State of the Art
Strategies for Human Milk Fortification to Achieve Optimal Health Outcomes in Premature Infants

Kari N Harris, MPH Candidate 2013
UNC Chapel Hill
Gillings School of Global Public Health
Nutrition Department

Abstract
Providing nutritional support to the preterm infant in the neonatal ICU has proved challenging despite improved survival rates among this vulnerable population. Premature infants are at higher risk for medical complications compared to term infants given their decreased birth weight and immature development. Human milk is associated with better health outcomes related to immune modulation, neurological development, and gastrointestinal function compared to preterm formula. However, extra-uterine growth failure still persists with the use of human milk. Human milk fortifiers are available to increase the macronutrient and micronutrient content of breastmilk to meet the increased needs of premature infants born weighing less than 1500g. Although adequate nutrient delivery can improve growth velocity, the most appropriate nutrition regimen to fortify human milk is not known. This review summarizes the benefits and limitations of available human milk fortification strategies as seen during my advanced clinical rotation in the Level III neonatal ICU at Children’s National Medical Center in Washington, D.C.

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BACKGROUND
What are the special nutritional needs of premature or growth-impaired infants?
Advancements in medical care within the past two decades have led to increased survival rates of premature infants. Nutrition interventions are an important aspect of care for these vulnerable infants and need to be continually re-evaluated as new research and clinical findings are available. However, meeting the nutritional needs of premature infants is challenging because of their limited nutrient reserves, need for rapid growth, decreased absorption of nutrients, immature organs, and increased risk for infection. Due to their low birth weight, it is known that infants born prematurely do not have sufficient protein and micronutrient stores because the majority of fetal nutrient accretion occurs during the third trimester of pregnancy. In general, premature (<37 weeks of gestation), low birth weight (LBW <2500g), and very low birth weight (VLBW, <1500 g) infants have higher needs of calories, protein, minerals (calcium, phosphorus, sodium, zinc, iron), and Vitamin D compared to term infants. Nutrient needs of extremely premature (<30 weeks gestation) are even greater. Fortification of breast milk is a practice used to reach these increased nutrient needs while providing many of the benefits of human milk.

Very preterm infants, who are typically too developmentally immature to suckle until approximately 34 weeks gestational age, are provided nutrition initially via parenteral nutrition (PN). Enteral feedings with expressed breast milk is initiated as soon as medically appropriate. Parenteral nutrition is tapered with the goal to discontinue PN as soon as full enteral feeds of expressed breast milk are reached in order to decrease risk of infection through the central line access. Thus the premature infant’s immunocompromised status has nutritional implications and necessary sanitary measures should be implemented to reduce risk of infections.

However, enteral nutrition for premature infants face unique challenges. Premature infants have an immature gastrointestinal (GI) tract, which leads to decreased motility and increased gut permeability. This makes providing adequate nutrition difficult because the premature infant’s GI tract has malabsorption of nutrients, increased risk of feeding intolerance, and increased risk of infection, especially after extended periods of no enteral intake. Feeding intolerance is a term used to summarize clinical symptoms of reflux, emesis, large gastric residual volumes, abdominal distention causing a decrease, delay, or discontinuation of feeds and/or visible abdominal loops on am. In addition, the premature infant’s immature GI tract and increased gut permeability in-
crease the infant’s risk of developing inflammation and severe tissue damage associated with necrotizing enterocolitis (NEC) once enteral feeds are initiated. NEC is a particularly high-risk adverse health outcome due to its high case-fatality ratio, high likelihood of requiring a surgical procedure and complications such as GI stricture, short-bowel syndrome, and cholestasis. Feeding intolerance and NEC are frequently the cause of delayed advancement of enteral feedings in premature infants. Delayed provision of full nutritional support and onset of these adverse clinical outcomes result in cumulative energy and protein deficits that in turn negatively impact postnatal growth outcomes.

Premature-LBW infants are born with decreased bone mineral density thus are already at increased risk for osteopenia of prematurity, fractures, and rickets. LBW infants have increased needs for calcium, phosphorus, zinc, and Vitamin D to promote bone mineralization. In addition, the risk of osteopenia, rickets, and collapsed vertebrae is elevated among LBW infants who require parenteral nutrition as sole source of nutrition for extended periods of time because parenteral nutrition regimens cannot provide adequate calcium and phosphorus due to osmolality and solubility limitations. Once enteral feeds become the primary source of nutrition, it remains important to monitor adequacy of mineral and micronutrient intake to prevent osteopenia of prematurity and anemia.

The best source of nutrition will meet the increased nutrient needs of premature infants, support growth, and limit micronutrient and macronutrient deficiencies while simultaneously decreasing risk for adverse health outcomes. See Table 1 for a description of select nutrient needs that have changed in recent years for LBW infants, which are similar to those born prematurely who do not meet LBW criteria.

To meet their special needs while providing breast milk, the American Academy of Pediatrics recommends fortifying breast milk for:

- Infants younger than 34 weeks gestation
- Infants weighing less than 1500g at birth
- LBW infants who require greater than 2 week of parenteral nutrition, as PN does not provide adequate micronutrients
- LBW infants with suboptimal growth or high acuity nutritional risks caused by medical conditions or complications of prematurity

Breast milk fortification is appropriate for these infants because they are born without adequate nutrient stores and have higher nutrient needs than breast milk can provide. Despite these recommendations, however, there is still substantial controversy about the most appropriate use of human milk fortifiers (HMF). There is still concern that in the attempt to meet premature infants’ nutrient needs for growth human milk fortifiers may actually counteract the immunoprotective benefits of breast milk by increasing the risk for other adverse clinical outcomes. This review summarizes the strategies of human milk fortification that have developed in recent years to promote growth in premature-low birth weight infants while minimizing adverse health events, such as necrotizing enterocolitis (NEC), feeding intolerance, late onset sepsis (LOS), osteopenia, metabolic acidosis, and anemia.

To evaluate the extent to which current evidence supports these strategies, this review aims to: (i) highlight the health outcomes that need be considered to identify optimal feeding regimens for these vulnerable infants; (ii) describe the evidence supporting the use of fortified human milk rather than enhanced infant formula; and (iii) review the evidence on benefits and risks related to the form, content, and administration of these fortifiers, which provides the scientific basis for current practice. There are, for example, debates related to the micronutrient and macronutrient content, use of liquid vs. powdered formulations to obtain ideal osmolality and to reduce risk of infection, and individualization of both dose and duration of these fortifiers. These factors and others appear to have an important impact on numerous health outcomes in these vulnerable infants.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>VLBW (&gt;1000g) Tsang, 2005</th>
<th>VLBW (&gt;1000g) ESGPHAN, 2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluid</td>
<td>135-190mL/kg</td>
<td>135-200mL/kg</td>
</tr>
<tr>
<td>Energy</td>
<td>110-130 kcal/kg</td>
<td>110-135 kcal/kg</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>7-17 g/kg</td>
<td>11.6-13.2 g/kg</td>
</tr>
<tr>
<td>Protein</td>
<td>3.4-4.2 g/kg</td>
<td>3.5-4.0 g/kg</td>
</tr>
<tr>
<td>Protein:Energy</td>
<td>---</td>
<td>3.2-3.6 g/100kcal</td>
</tr>
<tr>
<td>Fat</td>
<td>5.3-7.2 g/kg</td>
<td>4.8-6.6 g/kg</td>
</tr>
<tr>
<td>Minerals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>100-220 mg/kg</td>
<td>120-140 mg/kg</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>60-140 mg/kg</td>
<td>60-90 mg/kg</td>
</tr>
<tr>
<td>Magnesium</td>
<td>7.9-15 mg/kg</td>
<td>9-15 mg/kg</td>
</tr>
<tr>
<td>Sodium</td>
<td>69-115 mg/kg (3-5mEq/kg)</td>
<td>69-115 mg/kg</td>
</tr>
<tr>
<td>Potassium</td>
<td>78-117 mg/kg (2-3 mEq/kg)</td>
<td>66-132 mg/kg</td>
</tr>
<tr>
<td>Chloride</td>
<td>107-249 mEq/kg</td>
<td>105-177 mg/kg</td>
</tr>
<tr>
<td>Trace Elements</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zinc</td>
<td>1000-3000 mcg/kg</td>
<td>1.1-2.0 mg/kg</td>
</tr>
<tr>
<td>Copper</td>
<td>120-150 mcg/kg</td>
<td>10-132 mg/kg</td>
</tr>
<tr>
<td>Iron</td>
<td>2-4 mg/kg</td>
<td>2-3 mg/kg</td>
</tr>
<tr>
<td>Vitamins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin D</td>
<td>150-400 IU/kg</td>
<td>800-1000 IU/d</td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>0.3 mcg/kg</td>
<td>0.1-0.77 mcg/kg</td>
</tr>
<tr>
<td>Folac acid</td>
<td>25-50 mcg/kg</td>
<td>35-100 mcg/kg</td>
</tr>
</tbody>
</table>
1. DEFINING OPTIMAL GROWTH AND DEVELOPMENT TARGETS

How can we assess whether infant feeding regimens are optimal for growth and health?

Defining the best source of nutrition that can meet these increased nutrient needs of premature-LBW infants has been difficult partly due to the conflicting data regarding the optimal growth rate for premature infants. It was once thought that optimal growth was synonymous with the growth rate that mimics the rapid body mass accretion that occurs during the third trimester. Growth charts for premature infants, such as the Fenton, are based on these intrauterine growth rate projections.16,17,18 Preterm formula was heralded as the growth success story because it often helped premature infants achieve much more rapid growth rates than breast milk.19,20 However, more recent data has shown that rapid catch-up growth in small for gestational age infants is a risk factor for adult onset cardiovascular disease,21,22 adult onset leptin resistance and obesity3 and insulin resistance as a teenager.18 While these observational studies were not focused on premature infants, the research findings from these studies does beg the question as to whether current criteria for evaluating optimal feeding regimens, based on rapidly achieving intrauterine growth, are ideal given uncertainty about long-term health outcomes.23 Even though some studies have associated early postnatal suboptimal weight gain with later low cognitive function, others have found that breastfed infants have improved neurodevelopment in spite of insufficient weight gain, a phenomenon termed the “apparent breastfeeding paradox”.23 Despite the limited and conflicting data, in the absence of clear alternatives, the general consensus still remains that achieving intrauterine growth rates of 15-20g/kg/d are both possible and ideal for premature infants with birth weights between 1000g and 1800g to achieve as their extra-uterine growth rate.13,17,18,20

Growth outcomes alone are not sufficient to assess the optimal nutrition source for premature and low birth weight infants. Nutrition goals should also seek to reduce the premature infant’s risk for adverse nutrition-related clinical outcomes including necrotizing enterocolitis (NEC), osteopenia of prematurity, metabolic acidosis, jaundice, anemia, late onset sepsis and other infections, prolonged feeding intolerance, compromised gut integrity, and death compared to term infants and infants >1500g.

2. NUTRITIONAL AND IMMUNE DEFICIENCIES OF THE PRETERM AND/OR GROWTH-IMPAIRED INFANT

What is the rationale for fortifying human milk vs. providing enhanced preterm formula?

Breast milk is considered the best source of nutrition for all newborns15 due to the high bioavailability of nutrients in breast milk and the immunoprotective benefits it bestows on the infant. For premature infants, specifically, consumption of human milk is associated with a more rapid achievement of full feeds, improved feeding tolerance, reduction in incidence of NEC & sepsis, shorter hospital stay, fewer hospital readmissions up to 30 months after discharge, and improved brain development and neurodevelopmental outcome when compared to a diet of preterm formula.4,5,24,25 Overall these improved health outcomes from breastfeeding result in reduction in healthcare costs for premature newborns.4 Indeed, breast milk is associated with better health outcomes than formula with the exception of growth outcomes defined using current criteria.6,14,15,19,24,25

The variability of nutrient content and inadequate levels of some nutrients in breast milk have associated it with poorer growth outcomes compared to infant formulas. In contrast, preterm formulas have the advantage of consistently delivering nutrients at pre-determined levels that can be easily manipulated. Preterm infant formulas have been re-formulated in recent years to contain adequate levels of minerals but they lack the growth hormones and immunological properties of breast milk.26 Human milk fortifiers (HMF) are added to breast milk in order to maximize the delivery of micro- and macronutrients as an addition to the other beneficial components of breast milk. In addition, human milk fortification is not associated with an increased risk of NEC unlike preterm formulas.24 However, despite the inability to support intrauterine growth rates, a meta-analysis founds no differences in feeding tolerance when comparing premature infants fed fortified human milk or unfortified human milk.19 Despite the improvements in preterm formulas, fortifying human milk is considered ideal for these at-risk infants.

What are nutrient deficiencies in premature maternal milk?

Despite initially high nutrient density, concerns remain about the adequacy of the breast milk mothers of premature infants for achieving
current criteria for optimal growth and development. At first premature milk is higher in fat and protein compared to term breast milk, which helps meet the high caloric demands of a premature infant. The exact content of energy and protein in breast milk, however, will vary throughout the day, within each expression, over the course of lactation, and from mother to mother. This reduces the certainty of nutrient intake and can result in deficiencies in the premature infant. In addition, premature milk does not contain adequate levels of calcium, phosphorus, Vitamin D, and iron to meet the needs of the premature infant. Exclusively breastfed preterm and LBW infants who do not receive adequate levels of calcium, phosphorus, and Vitamin D are at increased risk for osteopenia of prematurity. Two weeks after birth, breast milk from the mother of a premature infant will decline in content of fat, protein, sodium, and zinc to levels found in breast milk from the mother of a term infant. The protein content of premature breast milk will continue to progressively decline in the first 4-6 weeks. However, the nutrient needs for preterm infant remain elevated during this time of nutrient decline. Unfortified preterm human milk are unable to provide the general caloric requirements of 110-135 kcal/kg/d and minimum protein intake of 3.4 g/kg/d to support the optimal average weight gain of 15 to 20 g/day in preterm, LBW infants. Consequently, American Academy of Pediatrics (AAP), US Food and Drug Administration (FDA), American Academy of Nutrition and Dietetics (AND), World Health Organization (WHO), European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN), American Society for Parenteral and Enteral Nutrition (ASPEN), American Board of Physician Nutrition Specialists (ABPNS) currently recommend fortifying preterm milk.

What are the nutrient deficiencies in donor milk?

In instances where a mother’s own breast milk is unavailable donor, human milk is the best substitute but has increased nutrient deficiencies. Donor milk is often from mothers expressing milk for term infants, which is lower in protein and fat content compared to preterm milk. The donor milk is combined with several donors and pasteurized creating batches of donor milk with levels of minerals, protein, and fat that can vary across samples. The Holder pasteurization process for donor milk heats milk at 62.5°C for 30 minutes, which decreases the “activity of many of the functional bioactive factors in milk.” The increased handling and storage of donor milk decreases caloric content as fat adheres to the walls of each container. Protein content of donor breast milk diminishes through the pasteurization and changing temperatures during storage. Thus, there are greater concerns related to the adequacy of donor milk than preterm milk.

Despite these concerns, however, a study by Colaizy et al., (2012) found that there were “no statistically significant differences in the prevalence of neonatal morbidities that may influence growth”—including NEC and sepsis—between extremely premature-VLBW infants who received majority of their intake as donor milk and those who received majority of their intake as maternal milk. More studies are needed to determine whether the decrease in bioactive components in donor breast milk decreases the immunoprotective and neurological development benefits found with mother’s own breast milk for preterm infants.

Due to its limited availability in clinical practice, donor breast milk is preferentially provided to premature infants and infants with malabsorption, allergy, short gut, and chronic lung disease, which are conditions prevalent among premature infants. These disease states further increase the infant’s nutrient demands. Given the larger nutrient deficits of both calories and protein, donor breast milk would require greater nutrient fortification to meet the growth requirements for premature infants. It is not surprising that in trials studying the growth & health outcomes associated with different milk types the extremely premature-VLBW infants receiving predominantly pasteurized donor human milk required more nutritional supplements yet persisted with a slower rate of weight gain and had higher rates of small-for-gestational-age (SGA) at discharge than infants receiving formula or maternal milk. These findings indicate that donor breast milk may require a different fortification strategy than mother’s own breast milk in order to support growth in preterm infants. Nevertheless, UNICEF and WHO “have issued a joint resolution support the use of banked donor milk as the ‘first alternative’ for infants whose mothers are unable to breastfeed.”
3. OPTIMIZING THE NUTRIENT CONTENT DELIVERED BY HUMAN MILK FORTIFIERS

How is human milk fortified?

Commercial human milk fortifiers (HMF) are designed as multi-nutrient supplements to provide adequate macronutrients and micronutrients without exceeding safe limits of osmolality. Until recently, commercial HMF were powders added to expressed breast milk but recent HMF products have been developed in liquid form. Standard HMF mixing protocols increase the caloric content of breast milk from 20 kcal to 22kcal/oz or 24kcal/oz. HMF contain differing quantities of minerals but are intended to meet the increased needs of premature-LBW infant in order to promote adequate bone mineralization and prevent anemia.

Due to the high levels of micronutrients, additional fortification with HMF in order to increase caloric or protein intake would result in excessive intake of minerals. One particular case study also found that high levels of calcium fortification could lead to calcium fatty acid insoluble soaps in the infant’s gut thus inhibiting absorption and obstructing the bowel and leading to lower weight gain. There was also concern that that these multi-nutrient fortifiers would counteract the anti-bacterial properties in breast milk. It was found that the high iron content of formulas facilitate the growth of gram-negative bacteria associated with NEC. Translating this to HMF, the concern is that multi-nutrient fortifiers containing iron would reduce the breast milk’s innate inhibition of gram-negative bacteria. However, a recent study revealed that higher levels of iron within the multi-nutrient fortifier or addition of iron with multi-nutrient fortifiers does not affect bacterial growth in human milk.

Preterm infant formulas or modular supplements are used to increase the caloric content up to 27 kcal/oz or 30kcal/oz if needed. The caloric density of the feeding will depend on the infant's growth velocity, needs due to any medical complications, and fluid restrictions or volume tolerance. Individual modular supplements for protein, carbohydrates, multivitamins, and minerals are needed when the commercial human milk fortifiers cannot meet the nutritional needs of the infant. However, adding all of these nutrients as individual modular supplements to breast milk increases the chance of dose error and potential for contamination. Nutrients individually or collectively added in too high of a dose can result in an osmolality of breast milk above the recommended upper limit of 450mosm/kg. Hyperosmolar feeds increase the risk for NEC and more likely to result in diarrhea. The use of modular supplements in neonatal intensive care units varies across the country likely due to lack of evidence on efficacy and safety to guide best practices.

Are human milk fortifiers providing sufficient protein?

A central focus of current debate on HMF is on whether multi-nutrient fortifiers supply optimal protein energy content (g/kg/d) and a sufficient protein to energy ratio (grams protein per 100kcal) in order to optimize growth for premature infants. Optimal protein feeding for premature infants has been defined based on fetal nutrition. In utero, protein is the main energy source for fetal body mass accretion. Protein has been identified as a growth-limiting factor for preterm infants as these infants have a much higher need for protein compared to term infants, in order to achieve current criteria for optimal growth. However, breast milk provides a greater proportion of calories from lipids and glucose and lower amounts of protein than the fetus would receive in utero, creating concern about optimal postnatal protein nutrition. If total energy intake is limited then the available protein is used for energy production and lean body mass accretion is hindered. As overall calorie intake increases, protein is spared and nitrogen retention increases thus improving lean body mass accretion. If sufficient energy is provided yet protein intake is limited then nitrogen retention plateaus and the excess energy is used for fat deposition. Total daily energy intake, total daily protein intake, and the proper ratio of grams of protein per 100kcal is important for supporting growth in the preterm infant.

Assessments of the protein adequacy of HMFs is further complicated as recent studies have found that multi-nutrient fortification of breast milk according to manufacturers labels did not provide adequate protein content. Due largely to the variability in breast milk protein content, the actual protein content of the fortified preterm breast milk was frequently lower than recommended intake of protein and protein: energy ratio. Reports vary on the assumed protein concentration of human milk ranging from 1.2g/100ml to 1.4 to 1.6 g/100mL to 1.9g/100mL. These protein levels are likely derived from reports on protein content during dif-
different stages of lactation: 2-3g/100ml in colostrum, 1.3-7.8g/100ml in transitional milk, and 1.3-1.8g/100ml in later milk. This variation resulted in a wide range of protein concentration offered in various brands of powder HMF (between 0.7 and 1.1g protein/100ml). These protein concentrations did not account for the decrease in protein in breast milk over time thus yielding a protein energy ratio and total protein intake insufficient for VLBW/ELBW infants to achieve fetal weight gain rates.

Indeed, use of HMF has been associated with high rates of extra-uterine or postnatal growth failure, defined as growth <10th percentile on intrauterine growth projection at a given gestational age, among ELBW. Several studies have shown that very few VLBW infants—as few as 2% of VLBW infants born 30-35 weeks—are able to achieve intrauterine growth rates even while receiving fortified human milk. The National Institute of Child and Human Development (NICHD) Neonatal Research Network reported that “16% of extremely low birth weight infants are small for gestational age at birth, but by 36 weeks corrected age, 89% have growth failure.” Since majority (84%) of the birth weights of these ELBW infants are born appropriate for gestational age (i.e. their weights are ~50th percentile weight-for-gestational age) then their low birth weight is attributable to being born prematurely and is not indicative of intrauterine growth failure. However, after birth the growth failure indicates the infants are not receiving adequate nutrition in order to achieve intrauterine growth rates. Such growth failure is a concern because it has been associated with poor psychomotor development at 18 months and neurodevelopmental impairment at 18mo and 22mo corrected gestational age.

Table 2: Calorie recommendations for Low Birth-weight Infants

<table>
<thead>
<tr>
<th>Professional Medical Organization</th>
<th>Recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGAN), 1991</td>
<td>98-128kcal/kg/d</td>
</tr>
<tr>
<td>Canadian Paediatric Society (CPS) Nutrition Committee, 1995</td>
<td>105-135 kcal/kg/d</td>
</tr>
<tr>
<td>American Academy of Pediatrics (AAP) Committee on Nutrition, 1998</td>
<td>105-130 kcal/kg/d</td>
</tr>
<tr>
<td>Life Sciences Research Office (LSRO), 2002</td>
<td>110-135 kcal/kg/d</td>
</tr>
<tr>
<td>ESPGAN, 2010</td>
<td>110-135 kcal/kg/d</td>
</tr>
</tbody>
</table>

Studies implementing more aggressive protein concentrations in EBM fortification for extremely premature, VLBW infants:

In response to these findings, recommendations for calorie and protein intake for premature, VLBW infants have increased over the years. These recommendations suggest more aggressive nutrition protocols that aim to supply protein and calories earlier and in higher concentrations (Table 2 and Table 3). Though limited, several studies do not suggest strategies to improve growth outcomes with increased protein concentrations in fortifiers provided to VLBW infants, based on existing growth criteria. For example, two studies, Miller et al. (2012) and Kanmaz et al. (2012), compared growth outcomes among preterm-LBW infants fed expressed breast milk (EBM) randomly assigned to treatment groups of varying levels of protein fortification. Both studies enrolled infants with a median gestational age at birth ≤ 30 weeks and median birth weight <1200g. Given these demographics, these infants require 3.5-4.0g protein/kg/d and 3.2g protein:100kcal according to ESPGHAN recommendations.

Given two studies with infants of similar birth weights, gestational age, and protein supplementation, the growth outcomes in terms of weight gain rate did not improve with greater protein intake. Neither of the studies demonstrated significantly different weight gain rate or length increments across their respective fortification groups (Kanmaz et al. 3.6 v 3.3 v 3.0 g protein/kg/d and Miller et al. 4.2 v 3.6g protein/kg/d) (i.e. greater protein intake did not result in relative increases in weight gain rates). Yet all of the preterm, VLBW infants enrolled in the Kanmaz et al. (2012) study achieved intrauterine growth rates (15-20gm/kg/d) regardless of their protein intake. Even the infants receiving 3.0g/kg/d, which is below the recommended protein intake for infants between 1000g and 1800g, achieved intrauterine growth rates.

In terms of other growth outcome measures, Kanmaz et al. (2012) saw a significantly greater weekly increase in head circumference in those infants receiving 3.3 g protein/kg/d and 3.6 g protein/kg/d compared to those infants receiving 3.0 g protein/kg/d. The improvements in head circumference growth are important because this anthropometric measure has the strongest link to long-term improvement of neurocognitive development. Where Kanmaz et al. (2012) saw improvements in growth outcomes (achieving intrauterine growth weight gain and head circumfer-
Table 3: Protein and Protein: Energy Ratios recommendations

<table>
<thead>
<tr>
<th>Weight &lt;1000g</th>
<th>Weight &gt;1000g</th>
</tr>
</thead>
<tbody>
<tr>
<td>g protein/kg/d</td>
<td>g protein/100kcal</td>
</tr>
<tr>
<td>ESPGHAN, 1987-47</td>
<td>~</td>
</tr>
<tr>
<td>Kashyap &amp; Heird, 1994-2</td>
<td>~</td>
</tr>
<tr>
<td>LSRO, 2002-20</td>
<td>3.4-4.3</td>
</tr>
<tr>
<td>Tsang, 2005-14</td>
<td>3.8-4.4</td>
</tr>
<tr>
<td>ESPGHAN, 2010-57</td>
<td>4.0-4.5</td>
</tr>
<tr>
<td>Ziegler, 2011-3</td>
<td>4.0</td>
</tr>
</tbody>
</table>

Table 4: Growth Outcomes with varying levels of protein fortification

<table>
<thead>
<tr>
<th>Daily weight gain (g/d)</th>
<th>Weekly increase in head circumference, (cm)</th>
<th>Length at discharge/study end (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19.7 ± 4.43*</td>
<td>0.69 ± 0.21 b</td>
<td>41.7 ± 2.33 d</td>
</tr>
<tr>
<td>20.6 ± 5</td>
<td>0.92 ± 0.22 b</td>
<td>42.05 ± 2.17 d</td>
</tr>
<tr>
<td>21.4 ± 4.7</td>
<td>0.82 ± 0.21 b</td>
<td>41.7 ± 2.32 d</td>
</tr>
<tr>
<td>26 (24-28) c</td>
<td>0.95 (0.92-0.99) b</td>
<td>45.5 ± 3.0</td>
</tr>
<tr>
<td>24 (20-28)</td>
<td>(0.9-0.98) b</td>
<td>46.3 ± 2.1</td>
</tr>
</tbody>
</table>


- achieved 16.4 ± 2.2g/kg/d in 3.0g protein/kg/d, 17.1 ± 3.4 g/kg/d in 3.3g protein/kg/d, 17.8 ± 3.2g/kg/d in 3.6g protein/kg/d (P=0.38).
- Mean = SD (all such values)
- P<.001; only p-value <0.05
- Median; 25th-75th percentile in parentheses (all such values)
- from enrollment to study end; study end occurred when the infant was discharged from hospital or reached expected date of delivery, whichever occurred first.

The differing growth outcomes highlight the difficulty of defining protein recommendations for premature, VLBW infants. There was significantly less extra-uterine growth failure in terms of length, but not for weight, among infants receiving 4.2 g protein/kg/d compared to 3.6g protein/kg/d. Infants receiving 3.6g/kg/d achieved greater gains in head circumference increase rate, but not weight gain rate, than those infants who received a standard human milk fortifier containing 3.0gram/kg/d. Ultimately, these findings support the ESPGHAN recommendations because an increase of protein intake within the recommended range did achieve intrauterine growth rates and improvements in head circumference increments. Exceeding the recommended range of protein intake did not result in adverse clinical outcomes nor did it necessarily improve growth outcomes.

Some of the differences seen in weight gain rates and increases in head circumferences could be attributed to the differing methods of enteral and parenteral nutrition protocols for all of the infants. For example, Kanmaz initiated human milk fortification for all the infants once enteral feeding volume reached 90-100ml/kg whereas Miller initiated fortification at a median volume rate of 120ml/kg. In addition, Kanmaz specified that the parenteral nutrition protocol was started at a dose of 2.3g amino acids/kg/d immediately after delivery and reached 4g/kg/d on day 3 of life. These more aggressive nutrition protocols could have minimized the differences seen between the various human milk fortification groups in the Kanmaz study and facilitated meeting intrauterine growth rates even with lower protein intakes. The variations seen in these studies highlight the differences seen in clinical practice when defining enteral nutrition initiation practices, "full feeding" volumes, and balance between parenteral and enteral nutrition administration.7,26,41

In addition, it is important to note that these studies used preterm formula when human milk when breast milk was not available. This may have confounded the results but it is a reality of clinical practice that preterm breast milk or donor milk is not always available in sufficient supply thus making the results more generalizable to general clinical practice. In fact, a survey of US NICUs found that "breastmilk was administered as the first feeding the majority of the time (75.4%),
with almost all (89.6%) of respondents reporting the administration of infant formula "some of the time" at the time of first feeding. Even though evidence suggests premature infants should be provided breastmilk exclusively, the reality is that premature infants are receiving different sources of nutrition. Nutrition care teams need to prioritize supporting mothers of premature infants to provide breastmilk as early as possible and as much as possible to their child.

**Other clinical outcomes with higher protein intakes**

These studies often exclude infants for feeding intolerance, development of NEC or other GI-related complications. For instance, one patient in Miller et al. (2012) study dropped out of the study due to perceived feeding intolerance yet there was no statistically significant differences in the incidence of clinical outcomes including NEC, sepsis, length of stay, time to reach full enteral feeds, days feeding was interrupted, or number of days receiving TPN between the fortification groups (4.2g protein/kg/d v 3.6g protein/kg/d). Similarly, the extra protein supplied in Kanmaz et al. (2012) study (3.6 v 3.3 v 3.0g protein/kg/d) was not associated with detrimental health outcomes, such as metabolic acidosis, NEC, feeding intolerance, or serum indicators for osteopenia. Incidence of feeding tolerance and NEC was similar across all groups of protein fortification in both the Miller et al and Kanmaz et al study, which indicates feeding tolerance was still an issue. Other studies have found that exceeding 4g protein/100ml of breast milk does not provide any nutritional advantage in terms of growth and can result in uraemia or hyperaminoacidemia, which is associated with developmental delays. This raises the idea that perhaps it is the concentration of protein rather than total intake of protein per day that could be related to adverse clinical outcomes. Other evidence suggests that better management of enteral feeds can reduce feeding intolerance and other adverse health outcomes such as NEC. For example, the use of trophic feeds, early small enteral volumes, has been beneficial in maintaining intestinal integrity. Slow rate advancement to reach goal enteral feeding rate can also reduce the incidence of these adverse health outcomes.

**Are there benefits to individualizing breast milk fortification for preterm infants?**

The concern for extra-uterine growth restriction despite fortification and no reduction in adverse clinical outcomes when intrauterine growth rates are achieved has pushed the idea of individualizing additional fortification of breast milk. As the Miller & Kanmaz studies show, additional protein intake allows some infants to achieve intrauterine growth rates while extra-uterine growth failure persists in others. Researchers believe that individualization of intake will meet the infant's nutrient needs based on the wide variety of conditions and needs of premature infants.

Nutrition recommendations and NICU protocols have been individualized by weight of the infant rather than gestational age/degree of prematurity because weight is seen as a better indicator of the possible nutrient deficits among premature infants. Recommendations from European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) published in 2010 specified target protein intake based on the infant's weight category. ESPGHAN suggested a goal of 3.0-4.5g protein/kg/day for infants up to one kilogram and 3.5 to 4.0 gram protein/kg/day for infants weighing 1000gram to 1800grams. These recommendations suggest that some smaller infants have higher protein needs, which may not be met by fixed protein concentrations in standard multi-nutrient fortifiers.

NICUs have adopted the practice of providing additional fortifier to increase the caloric content of EBM for infants <2kg not meeting the goal weight gain rate of 15-20g/kg/d. In this way, calorie and protein concentration provided in the NICU is based on the infant's weight and weight gain rate. The hypothesis is that more individualized administration of protein and calories will prevent growth deficits. However, this strategy is not time sensitive because weight and height growth trends have to be established and considered within the context of the typical weight loss seen after birth. It may take up to two weeks or longer for premature infants to regain their birth weight, which is considered a normal physiologic process. If a baby is born small-for-gestational age, there is need for additional catch-up growth to reach the 50th percentile weight for gestational age, which represents appropriate-for-gestational age (AGA) weight. In essence, weight gain trends must be established over several weeks thus prolonging the time frame to determine whether additional fortification is required to support adequate growth. Thus, on the one hand, the disadvantage of fortifying based on growth trends is that the additional protein fortification may be initiated after a protein deficit has started to accrue making it more difficult to reach intrauterine
growth rates. Although the ideal time frame to reach AGA has not been identified for clinical practice, growth studies often measure percentage of infants who reach AGA or achieve intrauterine growth rates by discharge. On the other hand, fortifying based on weight and growth trends may run the risk of exceeding the infant's tolerable upper limit of osmolality or micronutrient needs thus potentially increasing the risk of adverse clinical outcomes.

**What indicators should be used to determine individualized fortification?**

The use of biomarkers as an indicator of protein status seems like a more specific means of determining adequacy of protein intake. Arslanoglu et al. (2013) \(^\text{49}\) individualized additional protein supplementation according to twice weekly measurements of blood urea nitrogen (BUN), a biomarker expected to indicate inadequate or excessive protein intake. If BUN was <9mg/dl additional protein was provided to the multi-nutrient HMF. This study enrolled infants with a wide range of birth weights (600-1750g) and gestational age (26-34 weeks). This strategy is potentially advantageous because it measures adequacy of intake and tolerance for the individual infant and is time sensitive without waiting to see a trend of weight gain related to intake.

This study found that protein modular supplementation according to BUN levels in addition to a standard multi-nutrient fortifier resulted in greater protein intake, greater weight gain (17.5 ± 3.0 v 14.4 ± 3.0g/kg/d), and greater increases in head circumference (1.4 ± 0.3 v 1.0 ± 0.3cm/wk) compared to those receiving breast milk supplemented with a standard multi-nutrient fortifier. Arslanoglu et al. (2013) reported: “Infants receiving the adjusted regimen had mean protein intakes of 2.9, 3.2, and 3.4g/kg/d, respectively, in weeks 1, 2, and 3, whereas infants receiving the standard regimen had intakes of 2.9, 2.9, 2.8g/kg/d, respectively.”\(^\text{49}\) This greater protein intake in the adjusted regimen group, including 3.2 and 3.4g/kg/d, did not result in BUN levels >20mg/dl, the upper limits of normality. These results refute prior claims from studies in the 1980s that protein intake of 3 g/kg/d at two weeks of age should be the upper limit of intake in order to prevent exceedingly high BUN levels.\(^\text{20}\) Even though it has been shown previously that fluctuations in BUN levels may occur in preterm infants regardless of the adequacy of their protein intake due to immaturity of the infant’s renal system\(^\text{35}\), this study supports the use of using BUN as a biomarker for safety of additional protein fortification because none of the infants in the study developed necrotizing enterocolitis, sepsis, hyperaminoacidemia, or BUN levels above the normal limits while also achieving better growth outcomes.

Rather than determining fortification based on metabolic effects, one strategy is to ensure the breast milk available from either mothers or donors is fortified to meet the nutrition recommendations. Using approximated or assumed levels of protein based on expected average composition of premature expressed breast milk plus fortifiers of known protein content (termed “blind fortification”) risks inadequate\(^\text{49}\) as well as excessive protein intake. Miller et al. (2012) measured the content of the EBM with infrared spectroscopy after fortifying the breast milk in order to obtain a more accurate estimation of protein provision and macronutrient distribution. Other studies have validated such a technique to be used in order to individualize fortification according to the content of breast milk thus reducing variability in nutrient intake to reach the nutrition recommendations.\(^\text{38,39}\) However, the use of additional equipment requiring well-trained staff in order to individualize macronutrient composition of feeds daily is neither practical nor financially feasible in most NICU settings. Both Miller et al. (2012) and Arslanoglu et al. (2013) used nutrient modular supplements to fortify with additional protein, which increases room for error and potential for contamination. Again, the variability of breast milk composition within both a single day and over weeks make it difficult to know exactly how much protein the infant receives at each feed if measurements are conducted only periodically.

A practical way to analyze the macronutrient and micronutrient content of fortified breast milk has yet to be identified. At this time, there lacks evidence of a strategy as to how best to individualize nutrient intake. While there is evidence to support increased protein content of the commercial human milk fortifiers, using fixed fortification according to manufacturer’s guidelines appears adequate to support greater short-term growth among some preterm infants and not in others. Studies individualizing protein fortification are heterogeneous in design (number of days receiving HMF, initiation of enteral nutrition, volume rate of continuous enteral feeds, criteria for enteral volume advancement(increase), accompanying TPN administration, goal volumes) and are thus difficult to interpret.
Given all of these variations in nutrition protocols, simply adhering to a defined protocol for standard fortification and enteral nutrition administration in itself can improve health outcomes. A 2009 nurses survey of Level II & III NICUs found that while 60.9% had an enteral nutrition protocol for the preterm infant, only 26.6% actually adhered to their protocol in daily practice indicating there was variation in nutrition care. Creating an evidence-based protocol with an algorithm to manage growth failure or feeding intolerance could reduce variability in decision-making as to whether additional fortification or other changes in the nutrition plan of care are necessary to optimize growth outcomes and minimize adverse clinical outcomes.

Are powder HMF safe to use for premature infants?

The majority of studies on multi-nutrient human milk fortifiers have used commercial powdered products rather than liquid formulations, which may undermine conclusions about the adequacy of nutrient content for optimal outcomes. Powdered fortifiers, such as Enfamil Human Milk Fortifier and Nestle FM 85, are well tolerated in preterm infants but have not necessarily facilitated better short-term growth rates compared to pre-term formulas. Cases of *E. sakazakii* infections leading to meningitis, bacteremia, and NEC in preterm infants fed powdered infant formulas prompted the CDC and FDA to release statements in 2001 and 2002, respectively, discouraging the use of powdered human milk fortifiers for preterm infants because they are not sterile. An in-vitro study on the growth of *E. sakazakii* in breast milk and breast milk fortified with powdered Enfamil HMF compared to powdered infant formulas concluded that "powdered infant formula yielded higher population densities [of E. sakazakii] than those in breast milk or breast milk with fortifiers at all temperatures [10, 23, 37°C]. This is worrisome considering parents of preterm often receive recommendations to fortify EBM with powdered infant formulas upon discharge from the NICU. Another study by Chan et al. (2003) found that while preterm human milk has anti-microbial properties against *E. coli*, *Staph, E. sakazakii*, Group B Strep, adding Enfamil HMF powder to breast milk removed the antibacterial action compared to Similac HMF powder or MCT oil. However, a more recent study by Santiago et al. (2005) failed to show any differences between the two powdered fortifiers on bacterial growth in human milk. Regardless of the conflicting exper-

imental data on the anti-microbial properties of powder HMF, the *E. sakazakii*-related incidences of NEC have phased out the use of powdered HMF in most NICUs in favor more sterile options.

**Liquid HMF concerns: Does the risk of metabolic acidosis (with use of acidified liquid HMF) warrant preferential use of non-acidified, lower protein liquid HMF?**

Older generation liquid HMF were not concentrated so their addition to breast milk diluted the bioactive components and nutrient content. New liquid fortifiers are highly concentrated to minimize this dilution. An updated liquid fortifier Enfamil Acidified Liquid HMF (Mead Johnson) was introduced in the US in 2012 followed by Similac concentrate liquid HMF (Abbott Nutrition) in early 2013. Enfamil Acidified Liquid HMF (LHMF) and Similac LHMF increases caloric content of EBM from 20kcal/oz to an estimated 24kcal/oz. In addition, a human-milk protein based fortifier named Prolacta was developed in 2010. Prolacta is a fortifier derived from concentrated donor human milk. See Table 5 for a comparison of popular US human milk fortifiers. All of these liquid fortifiers are considered sterile but have presented drawbacks of their own.

The liquid formulation of Enfamil HMF uses the naturally acidic whey protein isolate hydrolysate, which results in an acidic pH of ~4.3-4.7. While this pH is similar to the gastric pH of a premature infant there are concerns that the Enfamil LHMF increases risk of metabolic acidosis, NEC, feeding intolerance, reflux, and loose stools. Gastric acidity becomes a concern when gastric pH drops below 4, an occurrence seen in infants with gastroesophageal reflux disease. Nonetheless, metabolic acidosis can lead to impaired growth and decreased bone mineral density due to the excessive chronic acid interfering with calcium absorption. Metabolic acidosis occurs more frequently in premature infants because of the limited capacity of their immature kidneys and greater endogenous acid production. A recent trial among 150 preterm-LBW infants comparing the use of Enfamil powdered HMF to Enfamil liquid HMF showed significantly higher HCO3 on two days of the twenty-eight day study. However, only one of those infants was diagnosed as having acidosis and it did not require clinical intervention.

Metabolic acidosis from intake of Enfamil LHMF could be attributed to the pH or due to the higher protein content. The trial using the Enfamil liquid HMF, which has a higher protein content than the powdered formulation, showed that feed-
ing tolerance was not an issue among preterm-LBW infants. In terms of growth outcomes, weight gain rate with use of Liquid human milk fortifier was similar to powder HMF but length growth rate was greater in those infants receiving the liquid human milk fortifier. By study day 28, infants receiving >80% of energy intake from breast milk fortified with liquid HMF achieved absolute greater weight and length than the powder HMF.

No study is yet available comparing growth and health outcomes associated with the use of an acidified liquid HMF, such as Enfamil LHMF, versus a liquid HMF with a more neutral pH but lower protein content, such as Similac LHMF. The results of a trial comparing Similac LHMF and Enfamil LHMF would be interesting to see also given the difference in nutrient content between these two LHMFs. Such a study would edify clinical practice by determining whether the benefit of higher protein content of Enfamil on growth outcomes outweighs the risk of metabolic acidosis and feeding intolerance due the product’s acidic pH profile. Enfamil LHMF provides enough protein in order to reach the minimal protein to energy ratio of 3g protein:100kcal for LBW infants and a protein intake between 3.3 and 3.8g/kg/d. This protein level in Enfamil LHMF contains ~20% more protein than the Enfamil powder HMF (Mead Johnson), Similac Powder HMF (Abbott), and the Similac Liquid HMF (Abbott). Similac LHMF provides approximately 15-20% less protein, significantly lower levels of iron and vitamin D, and significantly higher levels of other minerals and trace elements compared to Enfamil LHMF. The protein content in Similac LHMF is not adequate and requires additional protein modular supplement to meet protein needs of the premature infant. The infant receiving Similac LHMF, as opposed to Enfamil LHMF, would also require an additional iron supplement to meet the minimum intake of 2 mg iron/kg. As mentioned earlier, use of modular supplements does increase risk of infection and NEC due to potential contamination during preparation. However, Similac LHMF may have the advantage of promoting adequate bone mineralization to prevent osteopenia and fractures given the much higher levels of calcium, phosphorus, magnesium, and zinc. On the contrary, these very high levels of calcium could impair fat absorption and lead to feeding intolerance as cautioned earlier. Enfamil LHMF provides the advantage of higher nutrient content thus reducing the need for supplements. Currently the debate is whether to use the acidified liquid fortifiers, such as Enfamil LHMF, risking metabolic acidosis and feeding intolerance or use Similac plus a modular iron and protein supplement to meet protein needs, which may yet risk infection and NEC.

**Does a human milk-based fortifier confer advantages over bovine milk-based fortifiers?**

Similac LHMF is not the only alternative to Enfamil LHMF. The introduction of a human milk protein-based HMF offers the potential of reducing the issues with metabolic acidosis, feeding intolerance and NEC. Prolacta HMF is a liquid human milk protein-based fortifier that when provided with an exclusive breastmilk diet has been shown to reduce odds of NEC by 77% compared to bovine milk protein-based fortifiers. Yet in this randomized clinical trial the rate of weight gain was greater among the premature-LBW infants that received bovine protein-based HMF compared to the human milk protein-based fortifier (16.0 ± 7.8 vs 14.3 ± 3.8g/kg/d, P=.051). This rate of weight gain with Prolacta HMF is close to meeting the minimum goal of 15g/kg/d. Additional analyses reported a 11-14% reduction in likelihood of re-initiating TPN after full enteral feeds were achieved of breastmilk fortified with human milk-proteins compared to human milk supplemented with bovine milk-based fortifier or pre-term formula. It is assumed that feeding intolerance and/or metabolics acidosis issues would be improved using a human milk-based fortifier due to the similar whey & casein protein content as breast milk. However, further studies are needed to evaluate these outcomes in addition to the effects on bone mineral density in premature infants.

Despite these advantages, the cost of Prolacta HMF may be beyond the financial capabilities of many hospitals. The cost of the Prolacta HMF, estimated to be $6.25 per ml, is far more expensive compared to the $1.30 per packet of Enfamil or Similac powder HMF. A cost-saving effectiveness analysis showed that the cost of NEC management and treatment and costs of hospitalization were greater than the cost of Prolacta as HMF and use of donor milk to provide an exclusively human milk based diet to premature-LBW infants. The use of an exclusively human-milk based diet reducing probability of NEC and surgical NEC, length of stay, and use of medical intervention resulting in an estimated net direct savings of $8,167 per extremely premature infant. These cost-savings in the long term may be appealing if standard fortification of breast milk with human
milk-based fortifier can consistently deliver improved health outcomes.

**Conclusion & Summary**

Following current scientific evidence, it has become standard practice to fortify breastmilk for premature-LBW infants. The aim is to provide immune benefits of breast milk and increase nutrient content of premature and mature donor milk when needed to support growth, bone mineralization, gastrointestinal function, and immune modulation. Evidence supports that while breast milk fortification can decrease morbidity and mortality in these at-risk infants, higher protein content is needed in the human milk fortifiers in order to achieve short-term intrauterine growth rates for the sake of long-term health benefits, such as neurocognitive development. In addition, criteria for evaluating optimal growth and development primarily in terms of weight gain rate may need to be revised to take into account long-term growth. Other anthropometric measures, such as weekly increase in head circumference, have shown to be better predictors of long-term outcomes, such as neurocognitive development, than achieving intrauterine weight gain. Identifying ideal growth parameters that are related to longer-term outcomes, such as bone mineralization, growth, chronic diseases, and allergies in later childhood and adulthood, can help establish more appropriate criteria for human milk fortification strategies in preterm-LBW infants.

Current evidence suggests fortifying with personalized criteria, such as plasma urea, to provide optimal protein and micronutrients without increasing risk of NEC, feeding intolerance, metabolic acidosis, or hyperaminoacidemia. Individualizing protein intake seems like the way forward in order to manage the many health complications and unique nutrient needs for premature infants. This approach may be beneficial in promoting growth but, again, effective strategies based on growth outcomes or biomarkers need to be defined. Research is needed to determine the best fortification strategies when using donor milk versus preterm mother’s own milk to ensure adequate intake. Similarly, fortification algorithms should be delineated to help manage feeding intolerance issues and other common medical issues in preterm infants. In the meantime, BUN levels can be used as a guidance to ensure protein is not provided in excess protein.

With the recent advent of liquid human milk fortifiers, issues with sterility and inappropriate osmolality of commercial multi-nutrient fortifiers have been minimized. However, further research is needed to assess the efficacy of higher protein fortifiers and whether the protein levels in the fortifiers can reach the current recommendations without additional modular supplements. In addition, there is no evidence that acidified liquid HMF is associated with clinically significant detrimental health outcomes but limiting the protein content of liquid HMF should be avoided.

Human milk fortification is just one aspect of nutrition care for premature infants and must be integrated into best practices including aggressive parenteral nutrition, early trophic enteral feeding, breastfeeding and lactation support for mothers, and clinical algorithms for common medical issues among premature-LBW infants. NICU practice guidelines need to be updated to meet evidence-based recommendations for enteral nutrition regimens in order to reduce extrauterine growth restriction among premature-LBW infants.
Table 5: Nutrient Composition of Preterm Human Milk and Human Milk Fortifiers

<table>
<thead>
<tr>
<th></th>
<th>Energy</th>
<th>Carbohydrate (g/dL)</th>
<th>Protein (g/dL)</th>
<th>Fat (g/dL)</th>
<th>Calcium (mg/dL)</th>
<th>Phosphorus (mg/dL)</th>
<th>Magnesium (mg/dL)</th>
<th>Potassium (mg/dL)</th>
<th>Sodium (mg/dL)</th>
<th>Zinc (mg/dL)</th>
<th>Iron (mg/dL)</th>
<th>Vitamin D (IU/dL)</th>
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<tbody>
<tr>
<td>Human milk pre-term&lt;sup&gt;14&lt;/sup&gt;</td>
<td>68 kcal/ml</td>
<td>10</td>
<td>1.4</td>
<td>5.8</td>
<td>28</td>
<td>14</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
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<td>*</td>
</tr>
<tr>
<td>Enfamil Powder HMF&lt;sup&gt;55&lt;/sup&gt;</td>
<td>14 kcal/4 packets add 100 ml BM</td>
<td>&lt;0.4</td>
<td>1.1</td>
<td>1.05</td>
<td>90</td>
<td>50</td>
<td>1</td>
<td>29</td>
<td>16</td>
<td>0.72</td>
<td>1.44</td>
<td>150</td>
</tr>
<tr>
<td>Similac Powder HMF&lt;sup&gt;55&lt;/sup&gt;</td>
<td>14 kcal/4 packets</td>
<td>1.8</td>
<td>1.0</td>
<td>0.36</td>
<td>117</td>
<td>67</td>
<td>7.0</td>
<td>63</td>
<td>15</td>
<td>1.0</td>
<td>0.35</td>
<td>120 IU</td>
</tr>
<tr>
<td>Enfamil Liquid HMF&lt;sup&gt;55&lt;/sup&gt;</td>
<td>30 kcal/4 vials</td>
<td>&lt;1.2</td>
<td>2.2</td>
<td>2.3</td>
<td>116</td>
<td>63</td>
<td>1.84</td>
<td>45</td>
<td>0.96</td>
<td>1.76</td>
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<tr>
<td>Similac Liquid HMF&lt;sup&gt;58&lt;/sup&gt;</td>
<td>27.4/4 packets</td>
<td>3.2</td>
<td>1.4</td>
<td>1.06</td>
<td>140</td>
<td>80</td>
<td>8.64</td>
<td>83</td>
<td>22</td>
<td>1.22</td>
<td>0.428</td>
<td>140</td>
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<tr>
<td>Prolact +4HMF&lt;sup&gt;52&lt;/sup&gt;</td>
<td>82 kcal/dL (10 ml HMF/40 ml BM)</td>
<td>0.9 g/10 mL</td>
<td>0.6 g/10 mL</td>
<td>0.9 g/10 mL</td>
<td>51.5 mg/10 mL</td>
<td>36 mg</td>
<td>*</td>
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<td>*</td>
<td>0.3 mg/10 mL</td>
<td>*</td>
<td>13</td>
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*limited nutrient information available
References:


Bowen AB, Braden CR. Invasive Enterobacter sakazakii Disease in Infants. CDC Emerging Infectious Diseases 2006; 12(8):1185-1189.


