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**Original Article** 

# Preterm human milk composition and dietary intake of breastfeeding mothers in the Indonesian population

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#### Abstract

**Background** Human milk consumption is a protective factor against infection. However, the high variability of human milk composition makes it difficult for clinicians and researchers to assess the adequacy of nutritional intake. **Objective** To identify the nutritional profiles and composition of premature human milk and dietary intake of breastfeeding mothers.

**Methods** Human milk specimens were collected from mothers who gave birth to very premature and/or very low birth weight infants. Infants had been admitted to the Neonatology Unit of Dr. Cipto Mangunkusumo Hospital, Jakarta. The milk specimens were analyzed with a midinfrared milk analyzerTM (MIRIS) for four weeks, and maternal dietary macronutrient intake was determined with food record questionnaires on food consumption frequency and food recall.

**Results** Significant changes in nutritional composition of human milk were observed, with the fat concentration and calories increasing with time, and protein concentration decreasing with time. There were no significant differences observed in carbohydrate concentration of milk over the four weeks. Maternal intake of protein, carbohydrates, fat, and calories were lower than the recommended values for breastfeeding women.

**Conclusion** The macronutrient (protein and fat) and energy contents of human milk change weekly. Dietary intake of breastfeeding women are lower than recommended values. [Paediatr Indones. 2021;61:20-4 ; DOI: 10.14238/pi61.1.2021.20-4 ].

**Keywords:** macronutrient; human milk; very premature infants; VLBW infants

ndonesia ranks fifth worldwide, in terms of countries with the highest number of estimated preterm births.<sup>1</sup> Dr. Cipto Mangunkusumo Hospital, Jakarta, a tertiary hospital center in Indonesia, houses a busy neonatal intensive care unit (NICU). In 2019, 48.8% of infants were born prematurely, and 22% of these were considered to be very premature (below 32 weeks of gestational age).

Very premature (VP) infants and very low birth weight (VLBW) infants require adequate nutrition to achieve optimal growth at a rate similar to intrauterine growth. Human milk is the ideal nutrition for newborns. Human milk consumption is associated with lower rates of infection, particularly in VP infants and VLBW infants.<sup>2-4</sup> However, the high variability of human milk composition makes it difficult for clinicians and researchers to assess the adequacy

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of nutritional intake of babies. Milk macronutrient contents vary significantly among and within mothers, and can be influenced by various factors, including infant gestational age (GA) and post-natal age.<sup>5,6</sup> Preterm human milk shows significant changes in macronutrients over time. In order to determine the nutritional intake of breastfed infants, accurate data on the nutritional composition of human milk is required.<sup>5</sup> The aim of this study was to determine the nutritional composition of preterm human milk and assess for possible associations to dietary intake of Indonesian mothers. Our results may provide information on the adequacy of nutrition in human milk from mothers of VP and VLBW infants, to serve as a basis for further study in determining the need for milk fortification in such infants.

## Methods

This analytical descriptive study with longitudinal and multiple measurement design was conducted from July to October 2017; subjects were recruited by consecutive sampling. Of 48 babies born at gestational age <32 weeks (VP), and/or birth weight <1,500 grams (VLBW), and admitted to the Neonatology Unit of Dr. Cipto Mangunkusumo Hospital, Jakarta, 18 were excluded due to congenital maternal breast abnormalities, HIV infection, refusal to participate, or infants who died before 4 weeks of age. Hence, a total of 30 mother/infant pairs enrolled in this observational study.

Independent variables were infant gestational age, birth weight, chronological age of babies when breast milk specimens were taken, and birth measurements, as well as maternal food intake, nutritional status, economic status (below or above regional minimum wage, according to local government), frequency of breastfeeding/pumping, and education level (low: elementary/junior high school, middle: high school, high: bachelor degree). Dependent variables were the protein, carbohydrate, and fat content of breast milk specimens collected once per week for 4 weeks. Breast milk expression was supervised by a doctor or nurse in the hospital, by one of three possible methods: manual pump, electric pump, or manual hand-assisted expression. The mothers expressed milk for 15 minutes each side; 5 mL of breast milk was collected for analysis.

The mothers were also requested to pump every three hours and record the volume of breast milk collected. For quality control, mothers used one container per milk expression in the nursery and one container at home. Breast milk volume was measured using a syringe or a glass measuring cup. Health workers reminded mothers to deliver their breast milk to the hospital weekly. Maternal nutritional status based on body mass index (BMI) according to WHO criteria. A nutritionist assessed maternal nutritional intake using a food frequency questionnaire (collecting dietary profiles based on the frequency of food consumed per day, per week, or per month), food recall (recording food intake in 1 day prior), activities throughout the day, and weekly food record methods (mothers noted meals for 2 ordinary days and 1 day off/weekend). The 24-hour food recall method was supported by food frequency questionnaires to improve the accuracy of food intake data. Mothers recorded the type and amount of food and drinks consumed in household measurements, then the nutritionist ensured the weight of food ingredients using food models and authentic food samples. Total daily intake of macronutrients and calories taken by mothers then were compared to recommended dietary allowance. Recommended dietary allowance (RDA) was the daily dietary intake level considered to be sufficient by the Food and Nutrition Board of the Institute of Medicine to meet the requirements of 97.5% of healthy individuals, according to life-stage and sex.7 Macronutrient and calorie content in breast milk were measured by a MIRIS Human Milk Analyzer (MIRIS<sup>™</sup>), with macronutrient levels expressed in g/100mL and calorie content expressed in kcal/100mL.<sup>8</sup> Breast milk specimens were homogenized using a MIRIS sonicator and analyzed by MIRIS spectroscopy, with results available within 60 seconds. A previous study noted that MIRIS had good correlation coefficients to standard laboratory methods, with 0.997 for fats, 0.839 for proteins, and 0.776 for lactose.<sup>9</sup>

Statistical analyses were done using the Statistical Package for the Social Sciences (SPSS) version 23 software (IBM Corp., NY, USA). Changes in human milk composition were analyzed with ANOVA test with multiple measurements. Post-hoc tests were also conducted using Bonferroni correction, with P values <0.05 considered to be statistically significant. Informed consent was obtained from participants prior to the study. This study was approved by the Research Ethics Committee of the Universitas Indonesia Medical School.

#### Results

Thirty mother/newborn pairs participated, with 19/30 neonates born at 28-31 weeks' gestation and 23/30 neonates having birth weight of 1,000-1,499 grams (Table 1). There were significant changes in nutritional levels of milk during the 4-week lactation period, with a decrease in protein content, but increases in fat content and calories. There are no

Table 1. Characteristics of mothers and infants

Characteristics	(N=30)
Mean gestational age (SD), months	30.93 (2.09)
Gestational age by group, n <28 weeks 28-31 weeks >32 weeks	1 19 10
Mean birth weight (SD), grams	1285.83 (220.04)
Birth weight by group, n <1000 grams 1000-1499 grams >1500 grams	3 23 4
Mean maternal age (SD), years	29.37 (6.78)
Maternal nutritional status Normal weight Overweight Obese	12 6 12
Mean weight gain during pregnancy (SD), kg	10.00 (6.93)
Parity, n First pregnancy Subsequent pregnancy	16 14
Pre-eclampsia, n Yes No	11 19
Delivery method, n Vaginal Caesarean section	9 21
Socioeconomic status, n Below regional minimum wage Above regional minimum wage	18 12
Maternal education level, n Low Moderate High	4 18 8

changes in carbohydrate content during 4 week (Table 2). Based on food record methods, all macronutrient contents of maternal diet were lower than RDA (Table 3).

### Discussion

In our study, the mean age of mothers was 29.37 (SD 6.78) years. We found that breast milk calorie count and macronutrient contents were significantly different every week of observation. Calorie count and fat concentration of human milk increased gradually during the 4-week lactation period, whereas protein levels decreased. These findings were in agreement with previous studies.<sup>5,10,11</sup> In our study, the composition of protein was higher than the amount stated in a systematic review published in 2016.<sup>12</sup> Our study showed carbohydrate concentration in breast milk increased gradually with time in mothers with infants born at a gestational age of 32-35 weeks. The findings are similar to what have been reported by the previous studies.<sup>13</sup> However, in our study of mothers with infants born at gestational age of <32 weeks, there were no significant changes of carbohydrate concentration.

Breast milk protein concentration decreased with time, but was generally higher than that found in a previous study.<sup>12</sup> In addition, 60% of mothers were classified as overweight or obese, despite their lower than RDA dietary intake.<sup>14</sup> Studies linking maternal diet and breast milk protein content have not shown consistent results. A previous study found that human milk protein concentration was not affected by maternal diet. The protein concentration increased with maternal BMI, and decreased in mothers producing higher amounts of milk.<sup>6</sup> Protein concentration also reached its peak in breast milk from mothers aged 20-30 years. 15 The difference in fat concentration of breast milk between the first and second weeks was quite significant. Furthermore, the mean calorie count reached maximum values on the third and fourth weeks. In previous studies, the milk calorie count was generally higher, and thought to be due to higher maternal fat intake.<sup>10</sup> We also found that for almost all nutritional components studied, maternal dietary intake was below the RDA. In order to maintain breast milk quality, fresh breast

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Variables	Week I (n = 30)	Week II (n = 30)	Week III (n =30)	Week IV (n = 30)	P value
Protein, g/100 mL	2.35 (0.70)	2.05 (0.61)	1.82 (0.47)	1.80 (0.47)	0.0003
Carbohydrates, g/100 mL	5.08 (0.97)	4.82 (1.02)	5.01 (1.13)	5.13 (1.00)	0.447
Fat, g/100 mL	3.81 (1.86)	7.01 (2.43)	7.22 (2.29)	7.19 (2.13)	0.0004
Calories, kCal/100 mL	67.85 (16.61)	94.79 (22.24)	94.88 (20.51)	95.66 (19.81)	0.0006

Table 2. Changes in the mean nutritional components of subjects' breast milk over 4 weeks

Data are shown in mean (SD)

Table 3. Changes in maternal dietary intake by wee
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Dietary intake	Wee (n = 5	eek I Week II = 30) (n=30)		Veek II n=30)	Week III (n = 30)		Week IV (n = 30)		RDA
	Value	%	Value	%	Value	%	Value	%	-
Protein, grams/day	64.55 (15.90)	14.23 (2.42)	63.86 (13.07)	13.17 (10.00-18.00)*	62.01 (12.77)	13.5 (11.67-19.33)*	56.99 (13.74)	13.62 (2.24)	77
Carbohydrates, grams/day	253.31 (57.66)	55.83 (7.86)	270.56 (62.87)	55.93 (5.69)	246.75 (53.42)	55.11 (5.79)	240.69 (65.79)	55.65 (6.83)	368
Fat, grams/day	62.43 (17.51)	30.00 (6.59)	66.97 (16.31)	30.70 (5.99)	63.63 (16.47)	30.94 (5.18)	59.40 (17.19)	30.61 (5.81)	71
Calories, kCal/day	1,825.83 (332.29)		1,888.79 (306.25)		1,791.47 (347.97)		1,678.48 (326.95)		2,480

Data presented in mean (SD); \*Data were not normally distributed, and presented in median (range)

milk specimens were immediately checked to prevent time-related damage, despite the absence of a 'gold standard' for human milk sampling and the different approaches in various studies.<sup>16</sup> Past studies have found no correlation between maternal diet and breast milk composition.<sup>17</sup> In our study, we assessed maternal dietary intake from the time of birth, however, dietary intake should have been assessed since pregnancy.<sup>18</sup>

Changes in human milk composition began in the second week of breastfeeding, with decreased protein and increased fat concentrations, along with a subsequent increase in total calories. The macronutrient content of breast milk from mothers with VP and/or VLBW infants continued to change each week. Moreover, mothers did not achieve the RDA dietary intake.

A strength of this study was assessing for changes in nutritional content of breast milk from mothers of VP and/or VLBW infants in the first 4 weeks of life. We also considered maternal nutritional intake, in the form of dietary analysis. The limitation of our study was not collecting 24-hour data, so we could not have breast milk pump variation interpretation. Further study is warranted to identify the correlation between dietary intake of mothers and the composition of human milk.

In conclusion, mean maternal intake of protein, carbohydrates, fat, and calories are lower than the RDA. This study also find changes in the content of macronutrients every week in breast milk for VP and/or VLBW infants which do not fulfill the recommended needs by ESPGHAN. Human milk fortifier (HMF) can be considered to be given since second week to meet the needs of catching up.

#### **Conflicts of Interest**

None declared.

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