Variability in human milk composition: benefit of individualized fortification in very-low-birth-weight infants

Virginie de Halleux and Jacques Rigo

ABSTRACT
Background: Preterm infants fed fortified human milk (HM) grow more slowly than those fed preterm formulas. These differences could be related to the variability in the macronutrient composition of expressed HM, resulting in inadequate nutrient intake in relation to the estimated needs of the preterm infants.

Objectives: The aim of this article was to show the variability in HM composition from an infant’s own mother’s milk (OMM) or pooled HM from the milk bank. The second objective was to evaluate the advantages of individual fortification on nutritional intakes over standard fortification.

Design: The macronutrient composition of 428 OMM, 138 HM pools from single donors, 224 pools from multiple donors, and 14 pools from colostral milk was determined by using a mid-infrared analyzer. Individualized fortification was performed after analysis of the milk samples in 2 steps: adjustment of fat content up to 4 g/dL, followed by the addition of an HM fortifier to provide 4.3 g · kg⁻¹ · d⁻¹ according to the daily prescribed volume of feeding. Nutritional intakes resulting from the individualized fortification were compared with calculated intakes resulting from standard fortification (HM fortifier: 4 packets/dL).

Results: The variability in contents of fat, protein, and energy was high for all types of HM samples. Compared with standard fortification, individual fortification significantly reduced the variability in nutritional intakes, allowing the maintenance of protein intake and the protein:energy ratio in the range of the nutritional recommendations.

Conclusions: The variability in expressed HM with respect to its protein and energy content is high. This variability persists after standard fortification, possibly resulting in under- or overnutrition. Because both over- and undernutrition confer risks in later development, individualized fortification optimizes protein and energy intake.

Am J Clin Nutr 2013;98(suppl):529S–35S.

INTRODUCTION

Human milk (HM) is regarded as the gold standard in the provision of nutritional needs for all healthy and sick newborn infants during the first months of life (1). It contains nutrients necessary for growth and development but also numerous bioactive factors contributing to beneficial effects on host defense, gastrointestinal maturation (2, 3), infection rate (4–7), neurodevelopmental outcome (8–10), cardiovascular and metabolic disease (11, 12), and the infant’s and mother’s psychological well-being.

In preterm infants, there is a general agreement that the use of exclusive HM has short- and long-term beneficial effects on health and neurodevelopmental outcomes (1). However, preterm infants and particularly extremely-low-birth-weight (ELBW) infants are at risk of cumulative nutritional deficits and postnatal growth restriction during the first weeks of life up to the time of discharge or theoretical term (13, 14). It has been suggested that the neonatal period corresponds to a critical window when undernutrition does affect brain development (15–17). Preterm infants have higher protein, energy, mineral, and electrolyte requirements than term infants. Exclusive HM, even from an infant’s own mother’s milk (OMM) or banked HM cannot meet nutritional recommendations for ELBW infants (18, 19). Despite the benefits of HM fortification (20), growth in preterm infants fed fortified HM differs qualitatively and quantitatively from the optimal fetal growth and is also slower than that of preterm infants fed adapted preterm formulas (21–23). These differences could be related to the large variation in the nutritional value of expressed OMM or banked HM, particularly in terms of fat and protein contents (24–26). We recently suggested that the use of individualized HM fortification improves nutritional support and growth in very-low-birth-weight (VLBW) infants (27). As a result, since 2006, this procedure of fortification has been used for feeding VLBW infants in our neonatal intensive care unit (NICU).

The aim of the present study was to evaluate the variability in HM composition of both OMM and bank HM pools provided daily to our NICU. The secondary objective was to evaluate the influence of an individualized HM fortification procedure on nutritional intakes in preterm infants compared with standard fortification.

1 From the Department of Neonatology, University of Liege, Centre Hospitalier Universitaire de Liège, Centre Hospitalier Régional de la Citadelle, Liege, Belgium.
2 Address correspondence to V de Halleux, Service Universitaire de Néonatalogie, CHR de la Citadelle, Boulevard du Douzième de Ligne, 1, 4000 Liège, Belgium. E-mail: vdehalleux@chu.ulg.ac.be.
3 Address correspondence to V de Halleux, Service Universitaire de Néonatalogie, CHR de la Citadelle, Boulevard du Douzième de Ligne, 1, 4000 Liège, Belgium. E-mail: vdehalleux@chu.ulg.ac.be.
4 Abbreviations used: BUN, blood urea nitrogen; ELBW, extremely low birth weight; HM, human milk; MCT, medium-chain triglyceride; NICU, neonatal intensive care unit; OMM, own mother’s milk; VLBW, very low birth weight.
First published online July 3, 2013; doi: 10.3945/ajcn.112.042689.


529S
SUBJECTS AND METHODS

Validation of an infrared HM analyzer

HM analyses were performed with a mid-infrared analyzer (Milkoscan Minor; Foss) (27, 28). The instrument, originally developed for cow milk analysis in the dairy industry, requires additional calibration for HM use. It needs ~10 mL HM to provide data on protein, fat, and carbohydrate contents in 90 s. Results of 40 HM samples from our HM bank were analyzed in our laboratory, for comparison to chemical analysis for nitrogen (nitrogen analyzer EP Analyzer EP 428; Leco France) and fat (“Soxhlet” Soxtec Aventi 2055; Foss).

Variability in daily composition of OMM and of pools of HM from the milk bank

By using a mid-infrared analyzer (Milkoscan Minor), the macronutrient composition of 428 OMM samples used for individualized OMM fortification were obtained. In addition, data from HM pools from one single donor (5 L HM from one mother), pools from multiple donors (5 L from multiple-donor mothers), and pools of colostral milk (<8 d lactation, multiple donors) were also obtained at the milk bank of the NICU at the University of Liège, Belgium. HM was expressed at the hospital or at home, by manual expression or by using an electric pump, and transported under aseptic HACCP (Hazard Analysis Critical Control Point) conditions in accordance with written instructions to the mothers regarding mechanical expression, milk collection, storage, and transport. OMM provided by the mother was kept at 4°C and used within 72 h. A bacteriologic count was performed on the day of receipt to allow its use as raw milk or as requiring pasteurization or elimination. Milk samples of cytomegalovirus-positive mothers were also pasteurized. To allow individualized fortification, a sample of 10 mL was taken from the daily pool and analyzed before fortification. The surplus milk could be kept in the refrigerator to be used within 72 h of extraction or frozen for later use. All donor HM had been frozen and pasteurized by the Holder method (62.5°C for 30 min) and warmed by thawing to 37°C before analysis. The energy content was calculated by using the Atwater factors: 4 kcal/g for protein and carbohydrate and 9 kcal/g for fat.

Nutritional intakes resulting from individualized and standard HM fortification procedures

The individualized HM fortification protocol was designed in 2 steps to meet the current nutritional recommendations for premature growing infants (18, 19). This protocol has been routinely in use in the NICU for VLBW infants since 2006. First, the fat content of HM was adjusted up to 4 g/dL when necessary by using medium-chain triglycerides (MCTs; Liquigen Danone Nederland), a stabilized 1:1 mixture of MCTs and water (0.5 g/mL). Second, protein content was adjusted by using a complete powdered HM fortifier (Enfamil Human Milk Fortifier; Mead Johnson) to provide 4.3 g protein $\frac{kg}{d} \cdot \frac{d}{d}$ according to the daily prescribed volume of feeding. The nutritional composition of OMM, the MCT and the fortifier supplementation, the prescribed volume, and the infant’s body weight at the day of prescription were collected at the milk bank for calculating the

| TABLE 1 | Protein, fat, carbohydrate, and energy concentrations of own mother’s milk, single- and multiple-donor milk pools, and colostral pools |
|---------------------------------|----------------|----------------|----------------|----------------|
| Own mother’s milk | Single-donor milk pool | Multiple-donor milk pool | Colostral pool |
| (n = 428) | (n = 138) | (n = 224) | (n = 14) |
| Protein (g/dL) | 1.52 ± 0.28<sup>a</sup> | 1.34 ± 0.37<sup>b</sup> | 1.46 ± 0.24<sup>c</sup> | 2.00 ± 0.99<sup>d</sup> |
| Fat (g/100 mL) | 3.79 ± 0.73<sup>a</sup> | 3.45 ± 0.60<sup>b</sup> | 3.39 ± 0.48<sup>b</sup> | 2.92 ± 0.35<sup>c</sup> |
| Carbohydrate (g/dL) | 6.76 ± 0.27<sup>a</sup> | 6.93 ± 0.38<sup>b</sup> | 6.81 ± 0.20<sup>b</sup> | 6.51 ± 0.14<sup>c</sup> |
| Energy (kcal/dL) | 67.3 ± 6.5<sup>a</sup> | 64.1 ± 5.9<sup>b</sup> | 63.6 ± 4.5<sup>b</sup> | 60.3 ± 3.5<sup>b</sup> |

<sup>a</sup>All values are means ± SDs. Values not sharing a common superscript letter are significantly different, P < 0.05 (1-factor ANOVA with Bonferroni correction for multiple comparisons).

<sup>b</sup>Own mother’s milk: 28 ± 10 d of lactation.

<sup>c</sup>Colostral pool: donor milk <8 d.
nutritional intakes per kilogram of body weight per day. In addition, the theoretical nutritional intakes per kilogram of body weight per day corresponding to a standard HM procedure (4 packets complete HM fortifier/dL, providing 1.1 g protein, 1 g lipids, and 14 kcal energy; Enfamil Human Milk Fortifier) were also estimated.

Statistical analysis

The difference between infrared analyzer and chemical analysis for nitrogen and fat concentrations were evaluated by regression analysis and Bland-Altman plots (29) by using chemical analysis as the gold standard.

Macronutrient composition and variability in OMM and HM pools from a single donor, multiple donors, and colostral pools were compared by using 1-factor ANOVA with Bonferroni correction for multiple comparisons.

The variability in the nutritional content of the different milk groups and the nutritional intakes resulting from individualized or standard fortification were calculated as the mean value of the absolute difference between all individual values and the mean according to the following formula:

\[
\text{Variability} (\%) = \frac{\text{mean} |x(1 to n) - \text{mean}| \times 100}{\text{mean}}
\]

(1)

Nutritional intakes and variability resulting from individualized and standard fortifications were compared by using paired Student’s t test. All statistical analyses were performed by using Statistica software version 10 (StatSoft).

RESULTS

Validation of an infrared HM analyzer

Validation of the infrared HM analyzer was determined on 40 HM samples. A highly significant positive linear correlation was found between chemical reference values and infrared analysis (\(P < 0.001; r = 0.97\) and 0.99 for protein and fat, respectively). Both regression lines did not differ significantly from the identity line. With the use of chemical analysis as the gold standard, Bland-Altman plots (29) showed that the precision for nitrogen and fat estimation using infrared analysis corresponded to 6.7% and 4.3%, respectively, of the reference values (Figure 1).

Variability in daily composition of OMM and in HM pools from the milk bank

Mean (±SD) values for protein, fat, carbohydrate, and energy content of OMM (\(n = 428\)), single-donor HM pools (\(n = 138\)), multiple-donor HM pools (\(n = 224\)), and colostral pools (\(n = 14\)) are shown in Table 1. Significantly higher protein content and lower fat, carbohydrate, and energy contents were observed in the colostral pools (donor milk from 1 to 7 d of lactation) than in all the other groups. In OMM, mean protein, fat, and energy contents were significantly higher than in single- and multiple-donor milk pools. In addition, the protein content of single-donor milk pools was significantly lower compared with multiple-donor milk pools. Overall, of the 804 samples, 80% (\(n = 640\)) had a fat content <4 g/dL, whereas 51% (\(n = 413\)) had an energy content <65 kcal/dL. The protein content was <1.2 g/dL in 17% of samples (\(n = 141\)), between 1.2 and 1.6 g/dL in 50% of samples (\(n = 402\)), and >1.6 g/dL in 30% of samples (\(n = 243\)) (Figure 2).

The variability in protein, fat, and energy contents was high in the various groups (Table 2 and shown in Figure S1 under “Supplemental data” in the online issue). The variability in protein content was higher in single-donor pools and lower in colostral pools than in all other groups. The variability in fat content was higher in OMM than in all other groups, but the

FIGURE 2. Variability in protein (A), fat (B), and energy (C) concentrations of own mother’s milk and human milk pools (\(n = 804\)).
difference was not significant compared with the colostral pool ($P = 0.08$).

**Nutritional intakes and variability resulting from individualized and standard HM fortification procedures**

Between June 2006 and December 2011, 428 daily OMM individualized fortifications were performed in 24 VLBW preterm infants (mean ± SD birth weight = 1140 ± 230 g; gestational age = 28.6 ± 1.6 wk) over >3 wk. MCT supplementation was necessary in 64% (272 of 428) of daily OMM pools and HM fortifier was necessary in 99.5% (426 of 428) of daily OMM pools. The nutritional content of OMM after MCT supplementation and HM fortification is shown in Table 3. By comparison to standard fortification, protein intakes and the protein:energy ratio of individualized fortification were significantly lower, whereas the fat and the energy contents were significantly higher, with individualized fortification. The variability in nutritional intakes and protein:energy ratio were significantly lower using individualized compared with standard fortification. Thus, the variability in protein intake after individual fortification was reduced by 21% of the variability after standard fortification (Table 4 and Figure 3).

**DISCUSSION**

Several studies have shown an association between short- and long-term health, as well as neurodevelopmental outcomes, and cumulative intakes of HM during the early weeks of life in VLBW infants (20, 30). However, the use of HM as a sole source of nutrients is insufficient to cover the high nutritional requirements of growing preterm infants. OMM, with its higher protein content, improves growth compared with banked HM (31, 32), but remains suboptimal to support growth, especially lean body mass gain after the second or third week of lactation. Despite various HM fortifiers developed to increase protein, energy, minerals, electrolytes, trace elements, and vitamin supplies (20, 33), the use of fortified HM has failed to obtain postnatal growth in the range of fetal growth or that observed in infants fed preterm formulas (21–23).

In the present study, we showed that the macronutrient and energy composition of OMM and banked donor HM used for nutrition in preterm infants in the NICU are highly variable, leading to a high rate of protein and energy deficits compared with reference values.

As shown in Figure 1, protein, fat, and energy contents ranged from 0.8 to 2.4 g/dL for protein, from 1.8 to 6.6 g/dL for fat, and from 47 to 85 kcal/dL for energy. Furthermore, as shown in Figure 2, of all daily OMM and HM pool samples, 56% were <1.5 g protein/dL, whereas 79% were <4 g lipids/dL, and 67% were <67 kcal energy/dL (values frequently considered as reference values for preterm milk composition). These results differ from the recent reference values reported by Bauer and Gerss (34) who evaluated nutritional composition of OMM collected longitudinally from mothers of ELBW infants. In this study, they suggested that in OMM between 28 and 32 wk the protein content could be as high as 2.3–1.9 g/dL, whereas the fat and the energy content accounted for 4.4 g/dL and 77 kcal/dL, respectively.

Protein values of preterm mother’s milk are generally higher in the early postnatal period and decrease during lactation. However, a high variability remains between and within mothers (34). The present study confirms these 2 observations as shown in Figures S2 and S3 under “Supplemental data” in the online issue. Incomplete milk expression and manipulations of HM during expression, storage, transport, and processing are all additional factors influencing the high variability in expressed HM composition. Indeed, in clinical practice, it is not possible for mothers of preterm infants to follow the strict guidelines and methodology as proposed in a prospective study on HM composition (34). The fat content is highly related to manipulation and processing between expression and delivery to the preterm infants. As a result, the true energy and protein contents are unpredictable and differ significantly from those calculated by using a fixed composition for OMM or banked HM.

**TABLE 3**

Composition of OMM before and after individualized fortification with MCTs and HMF

<table>
<thead>
<tr>
<th></th>
<th>OMM (g/dL)</th>
<th>OMM + MCTs (g/dL)</th>
<th>OMM + MCTs + HMF (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>1.52 ± 0.28</td>
<td>1.52 ± 0.27</td>
<td>2.51 ± 0.14</td>
</tr>
<tr>
<td>Fat</td>
<td>3.79 ± 0.73</td>
<td>4.20 ± 0.45</td>
<td>5.09 ± 0.48</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>6.76 ± 0.27</td>
<td>6.76 ± 0.27</td>
<td>7.11 ± 0.28</td>
</tr>
<tr>
<td>Energy (kcal/dL)</td>
<td>67.26 ± 6.49</td>
<td>70.13 ± 4.52</td>
<td>82.66 ± 4.42</td>
</tr>
<tr>
<td>Protein:energy ratio</td>
<td>2.27 ± 0.37</td>
<td>2.17 ± 0.35</td>
<td>3.04 ± 0.19</td>
</tr>
</tbody>
</table>

1 All values are means ± SDs; n = 428. HMF, human milk fortifier; MCT, medium-chain triglyceride; OMM, own mother’s milk.

2 Fat concentration of human milk was adjusted up to 4 g/dL when necessary by adding MCTs.

3 Protein content was adjusted by using a complete HMF to provide 4.3 g protein · kg⁻¹ · d⁻¹ according to daily volume of feeding.
Growth differences between fortified HM and preterm formula-fed VLBW infants receiving an apparently similar energy and protein intake could also be related to a lower content of metabolizable protein and energy available for new tissue synthesis. Metabolic balance studies (35, 36) showed that nitrogen absorption as well as nitrogen utilization were lower in preterm infants fed fortified HM than in those fed preterm formulas. In all, the mean difference in nitrogen utilization accounted for 5.5% and could be related to nonnutritional proteins (lactoferrin, IgA) or nonprotein nitrogen content (urea) in HM. Net absorption of fat-derived energy was also frequently lower (78.3%) in infants fed HM than in those fed formula (88.4%), resulting in a higher fecal loss of energy. This difference could be increased by the use of pasteurized HM (37). Pasteurization of HM for high-risk preterm infants is frequently applied in milk banks and in neonatal units to reduce bacterial contamination and the risk of cytomegalovirus infection (38, 39). Pasteurization leads to inactivation of the bile salt–stimulated lipase of HM (40) as well as possible changes in the milk fat globule structure (41).

Standard fortification, adding a fixed amount of a fortifier as recommended by the manufacturer, is the most commonly used method to fortify mother’s milk. This method was not associated with a reduction in the variability in HM nutritional contents and often failed to meet the nutritional recommendations for preterm infants (42, 43). A more suitable fortification regimen was suggested to improve nutritional intakes and growth in preterm infants. Arslanoglu et al (44) adjusted the fortifier supply according to the values of blood urea nitrogen (BUN) considered to be a marker of protein adequacy in preterm infants. This BUN method, which was developed to avoid inadequate and excessive protein intake, is easy to apply and does not require daily milk analyses. However, it has been shown that BUN is not correlated to protein intakes during the first weeks of life but reflects the renal immaturity of ELBW and VLBW infants (45, 46). Therefore, the use of BUN as a threshold level to adjust protein intake is inadequate. Polberger et al (47, 48) have proposed analyzing, once or twice a week, the macronutrient content of 24-h OMM collections so as to adapt the fortification in the range of nutritional needs. Recently, Miller et al (49) suggested that an increase in the protein fortification from 1 g/dL to 1.4 g/dL produces a minimal benefit on growth in preterm infants. They found no significant increase in daily weight gain but a significant reduction in incidence of growth restriction in the higher protein group. However, such an increase in protein fortification does not compensate for the variability in HM composition. The risk of energy deficiency as well as of protein overload remains, with its potential long-term adverse effects. In 2007 we suggested that daily individualized HM fortification could provide nutritional supplies in the range of the nutritional recommendations and improve growth in VLBW infants (27).

In the present study, we confirm the high daily variability in the nutritional value of HM within a large number of samples of OMM, and that this variability could be reduced by daily individualized fortification. With standard fortification, protein deficiency or overload, and energy deficiency were frequently observed (Figure 3, A and B). By contrast, after individualized fortification, the range of protein intake decreased from 3.3–6.6 to 3.6–4.5 g·kg⁻¹·d⁻¹ and that of the protein:energy ratio from 2.4–4.7 to 2.4–3.8 g/100 kcal (Figure 3, A and C). With this technique, we showed that appropriate nutritional intakes could be provided daily in the upper range of recent ESPGHAN (European Society of Paediatric Gastroenterology, Hepatology, and Nutrition) recommendations (19). In addition, with individualized fortification, the mean use of fortifier was significantly lower (3.6 compared with 4.0 packets/dL), decreasing the osmolality of the fortified HM and the risk of gastric intolerance.

The currently available multicomponent HM fortifiers are not adequately designed for use in VLBW infants. In the present study, the relative fat deficit of expressed HM provided to the NICU was corrected with an MCT emulsion. However, the fatty acid profile of the fortified HM remains inadequate for preterm infants, especially in terms of long-chain PUFA content. Therefore, newer fortifiers providing high protein and energy intakes with adequate long-chain PUFA content, but without inducing a gastrointestinal osmotic load >360–400 mOsm/kg H₂O, need to be developed to improve the nutritional supply with minimal side effects for the preterm infants.

Although individualized fortification is time consuming and expensive and requires additional equipment and well-trained staff, the use of infrared technology to determine the macronutrient composition of HM is likely to expand its availability in NICUs. It could have practical application in HM banks for donor milk composition or to develop specific HM pools with higher protein and/or energy content.

<table>
<thead>
<tr>
<th>Intake</th>
<th>Individualized fortification</th>
<th>Standard fortification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (g·kg⁻¹·d⁻¹)</td>
<td>4.25 ± 0.13*</td>
<td>4.45 ± 0.51</td>
</tr>
<tr>
<td>Fat (g·kg⁻¹·d⁻¹)</td>
<td>8.6 ± 0.9*</td>
<td>8.1 ± 1.3</td>
</tr>
<tr>
<td>Energy (kcal·kg⁻¹·d⁻¹)</td>
<td>140 ± 9*</td>
<td>138 ± 13</td>
</tr>
<tr>
<td>Protein:energy ratio</td>
<td>3.04 ± 0.19*</td>
<td>3.24 ± 0.32</td>
</tr>
<tr>
<td>Variability (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>2.0 ± 2.3*</td>
<td>9.2 ± 6.8</td>
</tr>
<tr>
<td>Fat</td>
<td>6.6 ± 7.4*</td>
<td>12.1 ± 10.3</td>
</tr>
<tr>
<td>Energy</td>
<td>4.8 ± 4.5*</td>
<td>7.3 ± 6.1</td>
</tr>
<tr>
<td>Protein:energy ratio</td>
<td>4.5 ± 4.3*</td>
<td>7.6 ± 6.5</td>
</tr>
</tbody>
</table>

All values are means ± SD; n = 428. Intakes and variability resulting from individualized and standard fortifications were compared by using paired Student’s t test. *P < 0.05 when compared with standard fortification.
As a result of the lower energy and protein bioavailability of HM, an energy intake of 140 kcal $\cdot kg^{-1} \cdot d^{-1}$ and a protein intake of 4.2 g $\cdot kg^{-1} \cdot d^{-1}$ were estimated to be necessary to ensure an adequate growth. These values are slightly higher than those recently recommended by the ESPGHAN Committee on Nutrition in 2010 (19). These recommendations are more related to preterm infants fed formula than to those fed fortified HM, and recent studies suggest that specific recommendations for the use of HM are necessary. These new recommendations need to consider the lower metabolizable energy and protein content of fortified HM, the effect of pasteurization, and the additional nutritional losses suggested during continuous feeding (27, 50).

In conclusion, the macronutrient content of expressed preterm OMM and donor HM pools is widely variable, especially for protein, fat, and energy. Standard fortification, as recommended by the manufacturer, does not meet the high nutritional requirements of immature infants, thereby creating conditions for under- or overnutritional risks. Individualized fortification based on daily HM analysis improves and regulates the protein and energy intakes in preterm infants but requires equipment and a well-trained staff. Further studies are necessary to improve the fortifier formulation to meet individual needs and new recommendations, and studies particularly dedicated to ELBW and VLBW infants fed HM need to be developed.

We thank Michael Imeokparia for the English revision.

The authors’ responsibilities were as follows—VdH: was the principal investigator in the study and contributed to the conception and design of the study and acquisition, analysis, interpretation of data; drafted the manuscript; revised the manuscript for important intellectual content; and had final approval of the draft that was submitted for publication; and JR: contributed significantly to the conception and design of the study and analysis and interpretation of data, participated in drafting the manuscript and providing in-depth revision for important intellectual content, and had final approval of the draft that was submitted for publication. Neither of the authors had a conflict of interest to declare.

REFERENCES