Longitudinal analysis of macronutrients and minerals in human milk produced by mothers of preterm infants

Jacqueline Bauer a,*, Joachim Gerss b

a University Children’s Hospital of Muenster, Department of Pediatrics, Albert-SchweitzerStr. 33, D-48149 Muenster, Germany
b Department of Medical Informatics and Biomathematics, University of Muenster, Germany

Original Article

1. Introduction

Introduction of therapeutic measures such as surfactant replacement and antenatal steroid administration have led to dramatically improved survival rates of premature infants. Therefore, recent research focus has shifted towards factors influencing long-term outcome. Adequate nutritional supply during the first weeks of life has a major impact on neurodevelopment in premature infants.2 The appropriate nutrition for term neonates during the first 4–6 months of life is universally considered to be their own mother’s milk. Human milk provides not only the substrates needed for growth but also a large number of bioactive components that modulate neonatal development.2 The provision of human milk to premature neonates has demonstrated many beneficial effects including improvements in host defense digestion, absorption of nutrients, gastrointestinal function, neurodevelopment, and psychological effects for the mother.1,2 Feeding of mother’s milk to rapidly growing preterm infants, especially those who are extremely premature with a gestational age below 28 weeks, often results in suboptimal weight gain and nutritional deficits, due to premature infants need for large amounts of protein and energy to achieve appropriate growth.3,4 Additionally, inadequate substrate provision is associated with delays in long-term growth and in neurodevelopmental outcome until at least school age.1,2 Recent investigations on the effect of early postnatal nutrition indicate that the rate of weight gain in premature infants is influenced by the quantity of calories given. Gains in length and head circumferences on the other hand are affected by the amount of protein in the diet.4 Therefore, information about precise protein and energy contents of each individual mother’s milk are highly desirable when feeding premature infants.

Studies focusing on the macronutrient content of human milk fractions from mothers of extremely immature neonates are scarce and often cross-sectional.5,6 Various reports have revealed that protein concentrations of both preterm and term human milk decline through lactation, whereas the contents of fat, lactose and carbohydrate show no significant changes over time.5,6 In addition, considerable inter- and intra-individual variations have been detected in the course of lactation.5,6 In extremely premature...
infants, below 28 weeks of gestation, the lack of information about milk's macronutrient composition implies an obvious risk of undernutrition or overnutrition. However, the decision on how to fortify mother's milk for premature infants is arbitrary. Therefore, an individualized analysis of the composition of human milk is desirable to minimize tolerance problems and to prevent side effects related to fortification.

The main objective of our present study was to analyse macronutrients, energy and minerals in human milk samples from mothers with preterm infants (mean gestational age: 28.4 ± 3 weeks) and to compare the results with full-term milk samples over the first 8 weeks of lactation.

2. Subjects and methods

Breast milk samples were obtained from 113 healthy mothers giving birth prematurely between 23 and 33 weeks of gestation and having enough milk for their infants including a minimum of 3 mL milk extra for the study. Only participants that completed the entire study period of 8 weeks of lactation were evaluated for the study. Therefore, 11 mothers were later excluded from the study for various reasons; 2 were translated to other hospitals after delivery, 4 had insufficient milk supply, 4 mothers completed the milk production after 2 weeks for personal motives, and 1 moved far away. The analyses of these milk samples were not used for the calculations. Consequently, a total 102 participants were evaluated for the investigation: 4 mothers of 23 weeks of gestation, 8 mothers of 24 weeks, and 10 women per each gestational age group from 25 to 33 weeks, respectively. For comparison participants were divided in 3 groups according their gestational duration in: 1. extremely premature (below 28 weeks of gestation), 2. severely premature (28–31 weeks), and 3. moderately premature (32–33 weeks), respectively. The milk of additionally 10 term mothers was also analyzed. Milk specimens were collected longitudinally, at weekly intervals from the first to the eighth postpartum week. At admission all mothers received information about advantages of human milk nutrition for the infant, and were encouraged to initiate lactation immediately after delivery. Each woman obtained a comprehensive instruction of milk collection procedures including pumping and breastfeeding techniques by certificated lactation consultants (e.g. principal investigator, J.B.). Extended support was initiated to sustain and maintain milk production during the complete study period, as well as to avoid different ways of collecting samples that could influence milk nutrient content. All participants delivering prematurely and at term were visited at home weekly after discharge from the hospital to ensure a good adherence to the study protocol. Mothers at home were requested to collect once a week for 24 h their milk for analyses. To control for possible diurnal variations in human milk pooled probes only a collection period of 8:00 AM to 8:00 AM was used for analyses. Milk samples were collected from each participant on the same day each week. During the 24-h period mothers assemble their milk every 4 h. The collection of the 24-h samples started as soon as there was sufficient milk to feed the infant and to take an additional of 3 mL sample for analysis. Mothers pumped their breast mechanically (electric double-breast pump, Medela®, Germany) and collected the milk probes in sterile bottles until the end of the 24-h collection period. Probes were pooled and stored in a refrigerator at 4 °C until the end of the 24 h collection period. Samples for analyses were taken from an entire 24-h collection period, and stored immediately at −70°C until analyzed. Daily milk volume output was recorded in each participant. The Ethical Committees of the Medical Faculties of the Universities of Muenster and Heidelberg have approved the investigation and parental consent was obtained before studying each individual infant.

2.1. Laboratory methods

Breast milk samples stored at −70 °C were analyzed for caloric content in duplicate for each sample, using a ballistic bomb calorimeter (Parr® 6200 Oxygen Bomb Calorimeter, Parr Instrument Company, USA). The protein concentration in milk samples was determined according to the method of Lowry and later modified by Petersen.29 Carbohydrate content of the milk was analyzed by means of the Orcinol carbohydrate assay as detailed described by Polberger et al.31 Creatamotocrit values were determined using the technique presented by Lucas et al.30 Well mixed milk aliquots were placed into 2 glasses capillary tubes (75 μL), which were sealed at one end and centrifuged in a hematocrit centrifuge for 5 min at 12,000 rpm. The creatamotocrit was expressed as a percentage of the length of the milk column in the tube. With the described techniques a minimum of 3 mL milk per week was needed for analysis. Minerals concentration was measured by an absorption spectrometer and colorimetric assay with endpoint determination and sample blank (Roche® GmbH, Germany).

2.2. Statistical methods

Data were analyzed with the SPSS statistical software (Version 17.0 for Windows, SPSS Inc, Chicago, IL, USA) and SAS (Version 9.2 for Windows, SAS Institute Inc., Cary, NC, USA). Means and standard deviations (SD) of target parameters were calculated for each gestational age and lactation week. Correlations between target parameters and gestation as well as lactation week were described using Pearson’s correlation coefficient. In order to test for significant differences between gestational age groups, multivariate analyses were performed. The milk of extremely preterm infants were correlated against the severely, moderately and term milk. A mixed linear model was established for each target parameter that included a normally distributed random intercept (i.e., subject effect) as well as the fixed co-variables lactation week and gestational age group. The mixed model approach was chosen in order to account for intra-individual correlations among repeated observations collected from the same subject (longitudinal or clustered data). Data within each cluster (i.e., subject) cannot be regarded stochastically independent, in contrast to inter-individual data collected from different subjects. The model was fitted using a Restricted Maximum Likelihood approach. All statistical analyses were intended to be rather exploratory than confirmatory. P-values were considered statistically significant in the case of \( p < 0.05 \). No adjustment for multiple testing was carried out.

3. Results

All participants were healthy without suffering from hypertensive disorders or diabetes mellitus before, during or after pregnancy, and none of them was vegetarian or followed a special nutritional diet. Mother’s delivering prematurely had a mean gestational age of 28.4 ± 3.0 weeks (median 28 weeks), and the term group of 39 ± 0.9 weeks (median 39 weeks), respectively. The first collection started in all participants 6 ± 3 days after birth.

3.1. Human milk content

3.1.1. Protein

Protein values of both preterm and term human milk decreased linearly with progressive week of lactation (Fig. 1). The difference on protein content between the sub-groups of preterm milk samples is demonstrated on Table 1 showing remarkable higher protein values in the extremely immature milk in contrast with more mature human milk. A comparison between protein average
3.1.2. Fat

Average fat values of preterm milk were significantly higher compared to term human milk as demonstrated in Table 2. The Pearson’s correlation coefficient was used for comparing protein concentration between target parameters presenting a closed relation to lactation week \(r = 0.804\), but not to gestational week \(r = 0.152\). During the observation period average lipid concentration increased significantly \(p < 0.0001\) in preterm milk from initially 2.9 ± 0.3 g/dl to 6.8 ± 0.3 g/dl at 8 weeks of lactation, and in term milk from 2.9 ± 0.3 g/dl to 4.9 ± 0.2 g/dl, respectively. Lipid content in extremely preterm human milk versus term human milk increased by 0.322 g/dl (95% CI 0.226, 0.483, \(p < 0.05\)). In the comparison of severely preterm human milk versus term human milk lipid values differed by 0.375 g/dl (95% CI 0.219, 0.532, \(p < 0.05\)), and versus moderately preterm human milk by 0.76 g/dl (95% CI 0.594, 0.937, \(p < 0.05\)), respectively.

3.1.3. Carbohydrate

The carbohydrate average concentration of preterm milk was significantly higher compared to term milk values \((p < 0.05\), as showing in Table 2), and increased significantly \((p < 0.0001\) in the preterm and term milk during the observation period. In the preterm milk the minimal concentration was measured at the first 2 weeks with a mean of 6.3 ± 0.1 g/dl increasing to 8.5 ± 0.2 g/dl. In term milk a minimum of 5.0 ± 0.2 g/dl of carbohydrate concentration was detected in the first week reaching a maximum of 7.4 ± 0.3 g/dl at the end of the study. No pronounced correlation to lactation week \((r = 0.295)\) and to gestation week \((r = 0.021)\) was found during the study period. Extremely preterm human milk versus term human milk differed by 1.43 g/dl (95% CI 1.21, 1.65, \(p < 0.0001\)).

3.1.4. Energy

Positive correlations between lipid and energy values were detected in all lactation weeks in both preterm and term milk groups. Energy density increased in the preterm milk from 64.8 ± 0.5 in the first study week to 86.6 ± 1.9 kcal/dl during the study period, and from 65.3 ± 3.1 kcal/dl to 71.0 ± 3.5 kcal/dl in the term milk, respectively. Average energy content in preterm human milk showed significantly higher values than term milk during the study period \((p < 0.05\), see Table 2). No differences were observed between premature groups.

3.1.5. Minerals

Mineral contents were higher in the extremely immature milk compared to the moderately preterm milk, but without statistically significance (see Table 2). Sodium values of preterm milk are demonstrated in Fig. 2 showing higher values in the first study week than in the 8th week of lactation \((p < 0.05)\). No differences in the concentrations of potassium, phosphate, magnesium and calcium were established between the different preterm groups and the term milk group (see Table 2).

3.1.6. Milk output

In the first lactation week preterm mothers produced on average 254 ± 125 mL/d and on the eighth week a volume of 759 ± 325 mL/d, term mothers of 396 ± 258 mL/d at the first and

---

### Table 1

Protein content (g/dl) of human milk from 112 women delivering premature and at term.

<table>
<thead>
<tr>
<th>Lactation weeks</th>
<th>Gestational age (weeks)</th>
<th>23</th>
<th>24</th>
<th>25</th>
<th>26</th>
<th>27</th>
<th>28</th>
<th>29</th>
<th>30</th>
<th>31</th>
<th>32</th>
<th>33</th>
<th>Term</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mean</td>
<td>2.8</td>
<td>3.0</td>
<td>3.0</td>
<td>2.7</td>
<td>2.9</td>
<td>2.7</td>
<td>2.6</td>
<td>2.5</td>
<td>2.5</td>
<td>2.3</td>
<td>2.2</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>2</td>
<td>Mean</td>
<td>2.9</td>
<td>2.8</td>
<td>2.8</td>
<td>2.3</td>
<td>2.8</td>
<td>2.3</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.2</td>
<td>2.2</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>3</td>
<td>Mean</td>
<td>2.7</td>
<td>2.9</td>
<td>2.5</td>
<td>2.0</td>
<td>2.6</td>
<td>2.1</td>
<td>2.3</td>
<td>2.2</td>
<td>2.5</td>
<td>2.1</td>
<td>2.0</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>4</td>
<td>Mean</td>
<td>2.7</td>
<td>2.7</td>
<td>1.9</td>
<td>2.0</td>
<td>2.4</td>
<td>1.8</td>
<td>2.4</td>
<td>2.1</td>
<td>2.4</td>
<td>1.8</td>
<td>2.0</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>5</td>
<td>Mean</td>
<td>2.6</td>
<td>2.6</td>
<td>1.9</td>
<td>1.7</td>
<td>2.3</td>
<td>2.0</td>
<td>2.3</td>
<td>2.1</td>
<td>2.2</td>
<td>1.8</td>
<td>1.8</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>6</td>
<td>Mean</td>
<td>2.6</td>
<td>2.6</td>
<td>1.7</td>
<td>1.7</td>
<td>2.1</td>
<td>1.2</td>
<td>2.1</td>
<td>2.0</td>
<td>2.1</td>
<td>1.6</td>
<td>1.8</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>7</td>
<td>Mean</td>
<td>2.5</td>
<td>2.5</td>
<td>1.8</td>
<td>1.7</td>
<td>2.1</td>
<td>1.5</td>
<td>2.1</td>
<td>2.0</td>
<td>2.0</td>
<td>1.6</td>
<td>1.6</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>8</td>
<td>Mean</td>
<td>2.0</td>
<td>2.6</td>
<td>1.7</td>
<td>1.7</td>
<td>2.0</td>
<td>1.5</td>
<td>2.1</td>
<td>1.9</td>
<td>2.0</td>
<td>1.6</td>
<td>1.4</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.3</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Results are expressed as Mean ± SD.
985 ± 327 mL/d at the last study week, respectively. Comparing milk output of mothers of extremely preterm gestation to milk volume of women delivering at a moderately preterm gestation no significant difference was detected (p = 0.15).

4. Discussion

In the present investigation we confirmed as previously indicated in the literature that preterm human milk shows considerable differences in the macronutrient composition compared to term milk.11 Furthermore, we detected a significant difference in milk protein levels between women delivering extremely early (<28 weeks) compared to mothers giving birth moderately premature (32–33 weeks). This indicates an inverse relationship between protein content and gestation. It is remarkable that mothers of extremely premature infants are in fact able to produce higher protein levels despite the exceptionally short gestation. The present data reveal a pronounced effect of gestational age and lactation week on protein content in preterm human milk compared to values indicated in the literature.12 It is not a new observation that preterm human milk differs from term milk. It is remarkable that carbohydrate and fat concentrations increased during lactation. The high fat content of preterm milk is not completely understood.19,20 It seems likely that the discrepancies are due to differences in hormonal balance and metabolic regulation associated with shorter gestational period and certain aspects of immaturity of the mammary gland.19–21

More advanced gestation (32–33 weeks) concentrations ranged from 1.8 to 2.3 g/dl. Only few studies investigating the milk protein content of extremely immature milk (below 28 weeks) demonstrated a relation between milk composition and degree of prematurity.13 Most investigators have analyzed more mature milk (over 28 weeks of gestation) indicating protein concentrations ranging from 2.8 to 1.5 g/dl,11,13–16 which are similar to our results detected in severely and moderately preterm human milk during the first month of lactation. Theses compositional differences can only partly be explained by a compensation for the reduced milk volume produced by women giving birth prematurely. In fact, the protein production per 24-h sampling period is higher in mother’s milk of extremely premature infants suggesting an adaptation to the higher protein requirements of these infants.

In the present study, carbohydrate concentration of human milk increased gradually during the postpartum weeks of lactation, as well as fat content independent of milk volume. Faerk et al.15 demonstrated that carbohydrate and fat concentrations increased after 2 weeks of lactation reaching a constant level after this period. Preterm milk contained significantly more fat than term milk and showed a further increase during lactation. The high fat content was responsible for the high energy density of preterm milk. Changes in electrolyte composition were only seen in the first week of lactation. Especially a decline in sodium concentration was noted starting in the second week of lactation in all preterm groups. If the initial high sodium concentration simply reflects an adaptation to the small breast milk volume produced in the first days after birth, one would expect electrolyte concentrations to be altered in every woman with minimal milk output independent of the length of gestation. Accordingly, an inverse relationship between milk volume and sodium levels has been observed and reported by several researchers.17 The finding that mothers who had low breast milk sodium concentrations on day 3 of lactation had a great chance of nursing successfully was explained by low milk sodium concentrations, which might be a marker of milk production.18 Milk volumes from both preterm and term mothers were not significantly different in our study, and no differences in sodium concentration were detected when comparing preterm term and human milk at any week of lactation. This stresses that our understanding of the physiological processes responsible for the nutrient composition in human milk is still limited. Despite increasing evidence that a lactogenic hormone complex is required for the initiation of lactation at parturition, the origin of higher protein content and variations in mineral content in preterm human milk is not completely understood.19,20 It seems likely that the discrepancies are due to differences in hormonal balance and metabolic regulation associated with shorter gestational period and certain aspects of immaturity of the mammary gland.19–21

![Graph showing sodium values of preterm human milk (23–33 weeks of gestation). Box plots illustrating median, quartiles, range and outliers (○) and extremes (+).](image-url)
Studies in this field still yield conflicting results, and further research in hormonal regulation of lactation in prematurity is needed before a more precise picture of this complex process may arise. It may be suggested that the mammary gland of the mother delivering prematurely is functionally developed and undergoes regular maturational processes, which are responsible for changes in milk composition.\(^3\)\(^{-25}\)

Inadequate protein intake due to declining levels in human milk may lead to inappropriate growth in premature infants.\(^22\)\(^,\)\(^23\) therefore a supplement is routinely added. At the same time, the currently available milk fortifiers need to be improved to better mimic human milk.\(^24\) The higher protein levels found in our preterm human milk samples might be of advantage to the rapidly growing premature infant, whose protein requirement has been estimated to be greater than that of the more mature infant.\(^4\) Furthermore, our results of a high variability in milk protein content from mothers of both extremely short gestational age and of more advanced pregnancy should guide us towards more individualized nutritional regimens. With the recognition that extremely preterm human milk (< 28 weeks) has initially higher nutrient concentrations (e.g., protein, carbohydrate, fat) than previously estimated together with recently published recommendations on nutrient needs of premature infants,\(^25\) new calculations for supplementation will be useful. Recent studies indicate that the rate of weight again in preterm infants is affected by the amount of calories, whereas gains in length and head circumference are dependent upon the quantity of protein in the nutrition.\(^4\) Consequently, inadequate substrate supply results in changes in body composition, which might have effects on later life.\(^26\) This intriguing observation of higher human milk protein levels suggests that milk of mothers of extremely premature infants are adapted to the growth requirements of these neonates. The practical significance of this finding in view of nutritional or immunological and metabolic properties of the produced proteins remains to be established.

Human milk protein represents a heterogeneous mixture of compounds and no assay can satisfactorily measure all of its components. Therefore, we are well aware that our method of estimating protein content has its limitations. The Lowry method had low variability for whey and skimmed milk samples, but this method yielded analytical values closest to Kjeldahl protein values.\(^27\) Another limitation of the present study is the high degree of subjectivity regarding the creatamotricity using a caliper. This concern leads to our decision to determine energy density in human milk.

Several factors such as weeks of lactation, breastfeeding time, gestational age, genetic factors and dietary habits are responsible for inter-individual changes found in human milk components.\(^3\)\(^,\)\(^5\)\(^,\)\(^6\) Studies comparing preterm and term human milk have shown differences for a number of nutrients, although some of the results are contradictory.\(^12\)\(^,\)\(^20\) Different sampling and processing methods can result in variations in the quantity and in the composition of human milk.\(^3\)\(^,\)\(^12\)\(^,\)\(^20\) To minimize variables (e.g. sampling methods) that may affect milk composition, each participant received professional support by lactation consultants providing instruction in sampling milk during the entire investigation including home visits after discharge. From macronutrient analyses it is known that human milk shows a large change in protein and fat content during lactation.\(^29\)\(^,\)\(^15\) Studies focusing on the nutrient composition of human milk from mothers of extremely preterm neonates, particularly under 28 weeks of gestation, are limited due to hormonal and psychological factors that cause insufficient milk output. Clearly, mothers experience a greater degree of psychological distress after giving birth to a premature infant who remains hospitalized for a long period of time compared to mothers who deliver a healthy term neonate. In general, it is quite difficult to find an adequate number of mothers who are not only able to feed their preterm infants for an extended period and are also willing to participate in a clinical study when undergoing an enormously stressful experience. Trials have demonstrated that maternal anxiety and stress during pueroerum have a negative impact on breastfeeding outcome suggesting that counseling and supporting anxious mothers of high-risk infants may contribute to increased lactation initiation and maintenance.\(^30\) Lactation consultants take on a very important role for the promotion of human milk feeding in modern medicine providing special support to high-risk groups who have not been breastfeeding in recent decades. Our study pointed out that appropriate management of lactation and information about the essential role of human milk in the nutrition of preterm infants are helpful to ensure lactation in women delivering extremely early. The professional support for all lactating mothers and the effort to promote skin-to-skin care in our unit facilitated the milk production after birth. Non-nutritive sucking at the empty breast and oral stimulation initiated as soon as possible contributed favorable to establish milk supply during the entire study period.

In summary, a longitudinal collection of milk samples from the same preterm and term mothers allowed us to observe compositional pattern changes for specific nutrients with progressing weeks of lactation. Individualized protein fortification of breast milk is highly desirable to improve nutritional management in vulnerable premature infants and facilitates a more precise supplementation of human milk than currently possible. These data provide a more detailed insight into nutrient intake of preterm infants fed breast milk, whereas further research is needed on the topic of nutrient utilization in this area.

Conflict of interest statement
None of the authors have a conflict of interest.

Acknowledgments
We particularly thank the participating mothers, without whom this study would not have been possible, for generous contributions and understanding at time when they were undergoing an enormously stressful experience.

The authors gratefully acknowledge the technical support of Thorsten Marquardt from the University Children's Hospital of Muenster, of Michael C. Frühwald for manuscript review from the Klinikum Augsburg, Pediatrics 1, and of Manfred Fobker and Jerzy-Roch Nofer from the Institute of Clinical Chemistry and Laboratory Medicine, University of Muenster, Germany.

The authors’ responsibilities were as follows — JB: responsible for the overall study design, involved in subjects’ recruitment, data collection and analysis, performed the measurements, and wrote the manuscript. JG: performed the statistical analysis of the data, participated in the discussion of the results, and in the correction of the manuscript.

References