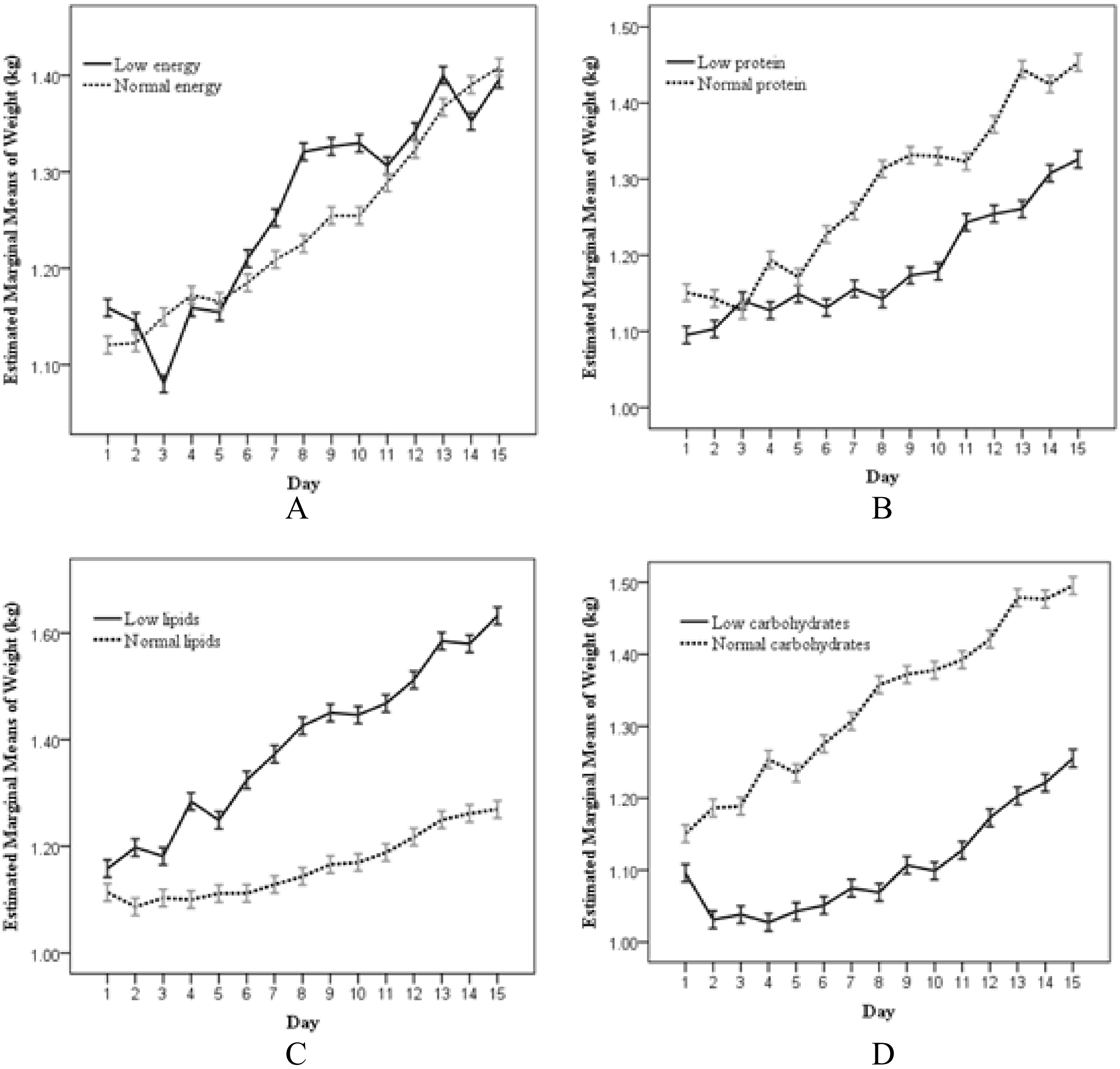

Figure 1: The effect of (A) energy, (B) protein, (C) total lipid and (D) total carbohydrate contents of milk and different follow up from the first day of full feed to 15 days on preterm infants daily weight gain.

* Covariates appearing in the figures are evaluated at the following values: Sex = 0.471, No. of preterm per mother = 0.529, Method of delivery = 0.706, Place of delivery = 1.882, Weight (kg) at full feed = 1.0994, Gestational age at full feed = 30.359, Days received fortified milk = 3.059, Days received formula milk = 5.412

small sample size[7,26]. The method of delivery was found to affect the protein content of the milk, where mothers delivered by NVD or SVD had a higher amount of protein in their milk (2.24 ± 0.60 g/dL) than mothers undergone LSCS (2.04 ± 0.24 g/dL). Dizdar et al. compared colostrum milk content of mothers delivered term babies either by LSCS or vaginal delivery and found that protein content of vaginally delivered mothers (Median = 3 g/dL, Range = 0.5 – 6.3 g/dL) was significantly higher than LSCS group (Median = 2.4 g/dL, Range = 0.3 – 6.4 g/dL). While energy, fat, and carbohydrates were the same[27,28]. In mothers delivered vaginally, labor and uterine contractions induce milk production by activation of some hormones such as prolactin and oxytocin that may affect the protein content of the milk, unlike in LSCS in which mothers do not undergo labor[29,30].

Similar to Bauer & Gerss, total lipids were highly and positively correlated to the energy content of the milk. In contrary to their study, total lipid content decreased with increasing gestational age at birth[7]. Total lipids showed a significant inverse relationship with birth weight, which was similar to Hsu et al. study. However, they did not found significant influence[26]. As birth weight and gestational age decreased, higher energy demand is required to provide the preterm infants with the required thermal protection by providing more energy and deposition of fat under their skin[6].

In this study, total carbohydrate content was found to be positively correlated to gestational age at birth opposite to other studies where they found a negative correlation[7, 26].

It was found that infants who received milk with energy, protein, and total carbohydrates that were in the recommended ESPGHAN range exhibited steadier and faster daily weight gain than infants who received milk with content lower than the recommended values. On the other hand, total lipids content showed an inverse relationship with weight gain. Infants who received milk with lipid content lower than the recommended ESPGHAN range for lipids exhibited faster and showed a better increase in their daily weight gain. Prentice et al. assessed % energy and macronutrient contents with weight gain for term infants and found that both % energy and fat had an inverse association with weight gain, while % protein and carbohydrates had a positive relationship. This inverse relationship between lipids and weight gain can be explained by those infants who receive less lipids milk may drink more volumes of milk since they do not reach satiety in which lipids are responsible for it[31]. Since the protein to energy ratio was found to be important for weight gain, Kashyap et al. found that the source of energy matters. Energy from carbohydrates was found to give better utilization of protein by preterm infants than if it was supplied from fat in milk formulas due to reduced protein oxidation[32]. This may explain why infants with low total lipids in this study had a better weight gain rate.

The main limitations of the study include the low sample size of the subjects due to the low number of eligible mothers and preterm infants for the study criteria and low participation rate. Unavailability of the mid-infrared human milk analyzer that can solve several problems in the study by analyzing small amounts (1 - 2 ml) of milk in a few minutes for energy and macronutrients, solve the problem of low participation rate, more samples can be taken more than two times per day to reflect a 24-hour representative collection of the milk and quantify lactose content instead of total carbohydrates due to the high cost of the kits and reagent required for lactose quantification. Anthropometric measurements written in preterm infant`s records were taken by different nurses. This may lead to errors in some measurements since it is preferred to be taken by one expert person.

To achieve better growth outcomes of preterm infants at the NICUs, it is recommended to have a bedside Human milk analyzer and to apply target fortification of human milk according to the ESPGHAN recommendations is favorable for energy and macronutrients to overcome the problem of human milk shortage.

CONCLUSION

Although the mean energy content was higher than the assumed 20 kcal/ounce, most of the studied preterm infants fail to reach the recommended daily growth and head circumference increment rates even though some were receiving fortified milk or formula milk, which can be attributed to the low protein to energy ratio. Further study is needed to confirm that total lipid contents lower than the recommended ESPGHAN values are better to increase the daily weight gain rates. The availability of human milk analyzers at the NICUs and the use of target fortification may reduce the problem of growth restriction of preterm infants.

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REFERENCES

1. Ehrenkranz RA, Dusick AM, Vohr BR, et al. Growth in the neonatal intensive care unit influences neurodevelopmental and growth outcomes of extremely low birth weight infants. Pediatrics 2006;117(4):1253-61.
2. Ehrenkranz RA, Younes N, Lemons JA, et al. Longitudinal growth of hospitalized very low birth weight infants. Pediatrics 1999;104(2): 280-9.
3. Singhal A, Cole TJ, Fewtrell M, et al. Breastmilk feeding and lipoprotein profile in adolescents born preterm: follow-up prospective randomized study. Lancet 2004; 363(9421):1571-8.
4. World Health Organization. Optimal feeding of low-birth-weight infants: Technical review. World Health Organization; 2006.
5. Eidelman A, Schanler R. Breastfeeding and the use of human milk. Pediatrics 2012; 129(3): e827-41.
6. Agostoni C, Buonocore G, Carnielli VP, 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 (1):85-91.
7. Bauer J, Gerss J. Longitudinal analysis of macronutrients and minerals in human milk produced by mothers of preterm infants. Clin Nutr 2011;30(2):215-20.
8. Khan S, Hepworth AR, Prime DK, et al. Variation in fat, lactose, and protein composition in breast milk over 24 hours: associations with infant feeding patterns. J Hum Lact 2013; 29(1):81-9.
9. Kuschel CA, Harding JE. Multicomponent fortified human milk for promoting growth in preterm infants. Cochrane Database Syst Rev 2004;(1):CD000343.
10. Patel AL, Engstrom JL, Meier PP, et al. Calculating postnatal growth velocity in very low birth weight (VLBW) premature infants. J Perinatol 2009;29(9):618-22.
11. Bradford MM. Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976; 72:248-54.
12. Atwood CS, Hartmann PE. Collection of fore and hindmilk from the sow and the changes in milk composition during suckling. J Dairy Res 1992;59(3):287-98.
13. Mitoulas LR, Kent JC, Cox DB, et al. Variation in fat, lactose and protein in human milk over 24 h and throughout the first year of lactation. Br J Nutr 2002;88(1):29-37.
14. Boyce C, Watson M, Lazidis G, et al. Preterm human milk composition: a systematic literature review. Br J Nutr 2016;116(6):1033-45.
15. Atkinson SA, Anderson GH, Bryan MH. Human milk: comparison of the nitrogen composition in milk from mothers of premature and full-term infants. Am J Clin Nutr 1980;33(4):811-5.
16. Fomon SJ. Requirements and recommended dietary intakes of protein during infancy. Pediatr Res 1991;30(5):391-5.
17. Horwitz W, Latimer WG. AOAC official method 991.20 Nitrogen (total) in milk: Kjeldahl methods, In: Horwitz W, Latimer WG. eds. Official Methods of Analysis of AOAC International. 18th Ed. Gaithersburg, MD: AOAC International; 2005.
18. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 1957;226(1):497-509.
19. Dubois M, Gilles KA, Hamilton JK, et al. Colorimetric method for determination of sugars and related substances. Anal Chem 1956; 28:350-6.

20. Atwater WO, Woods CD. The chemical composition of American food materials. U.S. Department of Agriculture, Office of Experiment Stations, Bulletin No. 28. Washington, D.C.: Government Printing Office;1896.
21. Rochow N, Fusch G, Zapanta B, et al. Target fortification of breast milk: How often should milk analysis be done? Nutrients 2015;7(4):2297-2310.
22. Kashyap S, Schulze KF. Energy requirements and protein-energy metabolism and balance in preterm and term infants. Neonatal Nutr Met 2006;134-146.
23. Maas C, Wiechers C, Bernhard W, et al. Early feeding of fortified breast milk and in-hospital-growth in very premature infants: a retrospective cohort analysis. BMC Pediatr 2013;13(1):178.
24. Rochow N, Landau-Crangle E, Fusch C. Challenges in breast milk fortification for preterm infants. Curr Opin Clin Nutr Metab Care 2015;18(3):276-84.
25. Costa-Orvay J, Figueras-Aloy J, Romera G, et al. The effects of varying protein and energy intakes on the growth and body composition of very low birth weight infants. Nutr J 2011; 10:140.
26. Sauer PJ. Can extrauterine growth approximate intrauterine growth? Should it? Am J Clin Nutr 2007;85(2):608S-13S.
27. Hsu Y, Chen C, Lin M, et al. Changes in preterm breast milk nutrient content in the first month. Pediatr Neonatol 2014;55(6):449-54.
28. Dizdar EA, Sari FN, Degirmencioglu H, et al. Effect of mode of delivery on macronutrient content of breast milk. J Matern Fetal Neonatal Med 2014; 27(11):1099-1102.
29. Nissen E, Uvnäs-Moberg K, Svensson K, et al. Different patterns of oxytocin, prolactin but not cortisol release during breastfeeding in women delivered by caesarean section or by the vaginal route. Early Hum Dev 1996; 45(1-2):103-18.
30. Campbell-Yeo ML, Allen AC, Joseph KS, et al. Effect of domperidone on the composition of preterm human breast milk. Pediatrics 2010;125(1): e107-14.
31. Prentice P, Ong KK, Schoemaker MH, et al. Breast milk nutrient content and infancy growth. Acta Paediatr 2016;105(6):641-7.
32. Kashyap S, Towers HM, Sahni R, et al. Effects of quality of energy on substrate oxidation in enterally fed, low-birth-weight infants. Am J Clin Nutr 2001;74(3):374-80.