Paediatrica Indonesiana

p-ISSN 0030-9311; e-ISSN 2338-476X; Vol.59, No.3(2019). p. 130-8; doi: http://dx.doi.org/10.14238/pi59.3.2019.130-8

Original Article

Macronutrient content in preterm and full term human milk in the first three weeks after delivery

Dessy Shinta Murty, Hasriza Eka Putra, Sri Mulatsih, Neti Nurani, Tunjung Wibowo

Abstract

Background The macronutrients in human milk change dynamically and vary among mothers. Evaluation of macronutrient content in human milk is needed to improve nutritional management in preterm infants.

Objective To measure the macronutrient content in preterm and full term human milk during three lactation periods in the first three weeks after delivery.

Methods We conducted a prospective study among 80 mothers of infants who were hospitalized in the Department of Perinatology/NICU at Sardjito Hospital, Yogyakarta. Carbohydrate, fat, protein, and caloric content were measured using a MIRIS human milk analyzer, once per week for three consecutive weeks after delivery. A single, daytime human milk specimen was collected in the morning by directly expressing from the breast.

Results Median protein, fat, carbohydrate, and caloric contents of mature milk in the preterm group were 1.40 (IQR 0.38), 3.25 (IQR 1.00), 5.70 (IQR 0.80) g/dL, and 60 kcal/dL, respectively. Median protein, fat, carbohydrate, and caloric contents of mature milk in the full term group were 1.40 (IQR 0.35), 3.30 (IQR 0.77), 5.80 (IQR 0.75) g/dL, and 62 kcal/dL, respectively, at the third week after delivery. In both groups, protein content in the first week was significantly higher than in the third week ($P<0.001$) after delivery. In contrast, fat content in the first week was significantly lower than in the third week ($P< 0.05$) after delivery, in both groups.

Conclusions There are no significant differences in macronutrient and caloric content between preterm and full term human milk during the first three weeks after delivery. However, there are significant changes in fat and protein content in both preterm and full term human milk during early lactation, between the first and third weeks. **[Paediatr Indones. 2019;59:130-8; doi: http://dx.doi.org/10.14238/pi59.3.2019.130-8]**.

Keywords: *human milk; macronutrient; calorie; lactation; preterm*

Human milk provides macronutrients, micronutrients, and bioactive substances needed by full term and preterm infants in early life.[1] Human milk macronutrients play an important role in the growth and development of infants and they are needed daily in larger quantities than other substances. Macronutrients in human milk consist of carbohydrates, fats, and proteins.[2] The *American Academy of Pediatrics* has recommended both preterm and full term infants to receive human milk from their own mothers, or from donors after it has been pasteurized according to standard procedures.[3] Human milk is a better choice than formula milk because it reduces the risk of necrotizing enterocolitis and sepsis.[1]

Protein and fat vary in concentration in preterm human milk, especially in the first four weeks after delivery.[4] Protein in preterm human milk decreases from 1.9 g/dL on the first day to 1.5 g/dL on days 22-30 after delivery, while the protein and caloric requirements for preterm infants increase by age.[5] Macronutrient content in preterm and full term

From the Department of Child Health, Gadjah Mada University Medical School/Dr. Sardjito Hospital, Yogyakarta, Central Java, Indonesia.

Corresponding author: Tunjung Wibowo. Department of Child Health, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada/Dr. Sardjito Hospital, Jl. Kesehatan no. 1 Sekip, Yogyakarta, Central Java, Indonesia. Phone: +62274-55284/541565. Email: tunjungwibowo@ugm.ac.id.

Submitted July 10, 2017. Accepted June 18, 2019.

human milk is relatively similar by 4-6 weeks after delivery.[6]

There have been few studies on macronutrient and caloric content of human milk at early lactation, or on changes between lactation stages in preterm and full term human milk in Indonesian mothers. The estimated human milk macronutrient content in our practice usually refers to published values from developed countries. Detailed information about macronutrient and caloric content in human milk may allow targeted nutritional management and individual fortification, especially for preterm and low birth weight infants.[7] A human milk analyzer (HMA) is designed to measure macronutrients (carbohydrates, proteins, and fats) and calories in 60 seconds, with a minimum 1-2 mL specimen, based on the principle of mid-infrared spectroscopy.[8] The HMA is essentially a portable and simple device for analyzing human milk and can be used to provide rapid measurement of milk composition.[9] This machine is especially advantageous for mothers of preterm infants, who often produce only small amounts of milk in early lactation.[10]

The primary objective of this study was to investigate the difference in macronutrient and caloric content of preterm and full term human milk, in the first three weeks after delivery. The secondary aim of this study was to study the difference in human milk macronutrient content between the lactation periods of colostrum (in the first week) and mature human milk (in the third week). The results are expected to provide preliminary data on macronutrient content of human milk from mothers in Yogyakarta, Indonesia.

Methods

Study subjects were lactating mothers who gave birth to preterm infants (gestational age 28 to < 37 weeks) or full term infants at the Department of Perinatology/ NICU, Dr. Sardjito Hospital, Yogyakarta. From July 2016 to September 2016, a total of 80 lactating mothers were recruited who met the inclusion criteria. General information on demographic factors, anthropometry (height and body weight before pregnancy, and at the time of data collection), and information on health-related problems (hypertension, diabetes, or heart disease) was collected by interviewing the participants. Further data on pregnancy outcomes such as gestational age at delivery (weeks), infant diagnosis, and neonatal birth weight (g) was obtained from medical records.

Study protocols and consent forms were approved by the Medical and Health Research Ethics Committee of Universitas Gadjah Mada Medical School/Dr. Sardjito Hospital. All participants provided written informed consent to participate in the study. There were 39 women in the preterm human milk group and 41 women in the full term human milk group.

We examined colostrum (i.e human milk, produced in low quantities in the first few days postpartum, is rich in immunologic components such as secretory IgA and lactoferrin), transitional milk (i.e human milk, typically produced from 7 days to two weeks), and mature milk (i.e. human milk, produced from the fourteenth day after delivery). Each participant provided breast milk once per week on day 3-7 the first week, day 8-14 the second week, and day 15-21 the third week after delivery. Breast milk was obtained by hand or pump. After each expression, if not immediately processed, milk specimens were poured into sealed containers and stored at 4°C. All specimens were processed and analyzed within 24 hours. Specimens which are not stored in ice-packed container were analyzed within 2 hours of being expressed.

Human milk specimens were from one expression, during the day, from one breast that had not been nursed for 2-3 hours. The mammary glands were fully evacuated of all accumulated milk in order to prevent any differences between foremilk and hindmilk. Specimens were homogenized and aliquoted. Surplus milk was returned to the infant.

To minimize diurnal variation in milk fat, sample collection was performed in the morning between 6:00AM and 8:00AM.[11] Mothers were encouraged to breastfeed or express breastmilk 8-12 times per day to enhance milk production. If the baby or mother had been discharged from the hospital, we visited their home at the time of collection and took 10 mL milk specimens within 1 hour. Specimens were labeled and sealed in a clean bottle, stored in an ice-packed container (about -15 to 4°C), then taken immediately to Dr. Sardjito Hospital for evaluation.

Three mL of milk was heated in a 40°C water bath, homogenized by gentle inversion of the container,

then subjected to measurement of fat, protein, carbohydrate, and energy content by a MIRIS mid-infrared human milk analyzer (HMA). Daily internal calibration was performed on the HMA using the check solution provided by the manufacturer (MIRIS). Since we only use fresh milk samples, the MIRIS HMA was operated using the unhomogenized sample mode, according to manufacturer's recommendations. The milk specimen (1 mL) was injected into the flow cell and measured for 60 seconds.

Human milk protein, fat, carbohydrate, and caloric contents are presented in mean and standard deviation or median and interquartile range in the tables, with 95% confidence intervals. Given the non-normal distribution of data, statistical analysis was performed by non-parametric test. Mann-Whitney test was used to analyze differences between non-related groups. Friedman and Wilcoxon tests were used to analyze differences between related groups. We used the *Statistical Package for the Social Sciences* (SPSS) program *version 22* software for statistical analysis. Results with P values < 0.05 were considered to be statistically significant.

Results

A total of 226 fresh human milk specimens were collected in the study, including 80 specimens collected in first week, and 73 samples collected each week in the second and third weeks after delivery. Three infants in the preterm group died less than 7 days after delivery and there was insufficient data for 4 infants in the full term group due to their being lost to follow-up. Subjects' flow chart is presented in **Figure 1**.

Table 1 shows the baseline characteristics of subjects. The distribution of age and parity was relatively similar between groups. Infants' birth weights ranged from 760 to 3,000 grams in the preterm group and from 1,980 to 4,098 grams in the full term group.

Kolmogorov-Smirnov and Shapiro-Wilk normality tests revealed a non-normal distribution of data (P=0.000), so we used a non-parametric test for statistical analysis (P<0.05; 95%CI). **Table 2** shows the comparison of median macronutrient and caloric content of the preterm and full term groups

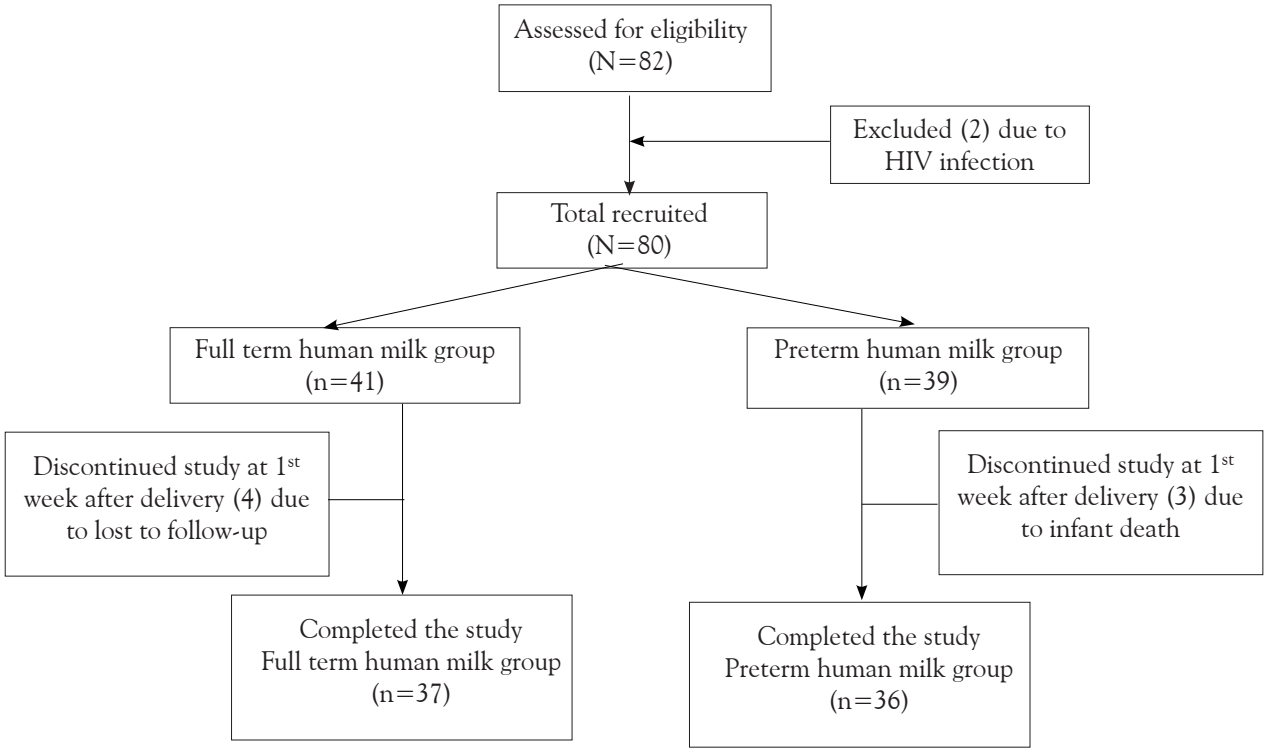

Figure 1. Study flow chart

at weeks 1, 2, and 3 after delivery. Based on non-parametric test, there was no significant difference in protein, fat, carbohydrate, or caloric content between the two groups, at any week during the study, respectively. In our study, there was high variability in the macronutrient content in both groups, on a week-to-week basis. In preterm human milk, the median protein contents (range) were significantly different on weeks 1, 2, and 3 [2.1 (1.0-6.1) g/dL, 1.5 (0.7-4.1) g/dL, and 1.4 (0.4-3.9) g/dL, respectively, (P=0.003)]. Median fat contents (range) in preterm human milk on weeks 1, 2, and 3 were also significantly different [2.3 (0.7-4.0) g/dL, 2.7 (1.1-5.3) g/dL, and 3.25 (1.4-5.6) g/dL, respectively, (P<0.001)]. However, median

Table 1. Subjects' baseline characteristics

Characteristic	Preterm group (n=39)	Full term group (n=41)
Median maternal age (range), years	30 (17-41)	31 (20-41)
Median parity (range)	2 (1-4)	2 (1-4)
Median gestational age (range), weeks	34 (28-36)	39 (37-41)`
Median maternal weight increase during pregnancy (range), kg	9 (0.5-23)	10 (3-28)
Infant birth weight, n (%)		
<1,000 grams	2 (5)	0
1,000-1,499 grams	13 (33)	0
1,500-2,499 grams	21 (54)	3 (7)
2,500-4,000 grams	3 (8)	37 (90)
>4,000 grams	0	1 (2)
Median body mass index (range), kg/m2	22.31 (16.65-32.47)	24.22 (16.64-34.22)
Delivery method, n (%)		
Ceasarian section	18 (46)	20 (49)
Vaginal	21 (54)	21 (51)
Birth location, n (%)		
Sardjito Hospital	36 (92)	36 (88)
Outside Sardjito Hospital	3 (8)	5 (12)

Table 2. Comparison of human milk macronutrient and caloric content

Parameters	Preterm group	Full term group	P* value
Median protein (IQR), g/dL			
Week 1	2.10 (1.0-6.1)	1.80 (1.3-4.2)	0.63
Week 2	1.50 (0.7-4.1)	1.60 (1.1-4.9)	0.41
Week 3	1.40 (0.4-3.9)	1.40 (0.8-4.2)	0.32
P**	0.003	<0.001	
Median fat (IQR), g/dL*			
Week 1	2.30 (0.7-4.0)	2.60 (0.9-6.7)	0.12
Week 2	2.70 (1.1-5.3)	2.75 ((1.3-5.9)	0.87
Week 3	3.25 (1.4-5.6)	3.30 (1.3-5.6)	0.84
P**	<0.001	0.045	
Median carbohydrate, g/dL			
Week 1	5.60 (3.1-8.8)	5.70 (4.8-7.2)	0.28
Week 2	5.70 (4.3-6.4)	5.70 (4.2-6.9)	0.75
Week 3	5.70 (4.3-7.9)	5.80 (3.9-7.1)	0.77
P**	0.365	0.382	
Median calories (IQR), kcal/dL			
Week 1	56.00 (41-84)	58.00 (43-102)	0.07
Week 2	58.50 (43-80)	58.00 (46-87)	0.95
Week 3	60.00 (46-100)	62.00 (43-87)	0.52
P**	0.029	0.032	

*Mann Whitney test, ** Friedman test, IQR = interquartile range

carbohydrate contents (range) in preterm human milk on weeks 1, 2, and 3 were not significantly different [5.6 (3.1-8.8) g/dL, 5.7 (4.3-6.4) g/dL, and 5.7 (4.3-7.9) g/dL, respectively, (P=0.365)].

In full term human milk, median protein contents (range) significantly differed on weeks 1, 2, and 3 [1.8 (1.3-4.2) g/dL, 1.6 (1.1-4.9) g/dL, and 1.4 (0.8-4.2) g/dL, respectively, (P<0.001)]. Median fat contents (range) in preterm human milk also significantly differed on weeks 1, 2, and 3 [2.6 (0.9-6.7) g/dL, 2.75 (1.3-5.9) g/dL, and 3.3 (1.3-5.6) g/dL, respectively, (P=0.045)]. However, median carbohydrate contents (range) in full term human milk were not significantly different in weeks 1, 2, and 3 [5.7 (4.8-7.2) g/dL, 5.7 (4.2-6.9) g/dL, and 5.8 (3.9-7.1) g/dL, respectively, (P=0.0382)].

Table 2 shows that the macronutrient composition changed with increasing post-natal age. In both the full term and preterm groups, a significant decline in protein content occurred over the first 3 weeks. In contrast, fat and caloric content increased with post-natal age (P<0.05).

Table 3 presents the comparison of macronutrient and caloric contents between colostrum (week 1) and mature milk (week 3) in the preterm (n=36) and full term (n=37) groups. Human milk composition changes during the days of early lactation, as an effect of milk maturation. There were significant differences in protein and fat content between the first and third weeks after delivery, in both groups.

In **Tables 2** and **3**, the caloric content in the preterm and full term groups were 56-60 kcal/dL and 58-62 kcal/dL, respectively, for every 100 mL of human milk. Both were lower than published reference values.[12]

Discussion

The macronutrient composition of human milk varies in response to several factors, including maternal age, maternal condition, and infant condition.[1] Human milk macronutrient content may vary between lactation times both within an individual and among individuals. Macronutrient content has been associated with maternal characteristics, lactation/feeding frequency, lactation stage, storage period, and pasteurization process.[13,14] Lactation can be divided into three stages, based on the length of time after delivery as follows: 1) colostrum, produced in low quantities in the first few days postpartum, is rich in immunologic components such as secretory IgA and lactoferrin, 2) transitional human milk, typically produced from 7 days to two weeks, and 3) mature human milk, produced from the fourteenth day after delivery.[1] Human milk is considered to be fully mature in the fourth to sixth weeks after delivery, and in this period the macronutrient and caloric content is relatively similar over lactation times and among individuals.[2]

Table 3. Comparison of macronutrient and calorie content on weeks 1 and 3 after delivery

Parameters	Week 1 (colostrum)	Week 3 (mature human milk)	P*** value
Median protein (IQR), g/dL			
Preterm	2.10 (1.0-6.1)	1.40 (0.4-3.9)	<0.001
Full term	1.80 (1.3-4.2)	1.40 (0.8-4.2)	<0.001
Median fat (IQR), g/dL*			
Preterm	2.30 (0.7-4.0)	3.25 (1.4-5.6)	<0.001
Full term	2.60 (0.9-6.7)	3.30 (1.3-5.6)	0.03
Median carbohydrate, g/dL			
Preterm	5.60 (3.1-8.8)	5.7 (4.8-7.2)	0.12
Full term	5.70 (4.3-7.9)	5.80 (3.9-7.1)	0.64
Median calories (IQR), kcal/dL			
Preterm	56.00 (41-84)	60.00 (46-100)	0.01
Full term	58.00 (43-102)	62.00 (43-87)	0.01

*Mann Whitney test, ** Friedman test, IQR = interquartile range

In most previous studies, preterm breast milk had higher content of various nutrients, especially protein, compared to corresponding values in full term human milk.[15] Gidrewicz *et al.* performed a meta-analysis of 41 studies on human milk macronutrients and micronutrients, in North America, Europe, and Japan. They reported a significant difference in protein content between preterm and full term human milk on days 0-3 and the second week after delivery. The protein content in preterm human milk on days 0-3 and the second week is 2.7 g/dL and 1.1 g/dL, respectively. Whereas in full term human milk, the protein content on days 0-3 and the second week is 2.0 g/dL and 1.0 g/dL, respectively.[6] Bauer *et al.* undertook a longitudinal study in Germany for 8 consecutive weeks postpartum and reported that carbohydrate, lactose, calorie, and fat contents were significantly higher in preterm human milk than in full term human milk.[16] However, we did not find significant differences in protein, fat, carbohydrate, or caloric content between preterm human milk and full term milk in the first three weeks after delivery.

Paul et al. used a longitudinal cohort design to compare human milk content on days 1, 7, 14, and 21 after delivery and reported no significant difference in macronutrients between the full term and preterm groups. The study involved 52 subjects, consisting of 23 mothers of full term infants and 29 mothers of preterm infants in India. They also reported a significant decrease in protein content and a significant increase in fat content in the third week after delivery.[17] Our study was of similar design, in that our sampling method was a single expression of milk, not pooling of milk in a 24-hour period. Hsu *et al.* in Taiwan reported that 17 mothers with infants of less than 35 weeks' gestational age and 15 mothers with infants of full term gestational age had no significant differences in protein, fat, lactose, caloric, calcium, or phosphate content of human milk in the first week and fourth week after delivery.[18]

In our study, high variability in the HMA results in both groups lead to non-normal data distribution. Kreissl *et al.* in Austria reported that HMA results on 83 human milk specimens from mothers of preterm infants on the second to the fourth weeks after delivery also had a non-normal data distribution, with median protein, fat, carbohydrate, and caloric content of 1.0 (0.2-2.2) g/dL, 3.1 (1.1-6.1) g/dL, 6.6 (5.5-8.0) g/dL, and 59(39-94) kcal/dL, respectively, for every 100 mL of human milk.[19] The similarity between our study and theirs was the homogenization technique, which was performed manually, while the differences were subject demography (enrolled from a developed vs. developing country) and the 24-hour pooling sampling method in their study.

Our measurement of carbohydrate content by the MIRIS HMA resulted in data with fairly high variation, similar to previous research by Gidrewicz *et al.*,[6] Silvestre *et al.*,[20] and Fusch *et al.*[21] Fat content was the most varied component of human milk macronutrients in 24 hours compared to protein and carbohydrate contents in an individual.[21,22] Fat is the major component of calories in human milk, since 1 gram of fat contains an equivalent 10 kilocalories. As such, calculation of calories in milk depends on factors that affect fat examination. Khan et al. reported the effect of a 24-hour pooling sample method on the results of caloric and fat content. Fat content was higher in specimens collected from mothers who frequently breastfed and by fully evacuating the breast of milk.[21]

Protein and carbohydrate contents in milk were not related to the volume of milk produced, the nursing process, diurnal variation, or maternal nutritional status.[22] When collecting human milk only a single time in 24 hours, the sampling time and lactation interval should be standardized. The best time to collect human milk is 6:00 to 8:00AM when the fat content is most in accordance to a 24-hour pooled milk specimen.11 This sampling method, however, explains only 55% of the total variance in the 24-hour fat content of breast milk. A standard sampling time and lactation interval could reduce fat content variation among individuals.[22]

The recommended homogenization technique prior to macronutrient measurement by HMA is using an ultrasonic vibrator for at least 30 seconds per 1 mL sample, to minimize the presence of an unexamined fat matrix.[21] However, in our study, the fresh milk specimens had never been frozen or thawed, so according to the HMA MIRIS instructions, homogenization should be done manually by gentle inversion for 30-60 seconds.[23]

In addition, the variability in the milk composition may have been due to different laboratory methods. As such, the fat and carbohydrate contents in our

study were lower than the references. The HMA cannot distinguish between nutrient and non-nutrient content, as it only measures total protein, total fat, total carbohydrate, and total calories.[19] However, Groh-wargo *et al.* showed that results from a mid-infrared spectroscopy analyzer did not significantly differ compared to conventional methods for macronutrient measurement, i.e., Kjehdahl for protein, Mojonnier for fat, and liquid chromatography for lactose.[8]

We found a significant decrease in protein content and significant elevation in fat and caloric content in mature human milk compared to colostrum milk. Protein and fat content were strongly associated with lactation stage.6 High protein content in colostrum was consistent with the physiological stage of the first week, in which secreted milk contains a lot of protein in the form of immunoglobulin.[2] Increased fat content in more mature milk might be associated with increased intensity, frequency, and duration of nursing, in accordance with infant needs.[15] At the beginning of each lactation episode, foremilk consistency is usually more fluid and becomes more viscous at the end of lactation when hindmilk is produced.[5] There was a gradual increase in fat content in human milk in each episode of lactation, but there was no significant increase in protein and lactose contents. Fat content increases in the first 15-30 minutes of lactation, after which the fat content is relatively constant.[24]

Although the mechanism of differences in human milk protein content in preterm and full term infants is unclear, this difference was only significant in the first month after delivery. Variations in protein, fat, and mineral contents in human milk in the first four weeks after delivery were higher in the preterm than in the full term milk groups.[5] A meta-analysis after the fifth week after delivery found no difference in protein content between preterm and full term human milk groups.[6]

In our study, we calculated the mean of human milk protein content in the preterm on week 1, week 2, week 3, were 2.2 g/dL, 1.6 g/dL, 1.5 g/dL, respectively. These values were within the normal range, as per the *American Academy of Pediatrics* reference (week 1: 0.3-4.1 g/dL, week 2: 0.8-2.3 g/dL, week 3: 0.6-2.2 g/dL). In the full term, the mean of human milk protein content on week 1, week 2, week 3 were 1.9 g/dL, 1.6 g/dL, 1.5 g/dL, respectively. According to *American Academy of Pediatrics* reference (week 1: 0.4-3.2 g/dL, week 2: 0.8-1.8 g/dL, week 3: 0.8-1.6 g/dL), these values were also within the normal range. However, mean caloric content in mature human milk was lower than the references (preterm: 62.8 kcal/dL and full term: 62.7 kcal/dL, per 100 mL). In a meta-analysis conducted in developed countries, the mean caloric content of pretem and full term human milk were 66 kcal/dL and 77 kcal/dL, respectively.[6] In clinical practice, a reference standard of 65-70 kcal/dL per 100 mL of human milk is used for planning and evaluating infant nutrition,[12] which was a higher value than our findings.

We did not evaluate for an association between in macronutrient content and infant growth and development, nor the human milk volume produced day per day by mothers. In clinical practice, infant weight gain, especially in preterm infants, is an important parameter for evaluating sufficiency of human milk intake, as well as the response to changes in macronutrient content from colostrum to mature human milk.[15] Further studies are needed to investigate for possible associations. Another limitation was our sample size, however, it was estimated to have 80% power to detect a difference of mean between groups greater than one standard deviation. Differences in macronutrient content between preterm and full term milk groups might be detected with a larger sample size. The other limitations of our study were the sampling method and the duration of investigation. We did not perform 24-hour human milk pooling collection, as in other studies, although we attempted to standardize breast expression method, sample collection time, and lactation interval. Also, the observation period was not long enough, as full maturation of human milk is achieved at weeks 4-6 after delivery, so we may not have fully described the changes in macronutrient content over lactation stages in our Indonesian population.

Our results may be used as preeliminary data on macronutrient and caloric content of human milk in the first three weeks after delivery in the maternal population in Yogyakarta. It can also be the basis for further study with a greater number of subjects, longer data collection period, better sampling techniques and collection, and more complete analysis of maternal and infant characteristics and nutrition factors.

In conclusion, there is no significant difference

in protein, carbohydrate, fat, and caloric contents of preterm and full term human milk in the first three weeks after delivery. Mature milk has higher fat content, but lower protein content than colostrum milk. In our population, a lower caloric content in human milk than the published value is noted in both preterm and full term human milk. Therefore, monitoring of an infant's body weight gain might be indicated, especially for high risk populations, i.e., preterm and very low birth weight infants. If fortification of human milk is needed, it should be individualized according to the macronutrient variability in milk over lactation periods.

Conflict of interest

None declared.

Funding Acknowledgment

The authors received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

References

1. Ballard O, Morrow AL. Human milk composition: nutrients and bioctive factors. Pediatr Clin North Am. 2013;60:49–74.
2. Walker M. Breastfeeding management for the clinician - using the evidence. Sudbury: Jones & Bartlett Publishers; 2013. p. 708.
3. Eidelman AI. Breastfeeding and the use of human milk: an analysis of the American Academy of Pediatrics 2012 Breastfeeding Policy Statement. Breastfeed Med. 2012;7:323–4.
4. Anderson GH, Atkinson SA, Bryan MH. Energy and macronutrient content of human milk during early lactation from mothers giving birth prematurely and at term. Am J Clin Nutr. 1981;34:258–65.
5. 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–-5S.
6. 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.
7. Maas YG, Gerritsen J, Hart AA, Hadders-Algra M, Ruijter JM, Tamminga P, *et al.* Development of macronutrient composition of very preterm human milk. Br J Nutr. 1998;80:35-40.
8. Groh-Wargo S, Valentic J, Khaira S, Super DM, Collin M. Human milk analysis using mid-infrared spectroscopy. 2016;31:266-72.
9. Miller EM, Aiello MO, Fujita M, Hinde K, Milligan L, Quinn EA. Field and laboratory methods in human milk research. Am J Hum Biol. 2013;25:1-11.
10. 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.
11. Ruel MT, Dewey KG, Martinez C, Flores R, Brown KH. Validation of single daytime samples of human milk to estimate the 24-h concentration of lipids in urban Guatemalan mothers. Am J Clin Nutr. 1997;65:439-44.
12. Velonà T, Abbiati L, Beretta B, Gaiaschi A, Flaùto U, Tagliabue P, *et al.* Protein profiles in breast milk from mothers delivering term and preterm babies. Pediatr Res. 1999;45:658-63.
13. Nommsen LA, Lovelady CA, Heinig MJ, Lönnerdal B, Dewey KG. Determinants of energy, protein, lipid, and lactose concentrations in human milk during the first 12 mo of lactation: the DARLING Study. Am J Clin Nutr. 1991;53:457-65.
14. Bauer J, Gerss J. Longitudinal analysis of macronutrients and minerals in human milk produced by mothers of preterm infants. Clin Nutr. 2011;30:215-20.
15. 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.
16. Hsu YC, Chen CH, Lin MC, Tsai CR, Liang JT, Wang TM. Changes in preterm breast milk nutrient content in the first month. Pediatr Neonatol. 2014;55:449-54.
17. Kreissl A, Zwiauer V, Repa A, Binder C, Thanhaeuser M, Jilma B, *et al.* Human milk analyser shows that the lactation period affects protein contents in preterm breastmilk. Acta Paediatr. 2016;105:635-40.
18. Silvestre D, Fraga M, Gormaz M, Torres E, Vento M. Comparison of mid-infrared transmission spectroscopy with biochemical methods for the determination of macronutrients in human milk. Matern Child Nutr. 2014;10:373-82.
19. Fusch G, Rochow N, Choi A, Fusch S, Poeschl S, Ubah AO, *et al.* Rapid measurement of macronutrients in breast milk: how reliable are infrared milk analyzers? Clin Nutr. 2015;34:465-76.

20. Mitoulas LR, Kent JC, Cox DB, Owens RA, Sherriff JL, Hartmann PE. Variation in fat, lactose and protein in human milk over 24 h and throughout the first year of lactation. Br J Nutr. 2002;88:29-37.
21. MIRIS AB Holdings. Miris HMA TM human milk analyzer. MIRIS AB Holdings. Uppsala; 2010. p. 1-58.
22. Riordan J. Breastfeeding and human lactation. 3rd ed. Burlington: Jones and Bartlett Publishers; 2005. p. 103-105.
23. Crawford MA, Hall B, Laurance BM, Munhambo A. Milk lipids and their variability. Curr Med Res Opin. 1976;4:S7-17.
24. American Academy of Pediatrics Committee on Nutrition. Pediatric nutrition handbook. 6th ed. Elk Groove Village: American Academy of Pediatrics; 2008. p. 41-56.