Abstract

Human milk is the feed of choice for preterm infants. However, human milk does not provide enough nutrition, especially protein, for preterm infants to achieve target growth rates similar to those in utero (15–20 g/kg per day). Fortifiers for human milk, manufactured from bovine milk, are commercially available and routinely used for patients born <32 weeks’ gestation prior to discharge home. Recent recommended dietary intakes (RDI) have been revised. Up to 4.2 g of protein and 135 kcal/kg per day is recommended for infants born very preterm. Additional supplements are needed to current commercial fortifiers to achieve these RDI and reduce the incidence of ex-uterine growth failure. A human milk fortifier that is manufactured from donor human milk is available in some developed countries and may confer some clinical benefits, including a reduction in necrotizing enterocolitis. Fortification can be added in a standardized protocol as per manufacturers’ instructions. Human milk composition can be analyzed and fortification individualized to take into account the large variation from mother to mother. Alternatively, fortification can be increased in a stepwise manner based on assumed composition while monitoring blood urea levels for safety. The current aim is to prevent preterm infants dropping percentiles and falling below the 10th percentile at 36 weeks’ corrected gestational age or discharge home. More data are required on how best to fortify human milk for preterm infants to achieve optimal growth, development and health outcomes in the long term. There is an urgent need for well-designed and informed randomized clinical trials in this vulnerable preterm population.

The Problem

The majority of preterm infants survive (>95% from 28 weeks’ gestation and >80% from 24 weeks’ gestation; Department of Health, Western Australia). However, their progress in the neonatal intensive care unit (NICU) is compli-
cated by feed intolerance, necrotizing enterocolitis (NEC), infection, broncho-pulmonary dysplasia and poor growth. Subsequently, neurological disability occurs in about 10%, and the incidence increases with decreasing gestational age (GA) to 25% infants born <27 weeks’ gestation. In addition, very preterm infants have a higher incidence of behavioral problems, academic difficulties and hospital readmissions than children born at term. Preterm infants as adults may also have adverse metabolic outcomes including abnormal insulin sensitivity, lipid metabolism and blood pressure [1, 2].

Extrauterine growth retardation (EUGR) defined as weight <10th percentile at discharge home, remains a serious problem. Clark et al. [3] reported an incidence of 28% measured in 24,371 infants born at 23–34 weeks’ gestation from 124 NICU in the United States of America, with an increasing incidence with decreasing gestation. Dusick et al. [4] reported an incidence of EUGR of 99% in 4,438 very-low-birthweight infants (501–1,500 g; incidence of birthweight <10th percentile or being small for GA (SGA) was 22%). In Western Australia, the incidence of EUGR at discharge home infants born <28 weeks’ gestation is 50% (18% SGA at birth, Neonatal Database Women’s and Newborns’ Health Service).

EUGR is associated with long-term low growth and cognitive impairment [4, 5]. Ehrenkranz et al. [6] reported a dose-dependent association between early postnatal growth and neurodevelopmental outcomes. Infants with birthweights 501–1,000 g (n = 495) were followed to 18–22 months and divided into weight gain quartiles for in-hospital growth velocity rates with the lowest being 12 g/kg per day and the highest 21 g/kg per day. Bivariate analysis demonstrated that as the rate of weight gain increased, the incidence of cerebral palsy, abnormal neurological examination, and neurodevelopmental impairment fell. Patients in the lower quartile had more NEC, bronchopulmonary dysplasia, late-onset sepsis and received more postnatal steroids. Logistic regression including variables related to neonatal morbidity suggested that growth velocity during hospitalization of the ELBW infant was a significant factor determining neurodevelopmental outcome at 18–22 months’ corrected age.

The Solution

How we feed these vulnerable patients has a direct effect on their growth, health and development. Human milk is undoubtedly the best source of nutrition, and the immunological benefits are especially important for the preterm infant.
The nutrient requirements of preterm infants have been determined by factorial and empirical methods and are well reviewed with recommended intakes by Ziegler [7]. With decreasing GA, the recommended nutrient intakes to match fetal accretion rates increase to over 4 g/kg per day protein and cannot be met with human milk alone. Protein and energy levels in human milk vary widely from mother to mother and across time. McLeod et al. [8] analyzed 336 samples from 36 mothers of preterm infants by laboratory methods and reported median (range) protein 16.1 (13.4–27.6) g/l and energy 730 (630–895) kJ/l.

Fortifiers are available commercially to increase nutrient intakes of very preterm infants with the aim of achieving recommended intakes and growth rates similar to the fetus. In practice, despite efforts, recommended dietary intakes (RDI) are often not met [8, 9]. A recent detailed audit growth of extremely low GA infants in Sweden demonstrated growth falling down percentiles despite early human milk feeds with high levels of fortification [10].

To meet growth targets, it has been suggested that more protein is required than currently available in commercial fortifiers [7]. Miller et al. [11] conducted a randomized clinical trial (RCT) of commercial fortifier (additional 1 g/100 ml) versus an isocaloric high-protein fortifier (additional 1.4 g/100 ml) in 92 infants born <32 weeks’ gestation. Unfortunately, many infants in their study also received preterm formula (2.4 g/100 ml) confounding the results. Median protein intakes (IQR) at weeks 1–4 of the study were 3.6 (3.7–4) versus 4.2 (3.2) g/kg per day. The primary outcomes of length velocity were not significantly different between groups (p = 0.08), neither was there a difference in weight gain or %EUGR at approximately 35% (with 12–16% SGA).

Feeding human milk maximally fortified with a fortifier produced from donor milk also does not prevent EUGR. Hair et al. [12] fed human milk early to patients born ≤1,250 g and fortified when 80 ml/kg per day was tolerated with a product from donor milk. They targeted growth of 20 g/kg per day 1 cm/week and feeding 130–140 kcal/kg per day and 3.6–4.4 g/kg per day. Infants received up to 150 kcal/kg per day and 5.25 g/kg per day protein, with 80% infants gaining ≥20 g/kg per day. With this, the incidence of EUGR was 43% (21% were SGA, and they were all EUGR at discharge, 79% had birthweight appropriate for GA, and 22% were EUGR at discharge).

Body composition is influenced by diet and is emerging as a necessary measure of nutrition adequacy. Air displacement plethysmography technology is a safe and efficient means by which to measure body composition of infants. The data suggest that preterm infants are lighter and fatter than term infants when measured at an equivalent term age [13], but this abnormality may not persist into childhood [14]. However, young adults who were born preterm have cen-
Central adiposity and altered fat distribution measured by detailed MRI studies. The pattern of fat distribution reported in ex-preterm adults has been associated with risk of cardiovascular disease [15].

**The Benefits of Human Milk Fortification for Preterm Infants**

Growth (weight, length and head circumference gain) of infants (<1,800 g) fed fortified human milk (under 2 kg) is better than that of those fed unfortified human milk in the short term (meta-analysis of 13 studies) [16].

Fortified human milk can achieve intakes of protein 4–4.5 g and energy 110–135 kcal as recommended by the ESPGHAN Committee on Nutrition [17].

Fortifiers also supplement calcium, phosphorus and vitamin D with the aim of preventing osteopenia of prematurity, but the data are limited and do not show a benefit.

Improved growth is associated with better neurodevelopmental outcomes [6]. Increasing SD for weight and head circumference from birth to discharge has been associated with neuromotor outcomes at 5 years of age [18]. Increasing protein intake is associated with better growth and better neurodevelopmental outcomes [19].

**The Risks of Human Milk Fortification**

Fortifiers increase osmolality, especially if milk is fortified for a 24-hour period rather than at the bedside immediately prior to the feed (as recommended by the manufacturer). In many busy NICUs, feeds are prepared in a ‘milk room’ under strict management guidelines which may result in slow increase in osmolality as the enzymes in human milk digest nutrients in the fortifier. Osmolarities <450 mosm have been recommended as safe and not increasing the risk of NEC [20].

Adding fortifier to human milk may increase feed intolerance and the risk of NEC compared with feeding unfortified human milk. It has been suggested that cow’s milk protein present in many fortifiers may be a trigger for intestinal inflammation and contribute to the risk of NEC [21].

The added carbohydrate from fortifiers may result in raised plasma glucose levels and diuresis.

For SGA infants, faster weight gain may be associated with higher later blood pressure and risk of obesity [22, 23].

Why?

The aim of human milk fortification is to achieve RDI for preterm infants [17] and achieve growth rates similar to those achieved by the fetus in utero at similar GA [24]. The overall aim is to maintain growth along percentiles feeding human milk. If born SGA, the aim is to prevent further growth retardation. If born lean, it appears best to stay lean.

Who?

Current practice is to fortify human milk for infants born <32 weeks’ GA and sometimes <34 weeks’ GA. Preterm infants born later than this are usually not in hospital long enough to receive nutrient supplementation and often receive some feeds directly from the breast. There is increasing evidence that ‘late pretermers’ (34–37 weeks’ GA) have increased risk of developmental and behavioral problems, but any effect of human milk fortification in this group has not been extensively studied.

When?

Fortifiers are added at the clinicians’ discretion, and guidelines vary between units. Because of concerns about feed intolerance and NEC, fortifiers are commonly added once ≥100 ml/kg per day human milk is tolerated. As daily protein intakes may fall as patients are transitioned from parenteral to enteral nutrition, some clinicians may add fortifier earlier. Historically, fortifiers may be discontinued when a weight of 2 kg is reached but many units continue fortifying human milk fed by tube or bottle until term or discharge, whichever is earliest. The fortification is weaned as more feeds are taken directly as breastfeeds.

In general, catch-up growth is not promoted, although it may occur naturally for the breastfed infants who regulate their own intake. Supplementing feeds with gastric tube feeding after term rarely occurs unless infants have neurological impairments. Postdischarge fortification is uncommon, although anecdotally some clinicians may recommend alternating breastfeeding with a bottle of fortified expressed breast milk if growth is a concern.
What?

Powder or liquid fortifiers based on cow’s milk proteins (intact or hydrolyzed) and fortifiers manufactured from donor human milk can be used.

Clinicians need to make an informed choice about which fortifier to use for their preterm patients. Protein and energy intakes need to be met at volumes prescribed. Whether energy is provided, a carbohydrate, or carbohydrate and fat, may be important especially for the very preterm infant who may have problems managing plasma glucose levels.

Attention needs be paid to whether the fortifier is supplemented completely with vitamins to meet RDI. Additional vitamin D supplements are often required to meet recent RDI.

Attention needs be paid to whether the fortifier chosen is supplemented with iron or not. Some clinicians prefer to supplement their patients with iron independently and at a later age than when fortifiers are introduced.

Importantly, clinicians should know the osmolality of fortified human milk when fed to their patients as high osmolalities have been associated with NEC. In some NICUs, fortifier is added to human milk at the bedside immediately prior to feeding, and the osmolality will be as stated by the manufacturer. In large busy NICUs, feeds may be prepared daily in the ‘milk room’ and fed over the following 24-hour period. In our NICU, fortifier based on intact cow’s milk protein has an osmolality of 420 mosm after 24 h and one based on hydrolyzed protein, 490 mosm after 24 h. Medications, if added to milk feeds, will further increase the osmolality.

Fortifiers are available that contain intact cow’s milk protein or hydrolyzed cow’s milk protein. In formula-fed infants, hydrolyzed protein has been associated with slower weight gain and higher urinary excretion of essential amino acids [25].

Fortifiers are available as powders or liquids. Liquid fortifiers were introduced because of concerns about bacteria in powered feeds resulting in invasive infection. Use of liquid fortifiers in preterm infants has been associated with acidosis and poor growth [26].

In North America, fortifiers manufactured from donor human milk are available, but these are expensive. Feeding only human milk-based products has been found in an RCT to decrease NEC compared with feeding human milk fortified with cow’s milk products including formula supplements [27]. However, in this RCT, the incidence of NEC was high. The results may not be generalizable to many NICUs where fortifiers based on cow’s milk are used, and donor human milk, not formula, is used when supply of mothers’ own milk is inadequate to meet her baby’s requirements.
How?

Traditionally, fortification of human milk has been standardized. The composition of human milk is assumed and fortifier is added per manufacturers’ instructions. The aim is that assumed plus fortified nutrients meet RDIs. This may not occur for reasons including: volumes of milk fed and tolerated may be less than prescribed; the addition of fortifier may be delayed, or fluids may be chronically restricted.

In our NICU, there may be two levels of standardized fortified human milk with commercial fortifier and supplemental protein: level 1, fortified (2.6 g protein/100 ml) and fed at ≥150 ml/kg per day, and level 2 (3 g protein/100) for infants who are fluid-restricted or failing to thrive. Blood urea is measured at least weekly to provide some reassurance that high protein intakes are tolerated.

A potential problem is that human milk varies over the postnatal period and from mother to mother, so assumed composition may not be accurate [8]. This has led to the suggestion that protein and energy content of human milk should be measured and fortification individualized [28]. Evidence of clinical benefit of individualized fortification has yet to be demonstrated.

One reason for the lack of clinical benefit is that infants receiving standardized fortification may receive more protein than those fed individualized fortified milk. De Halleux and Rigo [29] measured the composition of mothers’ own and donor milk by mid-infrared spectroscopy and targeted fortification in two steps: fat content adjusted to 4 g/dl and addition of fortifier to provide protein 4.3 g/kg per day. Nutrient intakes were calculated for 24 VLBW infants and control infants fed standard fortification. Variability of protein intakes was reduced by 21% but protein intake was lower in infants fed individualized fortification compared with infants fed standard fortifier.

We conducted an RCT of fortifying milk on the basis of milk analysis and by targeting PE ratios and protein intakes according to postnatal age [30]. 40 pre-term infants with GAs from 23 to 29 weeks and birthweights from 480 to 1,475 g were randomized to either individualized or standard fortification. Mean milk composition for the intervention infants was determined weekly from daily milk samples using mid-infra-red technology, and milk was fortified with commercial HM fortifier, a protein and/or energy supplement to reach recommended protein and energy RDIs. Control infants received standard fortification as per unit protocol based on assumed composition of HM. Nutrition intakes of both groups were calculated retrospectively using measured composition of milk for all infants. During the intervention period (6 weeks), infants were fed less protein (3.2 ± 0.4 vs. 3.9 ± 0.3 g/kg per day, p < 0.001) and gained less weight, but
there was no difference between the groups in weight or body composition at discharge home (2,265 ± 342 vs. 2,464 ± 528 g, p = 0.175, and 13.6 ± 3.7 vs. 13.6 ± 3.5 %fat).

Rochow et al. [31] in Canada conducted a case-control study with similar results. Ten VLBW infants were fed human milk with fortification targeted at final contents of fat 4.4 g, protein 3 g and carbohydrate 8.8 g per 100 ml. Their growth was compared with 20 infants in a matched-pair analysis and was similar.

A totally different approach to fortification was trialed by Arsanoglu et al. [32]. Their feeding guidelines slowly increase fortifier and protein intakes provided blood urea is maintained in the normal range. This results in high nutrient intakes than when feeding standardized fortified human milk (2.9, 3.2 and 3.4 vs. 2.9, 2.9 and 2.8 g/kg per day weeks 1, 2, and 3 adjusted vs. standard fortification), and was associated in their preterm patients with better growth.

**Donor Human Milk**

Preterm infants receiving pasteurized donor human milk (PDHM) grow less well than those fed formula [33]. Michaelsen et al. [34] analyzed by mid-infrared spectroscopy 2,554 samples from 224 mothers donating to human milk bank in Copenhagen and reported relatively low protein levels, mean 9.0 g/l (95% CI 6.3–14.3) with energy 696 kcal/l (95% CI 500–1,155). These levels are similar to those in our human milk bank for pasteurized donor milk of 10.4 ± 2.2 g/l and 667 ± 86 kcal/l (n = 89, mean ± SD).

The quality of both protein and fat is altered through pasteurization. Infants fed PDHM may benefit from a higher level of fortification than prescribed for mothers’ own milk. It has also been suggested that the human milk bank may be the best place to introduce individualized fortification. Batches of PDHM could have protein and energy contents measured and fortification added to ensure known composition is dispensed from the human milk bank [29].

In conclusion, higher nutrient intakes are required than commonly delivered with human milk supplemented with commercial fortifiers to meet recent RDIs for preterm infants. Extra protein can be added to fortifiers to reach RDI-based on assumed composition of human milk but the limited evidence suggests that protein alone will not be adequate. Alternatively, supplementation can be increased until growth targets are met as per regime of Arsanoglu et al. [32] of measuring tolerance by blood urea nitrogen.
Individualized or targeted fortification of human milk based on measured composition, although theoretical preferable, has yet to demonstrate a benefit in an RCT. A fortifier produced from human milk rather than bovine products may be beneficial, and results of early trials need to be confirmed before being adopted more generally.

At a more fundamental level, optimal rate of growth and quality of growth (including body composition) needs to be determined. It may not be possible, let alone desirable, for very preterm infants to grow along fetal growth charts. There are some data from ex-preterm children suggesting that relative undernutrition in early life may have beneficial effects on insulin resistance [2]. Further, health outcomes may be worse if growth retardation is followed by a period of rapid catch-up growth. This was suggested from animal studies, when protein deficiency followed by a cafeteria diet reduced longevity significantly [35].

The current aim is to prevent preterm infants dropping percentiles and falling below the 10th percentile at 36 weeks’ corrected GA or discharge home. Moltu’s group randomized 50 neonates <1,500 g to a multipronged intervention of high-protein/-lipid and -vitamin parenteral nutrition and high-protein fortifier and ‘protein shots’ prior to discharge in an attempt to meet RDI and prevent EUGR. Growth velocity and z scores were improved, but the trial ceased prematurely because of an increased incidence of septicemia in the intervention group possibly related to electrolyte disturbances indicative of accelerated protein synthesis.

There is a gap in our knowledge about how best to fortify human milk for preterm infants to achieve optimal growth, development and health outcomes in the long term. A Cochrane review [16] concludes that there is unlikely to be further trials on fortified versus unfortified human milk, but further research is needed comparing different fortifiers with short- and long-term outcomes to determine the optimal fortifier for human milk. There is an urgent need for well-designed and informed RCTs in this vulnerable preterm population.

**Disclosure Statement**

The author declares that no financial or other conflict of interest exists in relation to the contents of the chapter.
References


