

The content of macronutrients in milk from mothers of very preterm infants is highly variable

Gitte Zachariassen¹, Jesper Fenger-Gron², Mette Vogn Hviid¹ & Susanne Halken¹

ABSTRACT

INTRODUCTION: The objective of this study was to determine the content of macronutrients in human milk (HM) from mothers who gave birth very prematurely, and to investigate possible associations between macronutrients and certain maternal and infant characteristics.

MATERIAL AND METHODS: Mothers of very preterm infants with a gestational age (GA) below 32 weeks expressed milk for analysis two weeks after birth, every second week until discharge, at term, at two, and at four months of corrected age. The milk was analyzed using mid-infrared transmission spectroscopy.

RESULTS: A total of 214 mothers delivered 736 HM samples for analysis. Two weeks after birth, protein content varied from 1.06 to 2.96 with a mean of 1.76 g/100 ml HM, and the mean protein content decreased significantly until eight weeks after birth ($p < 0.04$). Previous breastfeeding experience was associated with a lower protein content ($p = 0.04$) two weeks after birth. HM from mothers of extremely preterm infants (GA < 28 weeks) had a higher fat and energy content two weeks after birth than infants with a GA of 28–32 weeks ($p = 0.001$).

CONCLUSION: Protein content in human milk varies considerably between mothers, and decreases within weeks after very preterm birth. Previous breastfeeding experience and low GA were associated with a lower protein and a higher fat and energy content in HM, respectively. Inter-individual differences in human milk content possibly influences nutrition and this raises the question of the need for an individualized approach when fortifying human milk for preterm infants.

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particularly with human milk (HM), should be started within the first days of life, timely increased, and later fortified with a HM fortifier containing extra calories, protein, and minerals [5]. When fed an “adequate” volume, fortified HM is considered to satisfy the specific nutritional requirements of preterm infants and thereby improve growth [6, 7]. Nevertheless, it has been demonstrated that HM protein content decreases within the first weeks and months after birth [8, 9], whereas content of fat, lactose, and total energy content in HM are reported with different results in the literature [8–11]. The reported variations in the composition of HM makes it difficult to meet nutritional requirements using standard HM fortification, and individualized fortification has been proposed [12]. Human milk is a complex fluid and is recommended not only for infants born at term, but also for infants born preterm. The optimal nutrition of preterm infants still needs to be investigated, and the aim of this study was to determine the content of macronutrients (energy, protein, fat, and lactose) in HM from mothers who gave birth very prematurely, and to investigate possible associations between macronutrients and certain maternal and infant characteristics during the first weeks after birth.

MATERIAL AND METHODS

HM samples were collected and analysed as part of a prospective birth cohort study [13] on infants with a gestational age (GA) $\leq 32 + 0$ weeks, who were recruited consecutively from four neonatal units in Denmark from 2004 to 2008. Birth weight (BW), GA, and single- or multiple-birth(s) were recorded at birth. Information on the mother’s age, education, occupation, smoking habits, and previous breastfeeding experience was obtained by interviews and questionnaires before hospital discharge. Breastfeeding rates and more details are described elsewhere [13]. Milk sampling during hospitalization:

ORIGINAL ARTICLE

1) Hans Christian Andersen Children’s Hospital
2) Paediatric Department, Sygehus Lillebaelt Kolding

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The overall goal of feeding very-low-birth-weight infants is to achieve growth similar to foetal growth rates with similar body composition in addition to satisfactory functional development of very preterm infants [1]. Inadequate nutrition of preterm infants has been associated with neurodevelopmental impairment and bone-disease as well as growth failure [2–4]. Enteral feeding,

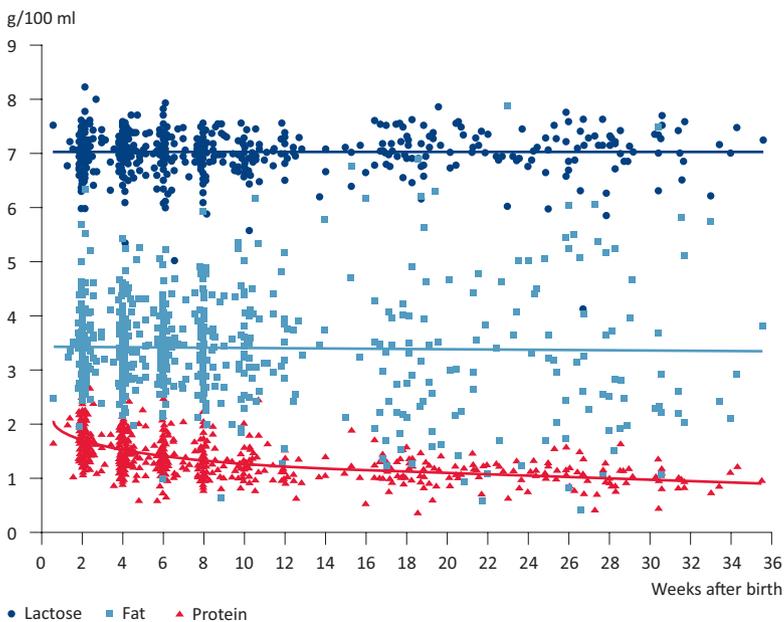


ABBREVIATIONS

BMI = body mass index
BW = birth weight
HM = human milk
HMA = human milk analyzer
GA = postmenstrual gestational age at birth (weeks and days)

FIGURE 1

Content of lactose, fat and protein in human milk.



Mothers were expressing milk within hours after birth using breast pumps available at all departments. The first milk sample for analysis of macronutrients was collected two weeks after birth, and then every second week until hospital discharge. Each time the mother expressed milk during 24 h, 2 ml were collected from the total amount of expressed milk, stored in a test-tube and frozen as soon as possible after 24 h of collection. After hospital discharge: Milk was sampled at term and at two and four months of corrected age (CA). The mother emptied one breast by hand or with a breast pump once in 24 h and stored 10 ml of this in a test-tube which was frozen as soon as possible. No specific time during the 24 h was defined for expressing HM during or after hospital stay. Analysis of HM samples in brief: The frozen HM samples were defrosted in a refrigerator, heated in warm water until reaching a temperature of 40 °C and homogenized before analysis. HM analyses were made using Human Milk Analyzer (HMA) from Miris AB, Sweden. The HMA measurement principle is based on mid-infrared transmission spectroscopy. The use of mid-infrared HMA has been described to afford good linearity and precision in application to protein, lipids, and lactose in HM [14]. The HMA used for analysing milk samples in this project was initially re-calibrated with reference milk analysed with the methodologies of Rose-Gottlieb (fat), Kjeldahl (protein), dry-oven (solids/lactose) at a certified laboratory for official controls of foods under the Danish Ministry of Food, Agriculture,

and Fisheries in Aarhus, Denmark. The energy content in HM samples was calculated from the individual fat, protein, and lactose values using the equation: energy = (9.25 kcal/g × g fat) + (4.40 kcal/g × g protein) + (3.95 kcal/g × g lactose) (Miris AB).

Statistics

Every HM sample contained HM for 2-5 analyses. A mean value was calculated for each HM sample. For all mothers' mean-values, standard deviation, and min.-max. values were calculated for energy, protein, fat, and lactose in the HM samples every second week since birth.

The cohort was characterised by GA (< 28 weeks or between 28 and 32 + 0), BW (< 1,000 g or ≥ 1,000 g), maternal smoking (yes or no), social group (high social group was defined as group 1 and 2, low social group as group 3 to 5) [15], body mass index (BMI) (< 25 kg/m² or ≥ 25 kg/m²), single or multiple birth, previous breastfeeding experience, and maternal age (< 25 or ≥ 25 years). The possible influence of the above mentioned binary variables on the content of energy, protein, fat, and lactose in HM two weeks after birth was analysed using t-tests and multiple logistic regression models.

To investigate if mothers with a content of macronutrients above the mean level two weeks after birth still had a high content later on, a binary variable was created labelling each mother as either "above mean level of macronutrients two weeks after birth" or not. This variable was used as the only explanatory variable in a regression model on content of energy, protein, fat, and lactose at four and six weeks after birth.

Ethics and trial registration: The study was approved in July 2004 by the Danish National Committee on Biomedical Research Ethics (J.no. VF20030208) and handling of data and registrations were approved in February 2006 by the Danish Data Protection Agency (J.no.2007-41-1349).

RESULTS

A total of 214 mothers delivered 736 HM samples (from two weeks after birth until six months CA). **Figure 1** shows the content of lactose, fat, and protein in all HM samples. Mean and "min. and max." values of energy, protein, fat, and lactose content in HM during the first 12 weeks after birth are described in **Table 1**. Two weeks after birth, the mean protein content was 1.76 g/100 ml HM with a variation from 1.06 to 2.96 g/100 ml HM. Energy content was closely related to fat content in the samples. Mean content of fat and lactose seemed stable during the entire period, while the protein content declined significantly until eight weeks after birth ($p < 0.04$).

A total of 151 (of 214) mothers delivered 155 HM samples two weeks after birth (four mothers delivered two milk samples during the first two weeks after birth). At four and six weeks, 124 and 71 of 151 mothers delivered HM samples, respectively. The characteristics of these 151 mothers are shown in **Table 2**.

Protein in HM: In a multiple logistic regression model looking at variables (GA, BW, smoking, social group, BMI, multiple birth, previous breastfeeding experience, and mother's age) possibly influencing protein content in mothers' milk two weeks after birth, mothers with previous breastfeeding experience had a lower protein content two weeks after birth than mothers with no previous breastfeeding experience ($p = 0.04$). The results are shown in Table 2. When comparing the protein content above the mean level in HM samples at two and four weeks after birth, a significant correlation ($p = 0.000$) was observed. However, six weeks after birth, the protein content above the mean level was no longer significantly correlated with the high content two weeks after birth ($p = 0.11$).

Lactose in HM: None of the analysed variables (described above) significantly influenced the content of lactose two weeks after birth. Mothers' milk with a high content of lactose two weeks after birth also had a high content four weeks after birth ($p = 0.000$), but the association was not significant later on.

Fat content and energy in HM: As shown in Figure 1, fat content showed large variations between samples. Of the analysed variables (described above) possibly influencing content of fat and energy in HM two weeks after birth, mothers of extremely preterm infants (GA < 28 weeks) had a significantly higher fat and energy content ($p = 0.001$) than mothers of preterm infants (GA \geq 28 weeks). HM from mothers with a BMI \geq 25 kg/m² also showed a significantly higher content of fat ($p = 0.02$) and energy ($p = 0.01$) two weeks after birth than HM of mothers with a BMI < 25 kg/m². Mothers' milk with a high fat and energy content two weeks after birth still had a high content of both fat and energy four and six weeks after birth ($p < 0.04$).

DISCUSSION

Using mid-infrared transmission spectroscopy, we have confirmed previous findings of considerable differences in preterm HM with regard to the content of macronutrients [8, 9], including a decrease in protein content during the first eight weeks after very preterm birth [9].

It is a weakness of this study that the number of mothers delivering HM samples decreased with time after birth. This was not unexpected since it appears to be easier to deliver HM samples during hospitalization than after hospital discharge when exclusively breastfeeding at home. Only 23% gave birth extremely prema-

TABEL 1

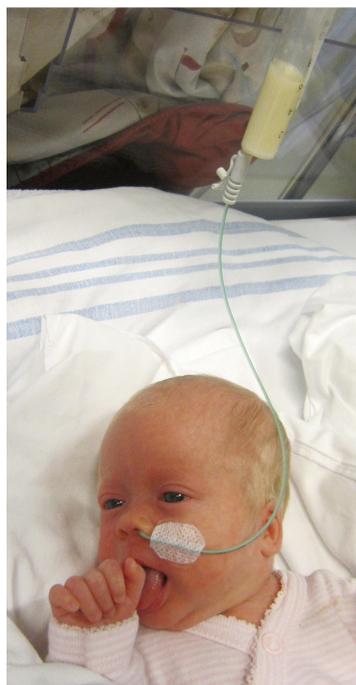
Mean energy, protein, fat, and lactose content in human milk the first 12 weeks after birth.

Weeks after birth, n	Milk samples, n	Mean \pm SD (min.-max.)			
		energy, kcal/100 ml	protein, g/100 ml	fat, g/100 ml	lactose, g/100 ml
2	155	62.1 \pm 8.9 (44.3-101.3)	1.76 \pm 0.37 (1.06-2.96)	3.46 \pm 0.72 (1.92-6.35)	6.97 \pm 0.44 (3.86-8.24)
4	175	60.8 \pm 7.4 (42.8-86.0)	1.50 \pm 0.30 (0.61-2.83)	3.39 \pm 0.65 (1.57-5.43)	7.09 \pm 0.31 (5.35-7.72)
6	110	60.4 \pm 7.1 (37.3-83.5)	1.42 \pm 0.31 (0.61-2.50)	3.39 \pm 0.63 (0.98-5.23)	7.02 \pm 0.39 (5.02-7.93)
8	90	61.0 \pm 8.7 (34.0-91.5)	1.33 \pm 0.29 (0.81-2.25)	3.45 \pm 0.77 (0.66-5.95)	7.03 \pm 0.33 (5.90-7.57)
10	48	62.4 \pm 11.4 (44.0-96.0)	1.30 \pm 0.29 (0.80-2.48)	3.58 \pm 0.95 (1.88-6.18)	6.94 \pm 0.33 (5.59-7.56)
12	21	59.8 \pm 10.2 (39.8-80.5)	1.15 \pm 0.22 (0.67-1.66)	3.37 \pm 0.95 (1.27-5.17)	7.07 \pm 0.23 (6.52-7.56)

SD = standard deviation.

turally (GA < 28 weeks), while 77% gave birth between GA 28 and 32 + 0 weeks. The mothers of infants born close to GA 32 weeks spent less time at the hospital and therefore delivered fewer HM samples. The breastfeeding rate was high at discharge, but decreased after hospital discharge [13] which may also explain the decrease in the number of HM samples after birth.

Another Danish study has previously shown that the protein content of preterm HM at the mean time of discharge (\approx 37 weeks postmenstrual age) had decreased to a level equivalent to HM 4-8 weeks after birth from mothers who gave birth at term [8]. Another study (us-



Preterm born twin receiving fortified mothers' milk.

TABEL 2

Comparison of variables possibly influencing protein content in mothers' milk two weeks after birth. Variables compared with t-test and a multiple logistic regression model.

Group	Mothers, n ^b	Protein content at 2 weeks, g/100 ml ± 1 SD	p-value (t-test)	p-value (multiple logistic regression) (n = 135)
A: GA < 28 weeks	32/151	1.84 ± 0.47	0.31	0.77
B: GA 28-32 weeks	119/151	1.75 ± 0.34		
A: BW < 1,000 g	31/151	1.84 ± 0.41	0.27	0.35
B: BW ≥ 1,000 g	120/151	1.75 ± 0.36		
A: smoking	23/143	1.88 ± 0.47	0.20	0.14
B: non-smoking	120/143	1.74 ± 0.35		
A: high social group ^a	56/143	1.76 ± 0.35	0.89	0.87
B: low social group ^a	87/143	1.75 ± 0.38		
A: BMI ≥ 25 kg/m ²	36/135	1.79 ± 0.42	0.64	0.57
B: BMI < 25 kg/m ²	99/135	1.75 ± 0.36		
A: multiple birth	36/151	1.85 ± 0.41	0.13	0.67
B: single birth	115/151	1.74 ± 0.36		
A: mothers < 25 yrs	13/151	1.79 ± 0.30	0.79	0.64
B: mothers ≥ 25 yrs	138/151	1.76 ± 0.38		
A: previous breast-feeding experience	51/139	1.67 ± 0.37	0.03	0.04
B: no previous breast-feeding experience	88/139	1.81 ± 0.37		

BMI = body mass index; BW = birth weight; GA = postmenstrual gestational age at birth (weeks and days).

a) Social group defined as high social group = 1 + 2 and low social group = 3 + 4 + 5.

b) Data available from 135 to 151 mothers depending on the variable.

ing the method of Lowry/Petersen) showed that the protein content of both preterm and term HM decreased with the progress of lactation, but that values in extremely preterm HM (GA < 28 weeks) were significantly higher than in moderately preterm and term HM [9]. In our study, we found that HM from mothers of extremely preterm infants (GA < 28 weeks) had a significantly higher fat and energy content than HM from mothers of preterm infants born between GA 28 and 32 + 0 weeks. It also had a higher protein content two weeks after birth, though the latter difference was not significant.

Mothers with previous breastfeeding experience had a significantly lower protein content two weeks after birth than mothers with no previous breastfeeding experience. This might be due to a larger milk yield at an earlier stage than among women with no breastfeeding experience. Hill et al found significantly greater milk yield in women with previous breastfeeding experience in one study, but no difference in a later study [16]. One study measured milk volumes 21 to 30 days after birth from 20 mothers who gave birth to infants born with a GA < 28 weeks. The total milk volume was 545 ml/24 h (median) (with min.-max. ranging from 224 to 1,817 ml/24 h) [17]. In this study, they found no significant difference in protein content in expressed volumes of fore- and hind-milk.

As already illustrated with protein, different laboratory methods and different HM content results were reported in the literature.

Lactose is the major carbohydrate in HM, and this disaccharide is an important energy source that has previously been described to be low in colostrums and to increase over time, especially in preterm HM [10, 11]. We found no similar increase in lactose content in HM. Fat content in HM has been reported to increase during the first weeks after birth [8, 11], but also to be high initially and then decrease within the first weeks after delivery [10]. We found fat content to be high among mothers who gave birth to the most preterm born infants, but also among mothers with a high BMI. One other study has looked into BMI and fat content and found no association [18]. We also found fat content to vary between samples during the entire study period. This may be due to sampling technique and diurnal variation in fat content. The latter has been described in an Australian study that found a low fat content at night and a high fat content during the day and evening in HM [19]. In the present study, we did not measure the total volume of HM during 24 h from each mother due to a risk of interfering with the normal breast feeding procedure. It might also be a weakness of the present study that HM samples for practical reasons had to be collected differently during hospitalization (when the infants were not breastfed or partly breastfed while practicing breastfeeding) and after discharge (when the infants were fully breastfed). During hospitalization, HM samples in the present study were collected by taking 2 ml from the total amount of expressed milk 6-8 times during 24 h, probably making the samples representative in terms of content of protein, but possibly not representative in terms of fat. We could have saved the total amount of expressed HM for 24 h, or collected a certain percentage of HM from the amount of expressed milk each time during 24 h which would have made the samples even more representative. However, the study was planned to minimize the impact on breastfeeding. These limitations illustrate the complexity of the HM production response, of collecting and evaluating, and of feeding HM to preterm infants. All these limitations concerning milk expression, sampling, storage, and analysis of contents in HM have recently been described very well by Miller et al [20].

CONCLUSION

In conclusion, our study confirms previous results showing a wide variation in human milk protein content between mothers, and a significant decrease in protein content within the first eight weeks after preterm birth. We also found previous breastfeeding experience and a low GA to influence protein, fat, and energy content in

human milk two weeks after birth. The content of macronutrients in HM is important when feeding preterm infants fortified HM during hospitalization, but agreements on protocols for HM sampling and analysis are definitely needed in future research on human milk in order to optimize nutrition of very preterm infants.

CORRESPONDENCE: *Gitte Zachariassen*, H. C. Andersen Børnehospital, Odense Universitetshospital, 5000 Odense, Denmark.
E-mail: gitte.zachariassen@rsyd.dk

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A full list of references is available from the authors.

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