Variability in human milk composition: benefit of individualized fortification in very-low-birth-weight infants[1–3]

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 $g \cdot kg^{-1} \cdot d^{-1}$ 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* doi: 10.3945/ajcn.112.042689.

INTRODUCTION

Human milk (HM)[4] 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 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, Liège, Belgium.

[2] Presented at the symposium "Bringing Science to Early Life Nutrition" held in Utrecht, Netherlands, 25 October 2011.

[3] Address correspondence to V de Halleux, Service Universitaire de Néonatologie, 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.

doi: 10.3945/ajcn.112.042689.

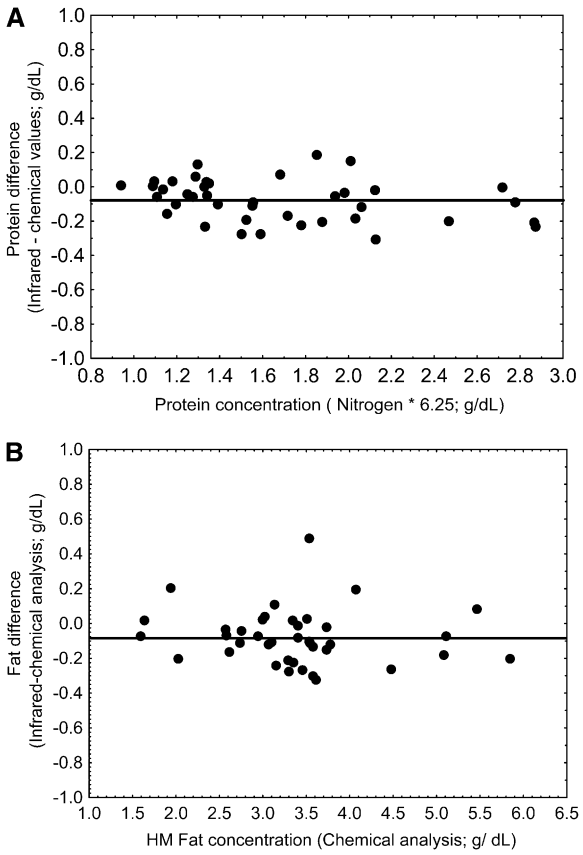

FIGURE 1. Accuracy of protein (A) and fat (B) determination in human milk (n = 40) with the use of infrared technology compared with chemical analysis as the gold standard using Bland-Altman plots (29). HM, human milk.

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 ("Soxhhlet" 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 $\cdot$ kg^{-1} $\cdot$ d^{-1} 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[1]

	Own mother's milk (n = 428)[2]	Single-donor milk pool (n = 138)	Multiple-donor milk pool (n = 224)	Colostral pool (n = 14)[3]
Protein (g/dL)	1.52 ± 0.28[a]	1.34 ± 0.37[b]	1.46 ± 0.24[c]	2.00 ± 0.09[d]
Fat (g/100 mL)	3.79 ± 0.73[a]	3.45 ± 0.60[b]	3.39 ± 0.48[b]	2.92 ± 0.35[c]
Carbohydrate (g/dL)	6.76 ± 0.27[a]	6.93 ± 0.38[b]	6.81 ± 0.20[a]	6.51 ± 0.14[c]
Energy (kcal/dL)	67.3 ± 6.5[a]	64.1 ± 5.9[b]	63.6 ± 4.5[b]	60.3 ± 3.5[b]

[1] 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).
[2] Own mother's milk: 28 ± 10 d of lactation.
[3] 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:

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

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

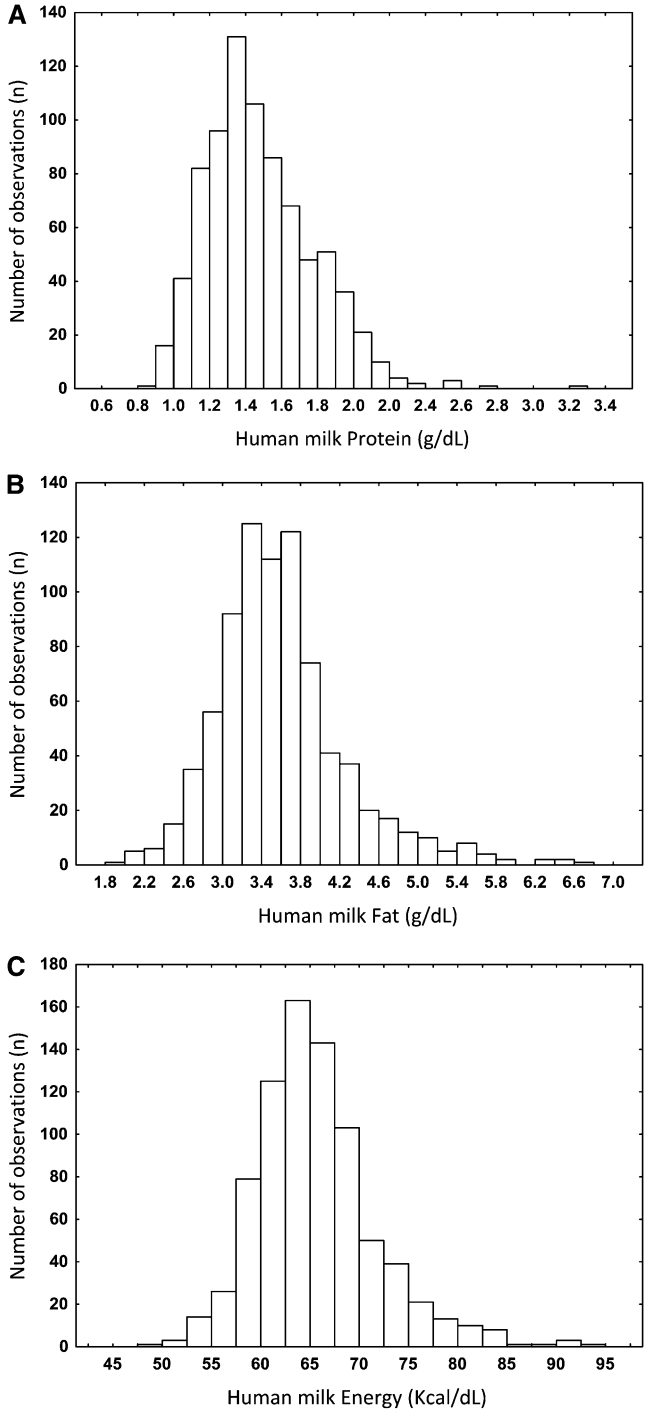

FIGURE 2. Variability in protein, fat, and energy concentrations of own mother's milk and human milk pools ($n = 804$).

TABLE 2
Variability in protein, fat, and energy contents of own mother's milk, single- and multiple-donor milk pools, and colostral pools[1]

	Percentage of variability[2]			
	Own mother's milk (n = 428)	Single-donor milk pool (n = 138)	Multiple-donor milk pool (n = 224)	Colostral pool (n = 14)
Protein	14.7 ± 10.6[a]	19.3 ± 19.4[b]	13.5 ± 9.9[a]	3.8 ± 2.4[c]
Fat	14.5 ± 12.7[a]	10.3 ± 8.4[b]	10.6 ± 9.4[b]	9.7 ± 6.5[a,b]
Energy	7.3 ± 6.26[a]	6.9 ± 6.0[a]	5.3 ± 4.7[b]	4.4 ± 3.6[a,b]

[1] 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).
[2] Variability(%) = mean[|x(1 to n) − mean| × 100/mean].

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[1]

	OMM	OMM + MCTs[2]	OMM + MCTs + HMF[3]
Protein (g/dL)	1.52 ± 0.28	1.52 ± 0.27	2.51 ± 0.14
Fat (g/dL)	3.79 ± 0.73	4.20 ± 0.45	5.09 ± 0.48
Carbohydrate (g/dL)	6.76 ± 0.27	6.76 ± 0.27	7.11 ± 0.28
Energy (kcal/dL)	67.26 ± 6.49	70.13 ± 4.52	82.66 ± 4.42
Protein:energy ratio	2.27 ± 0.37	2.17 ± 0.35	3.04 ± 0.19

[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.

TABLE 4
Comparison of individualized fortification intakes and percentage of variability with theoretical values obtained after standard fortification[1]

	Individualized fortification	Standard fortification
Intake		
Protein ($g \cdot kg^{-1} \cdot d^{-1}$)	4.25 ± 0.13*	4.45 ± 0.51
Fat ($g \cdot kg^{-1} \cdot d^{-1}$)	8.6 ± 0.9*	8.1 ± 1.3
Energy ($kcal \cdot kg^{-1} \cdot d^{-1}$)	140 ± 9*	138 ± 13
Protein:energy ratio	3.04 ± 0.19*	3.24 ± 0.32
Variability (%)		
Protein	2.0 ± 2.3*	9.2 ± 6.8
Fat	6.6 ± 7.4*	12.1 ± 10.3
Energy	4.8 ± 4.5*	7.3 ± 6.1
Protein:energy ratio	4.5 ± 4.3*	7.6 ± 6.5

[1] All values are means ± SDs; $n = 428$. 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.

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 $g \cdot kg^{-1} \cdot d^{-1}$ 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 H_2O, 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.

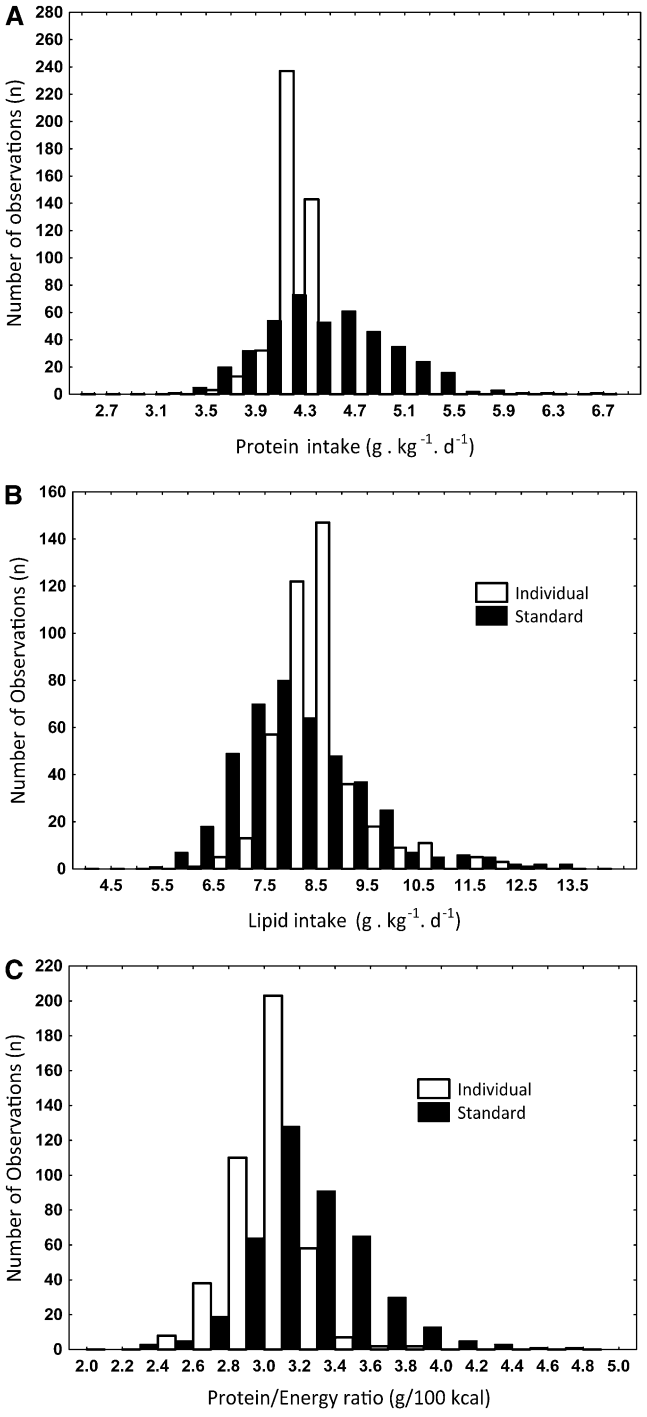

FIGURE 3. Protein (A) and lipid (B) intakes and protein:energy ratio (C) according to individualized or standard human milk fortification (n = 428).

As a result of the lower energy and protein bioavailability of HM, an energy intake of 140 kcal · kg^{-1} · d^{-1} and a protein intake of 4.2 g · kg^{-1} · 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

1. American Academy of Pediatric. Breastfeeding and the use of human milk. Pediatrics 2012;129:e827–41.
2. Donovan SM. Role of human milk components in gastrointestinal development: current knowledge and future needs. J Pediatr Nutrition and Gastrointestinal Tract Development and Function 2006;149:S49–61.
3. Taylor SN, Basile LA, Ebeling M, Wagner CL. Intestinal permeability in preterm infants by feeding type: mother's milk versus formula. Breastfeed Med 2009;4:11–5.
4. Rønnestad A, Abrahamsen TG, Medbo S, Reigstad H, Lossius K, Kaaresen PI, Egeland T, Engelund IE, Irgens LM, Markestad T. Late-onset septicemia in a Norwegian national cohort of extremely premature infants receiving very early full human milk feeding. Pediatrics 2005;115:e269–76.
5. Schanler RJ, Shulman RJ, Lau C. Feeding strategies for premature infants: beneficial outcomes of feeding fortified human milk versus preterm formula. Pediatrics 1999;103:1150–7.
6. Sisk PM, Lovelady CA, Dillard RG, Gruber KJ, O'Shea TM. Early human milk feeding is associated with a lower risk of necrotizing enterocolitis in very low birth weight infants. J Perinatol 2007;27:428–33.
7. Meinzen-Derr J, Poindexter B, Wrage L, Morrow AL, Stoll B, Donovan EF. Role of human milk in extremely low birth weight infants' risk of necrotizing enterocolitis or death. J Perinatol 2009;29:57–62.
8. Vohr BR, Poindexter BB, Dusick AM, McKinley LT, Higgins RD, Langer JC, Poole WK. Persistent beneficial effects of breast milk ingested in the neonatal intensive care unit on outcomes of extremely low birth weight infants at 30 months of age. Pediatrics 2007;120:e953–9.
9. Isaacs EB, Gadian DG, Sabatini S, Chong WK, Quinn BT, Fischl BR, Lucas A. The effect of early human diet on caudate volumes and IQ. Pediatr Res 2008;63:308–14.
10. Lucas A, Morley R, Cole TJ, Lister G, Leeson-Payne C. Breast milk and subsequent intelligence quotient in children born preterm. Lancet 1992;339:261–4.
11. Singhal A, Cole TJ, Lucas A. Early nutrition in preterm infants and later blood pressure: two cohorts after randomised trials. Lancet 2001; 357:413–9.
12. Lucas A. Long-term programming effects of early nutrition—implications for the preterm infant. J Perinatol 2005;25(suppl 2):S2–6.
13. Embleton NE, Pang N, Cooke RJ. Postnatal malnutrition and growth retardation: an inevitable consequence of current recommendations in preterm infants? Pediatrics 2001;107:270–3.

14. Senterre T, Rigo J. Reduction in postnatal cumulative nutritional deficit and improvement of growth in extremely preterm infants. Acta Paediatr 2012;101:e64–70.
15. Ehrenkranz RA, Dusick AM, Vohr BR, Wright LL, Wrage LA, Poole WK. Growth in the neonatal intensive care unit influences neurodevelopmental and growth outcomes of extremely low birth weight infants. Pediatrics 2006;117:1253–61.
16. Latal-Hajnal B, von Siebenthal K, Kovari H, Bucher HU, Largo RH. Postnatal growth in VLBW infants: significant association with neurodevelopmental outcome. J Pediatr 2003;143:163–70.
17. Belfort MB, Rifas-Shiman SL, Sullivan T, Collins CT, McPhee AJ, Ryan P, Kleinman KP, Gillman MW, Gibson RA, Makrides M. Infant growth before and after term: effects on neurodevelopment in preterm infants. Pediatrics 2011;128:e899–906.
18. Tsang RCUR, Koletzko B, Zlotkin SH. Summary of reasonable nutrient intakes (mass units) for preterm infants. In: Tsang R, Uauy R, Koletzko B, Zlotkin S, eds. Nutrition of the preterm infant scientific basis and practical guidelines. 2nd ed. Cincinnati, OH: Digital Educational Publishing, 2005:415.
19. Agostoni C, Buonocore G, Carnielli VP, De Curtis M, Darmaun D, Decsi T, Domellof M, Embleton ND, Fusch C, Genzel-Boroviczeny O, et al. Enteral nutrient supply for preterm infants: commentary from the European Society of Paediatric Gastroenterology, Hepatology and Nutrition Committee on nutrition. J Pediatr Gastroenterol Nutr 2010; 50:85–91.
20. Kuschel CA, Harding JE. Multicomponent fortified human milk for promoting growth in preterm infants. Cochrane Database Syst Rev 2004;CD000343.
21. Pieltain C, De Curtis M, Gerard P, Rigo J. Weight gain composition in preterm infants with dual energy X-ray absorptiometry. Pediatr Res 2001;49:120–4.
22. Henriksen C, Westerberg AC, Ronnestad A, Nakstad B, Veierod MB, Drevon CA, Iversen PO. Growth and nutrient intake among very-low-birth-weight infants fed fortified human milk during hospitalisation. Br J Nutr 2009;102:1179–86.
23. Sullivan S, Schanler RJ, Kim JH, Patel AL, Trawöger R, Kiechl-Kohlendorfer U, Chan GM, Blanco CL, Abrams S, Cotten CM, et al. An exclusively human milk-based diet is associated with a lower rate of necrotizing enterocolitis than a diet of human milk and bovine milk-based products. J Pediatr 2010;156:562–7.e561.
24. Weber A, Loui A, Jochum F, Buhrer C, Obladen M. Breast milk from mothers of very low birthweight infants: variability in fat and protein content. Acta Paediatr 2001;90:772–5.
25. Michaelsen KF, Skafte L, Badsberg JH, Jorgensen M. Variation in macronutrients in human bank milk: influencing factors and implications for human milk banking. J Pediatr Gastroenterol Nutr 1990;11: 229–39.
26. Saarela T, Kokkonen J, Koivisto M. Macronutrient and energy contents of human milk fractions during the first six months of lactation. Acta Paediatr 2005;94:1176–81.
27. de Halleux V, Close A, Stalport S, Studzinski F, Habibi F, Rigo J. Intérêt de la supplémentation du lait maternel “à la carte”. [Advantages of individualized fortification of human milk for preterm infants.] Arch Pediatr 2007;14(suppl 1):S5–10 (in French).
28. Michaelsen KF, Pedersen SB, Skafte L, Jaeger P, Peitersen B. Infrared analysis for determining macronutrients in human milk. J Pediatr Gastroenterol Nutr 1988;7:229–35.
29. Bland JM, Altman D. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986;1:307–10.
30. Morley R. Nutrition and cognitive development. Nutrition 1998;14: 752–4.
31. Montjaux-Régis N, Cristini C, Arnaud C, Glorieux I, Vanpee M, Casper C. Improved growth of preterm infants receiving mother’s own raw milk compared with pasteurized donor milk. Acta Paediatr 2011; 100:1548–54.
32. Stein H, Cohen D, Herman AA, Rissik J, Ellis U, Bolton K, Pettifor J, MacDougall L. Pooled pasteurized breast milk and untreated own mother’s milk in the feeding of very low birth weight babies: a randomized controlled trial. J Pediatr Gastroenterol Nutr 1986;5:242–7.
33. Kashyap S, Schulze KF, Forsyth M, Dell RB, Ramakrishnan R, Heird WC. Growth, nutrient retention, and metabolic response of low-birth-weight infants fed supplemented and unsupplemented preterm human milk. Am J Clin Nutr 1990;52:254–62.
34. 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.
35. De Curtis M, Senterre J, Rigo J, Putet G. Carbohydrate derived energy and gross energy absorption in preterm infants fed human milk or formula. Arch Dis Child 1986;61:867–70.
36. Rigo J. Protein, amino acid and other nitrogen compounds. In: Tsang RCUR, Koletzko B, Zlotkin SH, eds. Nutrition of the preterm infants scientific basis and practical guidelines. 2nd ed. Cincinnati, OH: Digital Educational Publishing, 2005:45–80.
37. Andersson Y, Savman K, Blackberg L, Hernell O. Pasteurization of mother’s own milk reduces fat absorption and growth in preterm infants. Acta Paediatr 2007;96:1445–9.
38. Hamprecht K, Maschmann J, Jahn G, Poets CF, Goelz R. Cytomegalovirus transmission to preterm infants during lactation. J Clin Virol 2008;41:198–205.
39. Vervoort A, Delsat L, Pieltain C, de Halleux V, Rigo J. Evaluation de la qualité bactériologique du lait maternel dans un service de néonatologie (NIC). [Evaluation of the bacteriologic quality of breast milk in a neonatology service in Belgium.] Rev Med Liege 2007;62:159–65 (in French).
40. Henderson TR, Fay TN, Hamosh M. Effect of pasteurization on long chain polyunsaturated fatty acid levels and enzyme activities of human milk. J Pediatr 1998;132:876–8.
41. Soderhjelm L. Fat absorption studies in children. I. Influence of heat treatment on milk on fat retention by premature infants. Acta Paediatr 1952;41:207–21.
42. Corvaglia L, Aceti A, Paoletti V, Mariani E, Patrono D, Ancora G, Capretti MG, Faldella G. Standard fortification of preterm human milk fails to meet recommended protein intake: Bedside evaluation by near-infrared-reflectance-analysis. Early Hum Dev 2010;86:237–40.
43. Arslanoglu S, Moro GE, Ziegler EE. Preterm infants fed fortified human milk receive less protein than they need. J Perinatol 2009;29:489–92.
44. Arslanoglu S, Moro GE, Ziegler EE. Adjustable fortification of human milk fed to preterm infants: does it make a difference? J Perinatol 2006;26:614–21.
45. Ridout E, Melara D, Rottinghaus S, Thureen PJ. Blood urea nitrogen concentration as a marker of amino-acid intolerance in neonates with birthweight less than 1250 g. J Perinatol 2005;25:130–3.
46. Roggero P, Giannì ML, Morlacchi L, Piemontese P, Liotto N, Taroni F, Mosca F. Blood urea nitrogen concentrations in low-birth-weight preterm infants during parenteral and enteral nutrition. J Pediatr Gastroenterol Nutr 2010;51:213–5.
47. Polberger S, Raiha NC, Juvonen P, Moro GE, Minoli I, Warm A. Individualized protein fortification of human milk for preterm infants: comparison of ultrafiltrated human milk protein and a bovine whey fortifier. J Pediatr Gastroenterol Nutr 1999;29:332–8.
48. Polberger S. New approaches to optimizing early diets. Nestle Nutr Workshop Ser Pediatr Program 2009;63:195–204; discussion 204–8, 259–68.
49. Miller J, Makrides M, Gibson RA, McPhee AJ, Stanford TE, Morris S, Ryan P, Collins CT. Effect of increasing protein content of human milk fortifier on growth in preterm infants born at <31 wk gestation: a randomized controlled trial. Am J Clin Nutr 2012;95:648–55.
50. Rogers SP, Hicks PD, Hamzo M, Veit LE, Abrams SA. Continuous feedings of fortified human milk lead to nutrient losses of fat, calcium and phosphorous. Nutrients 2010;2:230–40.