Target Fortification of Breast Milk with Fat, Protein, and Carbohydrates for Preterm Infants

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Objectives
Fortification of breast milk is an accepted practice for feeding very low birth weight infants, however, fixed dosage enhancement does not address variations in native breast milk. We therefore established the infrastructure for target fortification in breast milk by measuring and adjusting fat, protein, and carbohydrate content daily. We analyzed nutrient intake, growth, and safety variables.

Study design
Each 12-hour batch of breast milk was analyzed using near-infrared spectroscopy. Macronutrients were individually added to routine fortification to achieve final contents for fat (4.4 g), protein (3 g), and carbohydrates (8.8 g) (per 100 mL). Fully breast milk fed healthy very low birth weight infants (<32 weeks) were fed the fortified breast milk for at least 3 weeks. Matched pair analysis of 20 infants fed routinely fortified breast milk was performed using birth weight, gestational age, and postnatal age.

Results
All 650 pooled breast milk samples required at least 1 macronutrient adjusted. On average, 0.3 ± 0.4 g of fat, 0.7 ± 0.2 g of protein, and 1.2 ± 0.2 g of carbohydrate were added. Biochemistry was normal in the 10 target fortified infants (birth weight: 860 ± 309 g, 26.3 ± 1.6 weeks gestational age); weight gain was 19.9 ± 2.7 g/kg/d; and milk intake was 147 ± 5 mL/kg/d (131 ± 16 kcal/kg/d). Osmolality of fortified breast milk was 436 ± 13 mOsmol/kg. Matched pair analysis of infants indicated a higher milk intake (155 ± 5 mL/kg/d) but similar weight gain (19.7 ± 3.3 g/kg/d). No adverse event was observed. The linear relationship between milk intake and weight gain observed in study babies but not seen in matched controls may be related to the variable composition of breast milk.

Conclusions
Daily target fortification can be safely implemented in clinical routine and may improve growth.

There is consensus that fortification of breast milk is needed to meet the nutritional requirements of very low birth weight (VLBW) infants (birth weight <1500 g).1,2 The common strategy for breast milk fortification assumes an average composition of breast milk, and fortifiers are added in a fixed dosage. It is not routinely taken into consideration that the caloric and nutrient content of native breast milk varies between mothers and between individual samples from the same mother.3-6 In an individual infant, this could lead to deficiencies in calories and macronutrients and contribute to postnatal growth restriction. Indeed, recent studies indicate that up to 58% of VLBW infants fed with fortified breast milk have postnatal growth restriction.7 In preterm infants, provision of sufficient amounts of nutrients for growth is crucial for normal brain development. Postnatal growth restriction has been associated with altered brain function, impaired long-term neurodevelopment, and reduced IQ.8 Therefore, alternative concepts are needed to ensure that nutritional needs of preterm infants are met.9-12 Recently, we demonstrated that implementation of a nutritional program leads to appropriate postnatal weight gain and head circumference in VLBW infants.10 Adjusting protein fortification on the basis of blood urea nitrogen (BUN) levels also leads to significantly better weight gain.13

Because breast milk has considerable variation for all macronutrients, optimized fortification of breast milk would be achieved by adjusting for individual macronutrients. Breast milk should be individually tailored based on the nutrient composition of each single feeding. Such an approach requires real-time measurements using point-of-care devices but would result in a more balanced composition and consistent intake of fat, proteins, and carbohydrates. This practice could lead to better short- and long-term growth and neurodevelopmental outcome of preterm infants.

The aim of this study was to establish the infrastructure to safely perform target fortification of breast milk in neonatal intensive care unit (NICU) by measuring and adjusting fat, protein, and carbohydrate content on a daily basis.

BUN Blood urea nitrogen
ESPGHAN European Society for Pediatric Gastroenterology, Hepatology, and Nutrition
L2 Level 2
NICU Neonatal intensive care unit
VLBW Very low birth weight
Growth and nutrient intake were monitored daily, and osmolality of feedings was analyzed as a safety variable.

Methods

The study was a prospective, single-center clinical trial to test feasibility and safety of target fortification of breast milk. The trial was conducted in the NICU (level 3) at the McMaster Children’s Hospital in Hamilton, Ontario, Canada. Clinical data of infants enrolled in the trial were compared with data from infants who had been fed with routinely fortified breast milk using a matched pair analysis. The study was approved by the Research Ethics Board of McMaster University. Informed written parental consent was obtained prior to inclusion of infants in the study.

Healthy preterm infants (n = 10, birth weight <1500 g and gestational age <32 weeks based on last menstrual cycle or early ultrasound data) who were fed exclusively breast milk through March 2011 and November 2011 were enrolled in the study. Infants were enrolled after fortification of breast milk at recommended dosages was achieved. Infants fed formula and infants with gastrointestinal (ie, intestinal malformation, abdominal surgery, necrotizing enterocolitis ≥ Bell stage 2) or chromosomal disorders, or preceding episodes of sepsis, or small for gestational age (birth weight less than the 3rd percentile using somatic growth standards) were not eligible.

For matched pair analysis, 2 infants who exclusively received routinely fortified breast milk were identified per study infant. Infants admitted between January 2010 and December 2011 were screened for the matching criteria of gestational age (±1 week), birth weight (±100 g), and postmenstrual age when fortifier was initiated (±1 week). Exclusion criteria were the same as for infants with target fortification. Nutrient intake and weight gain were collected daily after 4 days of introduction to routine fortification.

Intervention Period

The minimum duration of intervention was 3 consecutive weeks, prior to a postmenstrual age of 36 weeks. Once infants completed 3 weeks of intervention, target fortification was continued until 36 weeks. At 36 weeks of postmenstrual age, target fortification was discontinued and routine fortification was provided as indicated. General care of the infants and their nutritional management was conducted according to the standard protocols of the NICU.

Intervention Products and Dosage of Target Fortification

The following commercially available products were used: Similac (Abbott Nutrition, Columbus, Ohio), a breast milk fortifier in recommended dosage of 3.6 g fortifier per 100 mL breast milk (0.36 g fat/100 mL breast milk, 1.0 g protein/100 mL, and 1.8 g carbohydrate/100 mL); Microlipid (Nestle Health Care Nutrition, Minneapolis, Minnesota), a safflower oil fat emulsion developed for enteral feedings (0.5 g fat/mL); Beneprotein (Nestle Health Care Nutrition), an instant whey protein powder (0.86 g protein/g); and Polycose (Abbott Nutrition) a glucose polymer powder (0.94 g carbohydrate/g).

The defined macronutrient concentration in breast milk was 4.4 g/100 mL of fat, 3 g/100 mL of protein, and 8.8 g/100 mL of carbohydrate to meet the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) guidelines (6.6 g/kg/d of fat, 4.5 g/kg/d of protein, and 13.2 g/kg/d of carbohydrate) assuming an intake of 150 mL/kg/d. The calculation for breast milk target fortification was done in 3 steps. First, the concentration of macronutrients was determined in native breast milk; in a second step, the fortification was done as usual using the routine fortifier. Third, the additional amount of fat, protein, and/or carbohydrate required to achieve target levels of macronutrients was calculated as addition = ESPGHAN recommendations − (native breast milk + increment by routine fortification) and was subsequently added. In cases where a macronutrient component in the routine fortified breast milk exceeded the target value, only the other deficient macronutrient components were adjusted according to the protocol.

To enhance feeding tolerance at the start of the intervention, target fortification was gradually introduced in a stepwise manner over a 3-day period (maximum target dosage of added fat, protein, and carbohydrate was at day 1: 0.3 g, day 2: 0.6 g, and day 3: 0.9 g per 100 mL breast milk). On day 4, the full amount of target fortification for each macronutrient was prescribed; this day marked the starting point of the intervention period.

Milk Analysis

Prior to analysis, breast milk samples were homogenized using a sonicator for 3 × 10 s (VCX 130; Chemical Instruments AB, Sollentuna, Sweden). The osmolality was measured with a freezing point osmometer (sample volume of 20 μL, 3320 Micro-Osmometer; Advance Instruments, Norwood, Massachusetts). The macronutrients of breast milk were measured using a near-infrared milk analyzer (sample volume of 1 mL, Spectra Star; Unity Scientific, Brookfield, Connecticut) and adjusted according to internal validation. Precision and accuracy of the milk analyzer was evaluated by comparison with traditional methods. The mean difference between milk analyzer and traditional methods was −0.04 g for fat and +0.03 g for protein. Measurements of macronutrients by breast milk analyzer and traditional method correlated with a R² of 0.91 for carbohydrates, 0.95 for fat and 0.95 for protein.

Algorithm for Target Fortification of Breast Milk with Fat, Protein, and Carbohydrate

In the clinical routine of the McMaster Children’s Hospital, the fortification of breast milk was usually done in the afternoon (15:00-17:00) for the following 12-hour night shift (20:00-7:59) and in the morning (5:00-7:00) for the following 12-hour day shift (8:00-19:59). For this study, the target fortification of breast milk was integrated into this schedule by measuring and adjusting fat, protein, and carbohydrate on a daily basis (Figure 1). Nurses, neonatal research laboratory staff, dietitians, physicians, nurse practitioners, and technicians from nutrition services were involved.
Twice per day (at 5:00 and 15:00), bedside nurses thawed suitable samples from the pool of breast milk stored for each baby to compose the batch for the following 12-hour shift. A 1 mL aliquot from each batch of native breast milk was used for macronutrient analysis in the NICU research laboratory. The remaining batch was then first fortified with the routine fortifier. Macronutrient analysis determined how much extra fat, protein, or carbohydrate was needed in the batch to obtain final target fortified breast milk.

Milk analysis was done each morning in aliquots from batches collected from that morning and the previous afternoon. The mean of both measurements was used to calculate the required amount of extra fat, protein, and carbohydrate for the following 2 batches of breast milk (night batch 20:00-8:00 and the following day batch of the next day 8:00-20:00) using a predefined Excel spreadsheet (Microsoft Inc, Redmond, Washington).

As a safety assessment to ensure that an appropriate amount of fortifier was added, osmolality of unfortified and fortified breast milk samples was measured. Bedside nurses were then informed whether osmolality of fortified milk was within the acceptable target range (400-480 mOsmol/kg) before the milk was allowed to be fed during the next 12-hour shift. Osmolality lower or higher than the defined target range was considered as a sample preparation error on the fortification, and a new batch of breast milk was prepared.

Prescription of target fortification was usually completed before noon. These prescriptions were then approved by the dietitian and physicians. Subsequently, individual additives were provided by nutrition services. Bedside nurses prepared batches of fortified breast milk including the additives for target fortification and divided it into single feeding portions to be given to infants.

**Outcomes and Statistical Analyses**

Data from breast milk analyses, breast milk fortification, and enteral intake were documented daily. Weight was measured daily. Acid-base-status, serum triglycerides, BUN, and glucose were determined weekly as part of the NICU routine protocol, and during study weeks 1-3 and, if applicable, every 2 weeks thereafter.

Average weight gain during the first 21 days of intervention was calculated from daily weight measures using an exponential regression model. Values were expressed as mean and SD; median; or minimum and maximum. The statistical analysis was performed using the SPSS v 19 software (IBM, Somers, New York).

**Results**

During the study period (March 2011- November 2011), informed consent was obtained for 23 infants. Thirteen infants were excluded from analysis; 5 infants were excluded prior to initiation of target fortification (3 infants were transferred to level 2 [L2] nursery, 1 developed sepsis, and 1 developed bloody stools). Eight infants did not complete 3 consecutive weeks (4 were transferred to L2 nursery, mothers of 2 infants had insufficient breast milk supply, and 2 infants developed sepsis). Ten infants (5 male, 5 female) with a birth weight of 860 ± 310 g and a gestational age of 26.3 ± 1.6 weeks completed the minimum 3-week intervention period.

![Figure 1. Algorithm for target fortification of breast milk in this study. TFO, target fortification; CHO, carbohydrate.](image-url)
Target fortification of breast milk was started on day of life 30 (13; 56) median (minimum; maximum), infants were on average 30.9 ± 1.5 weeks postmenstrual age old. The duration of intervention was 29 (21; 53) days. The feeding volume was 147 ± 5 mL/kg/d and the weight gain during the first 3 weeks of intervention was 19.9 ± 2.7 g/kg/d.

Nutrient content was measured for 650 batches of pooled native breast milk (average of 65 ± 19 samples per mother). Figure 2 gives the distribution of macronutrient content in: (1) native; (2) routine fortified; (3) target fortified breast milk; and (4) shows macronutrient levels that would have been achieved if an “ideal” target fortification would have been performed where the same batch was measured and fortified. For native breast milk, the range (minimum-maximum) was 1.3-9.3 g/100 mL for fat, 0.7-2.4 g/100 mL for protein, and 4.8-6.4 g/100 mL for carbohydrate content. Intra-individual variation of native nutrient content was normally distributed and was 21% for fat, 14% for protein, and 3% for carbohydrates. Compared with native breast milk, routine fortification increased the intake of macronutrients, but only target fortification met the recommendation for all infants, and “ideal” target fortification reduced the inter-day variability.

Figure 3 shows the concentration of macronutrients achieved by routine fortification only. It is of interest to note that all routine fortified breast milk samples required additional protein and carbohydrates, and 45% of routine fortified breast milk samples needed additional fat to achieve the recommended target levels of macronutrients.

Because the target fortification was added to batches of breast milk with a 24-hour delay, the actual nutrient concentration could vary from the “ideal” target concentration. However, on average, there was no deviation from the targeted macronutrient concentration (Δfat of 0.1 ± 0.5 g/100 mL).
mL, Δprotein of 0 ± 0.2 g/100 mL, and Δcarbohydrate of 0 ± 0.3 g/100 mL. The variation of macronutrients amongst fortified batches was not higher than those observed in unfortified samples.

The osmolality of breast milk was 298 ± 7 mOsmol/kg before fortification and 436 ± 13 mOsmol/kg after fortification, with a maximum of 477 mOsmol/kg. Fourteen percent of fortified breast milk samples had an osmolality between 450 and 480 mOsmol/kg.

No feeding intolerance (defined as gastric residual volume of more than 50% of the previous feeding volume, emesis or abdominal distension or both, decrease, delay, or discontinuation of enteral feedings17) was observed during the target fortification intervention period. The biochemical safety variables were within normal ranges. The blood measurements were for triglycerides: 0.7 ± 0.3 mmol/L; BUN: 3.9 ± 1.1 mmol/L; protein: 44 ± 3 g/L; albumin: 32 ± 4 g/L; and glucose: 4.7 ± 0.5 mmol/L. No metabolic acidosis or adverse event related to target fortification was observed.

The demographics for matched pair subjects were as follows: birth weight was 880 ± 200 g and gestational age was 27.1 ± 1.6 weeks. The 3-week observation period began when babies reached a gestational age of 30.4 ± 1.2 weeks and at day of life 21 (13; 54). Compared with the infants on target fortification, the mean feeding volume (155 ± 5 mL/kg/d) of matched pair infants on routinely fortified breast milk was 8.4 ± 6.5 mL/kg/d (P < .001) higher, but the mean weight gain was similar (19.7 ± 3.3 g/kg/d).

**Figure 4** compares the growth pattern for the infants of both groups. Infants with target fortification showed a significant correlation of weight gain with milk intake. This correlation was not found in infants of the match pair analysis.

### Discussion

In this study, we showed that target fortification of breast milk is a feasible clinical routine. We provided a defined enteral intake according to the ESPGHAN guidelines12; this was achieved by analyzing the composition of each 12-hour batch of pooled breast milk and adjusting macronutrients (fat, protein, and carbohydrate) accordingly. Infants on target fortification had growth rates, which were linearly correlated to the feeding volume. All safety measurements during the intervention were within normal ranges and plasma BUN levels suggested adequate protein intake.13

The new fortification strategy results in a defined and predictable intake of macronutrients. In the 10 infants who received target fortified breast milk, there was a significant linear relationship of growth with milk intake over the whole range of feeding volumes (140–155 mL/kg/d) (R² of 0.68). Infants (n = 20) on routine fortification did not show such correlation; indeed, growth rates seem to be independent from milk intake. This indicates that the known natural variation in breast milk composition might have an effect on growth rates achieved and highlights the potential efficiency of targeted fortification. It might be possible to achieve growth rates of 21-23 g/kg/d with an appropriate fat mass to lean mass ratio with 155-160 mL/kg of target fortified breast milk. Such growth rates are required in very immature infants (ie, <27 weeks of gestation) to achieve postnatal growth rates close to those in utero. For future studies, to evaluate healthy growth, not only weight gain, but also body composition, length, and head growth need to be assessed. We recently demonstrated that the introduction of a nutritional program increased weight gain as well as head circumference, but did not increase percent body fat.10 A future randomized controlled trial on target fortification needs to include body composition measurements such as skinfold thickness, air displacement plethysmography, or dual energy X-ray absorptiometry.18-20

For the present study all 3 macronutrients (fat, protein, and carbohydrates) were measured and individually adjusted on a daily basis. To date, studies investigating target fortification have added commercially available protein11,13,21 to routinely fortified breast milk, without considering the variation of other macronutrients in breast milk.11,13,19 In this study, none of the 12-hour-batches would have met the targeted nutrient composition for protein and carbohydrate, and only 45% of batches would have met the target for fat if milk had been fortified using only routine fortifier. As
a consequence, preterm infants could experience nutritional deficits, which might explain the inadequate weight gain sometimes seen in preterm infants fed recommended volumes of routinely fortified breast milk.22 In this regard, target breast milk fortification would be beneficial.

There is growing evidence that not only protein but also the composition of nonprotein calories influences protein accretion rates. Recently Kashyap et al showed that protein accretion of enterally fed low birth weight infants was more efficient when nonprotein calories were supplied as carbohydrates instead of fat because protein oxidation was reduced.23 Furthermore, at isoosmolar intakes a formula with a calorie ratio of carbohydrates and fat at 2:1 was more effective than a 1:1 or 1:2 in enhancing growth and protein accretion in enterally fed low birth weight infants.24 To adjust for the variation of macronutrients in breast milk, it may be preferable to provide individual additives of each macronutrient in addition to routine fortifier rather than only protein, a practice frequently used in NICUs.

Target fortification turns breast milk into a standardized product and significantly reduces intra-individual variations in macronutrient levels. Our data show (Figure 2) that average macronutrient content of individually fortified milk meets the target levels, but that there is still considerable variation between batches. This is due to the fact the breast milk analyzer and osmometers were located in our neonatal research laboratory and not in the NICU. As a consequence, measurements could be performed by our laboratory staff only during day time. Although this organizational drawback did not impact nutrient enhancement per se, the 24-hour delay between sample measurement and sample fortification limited a further reduction of individual variation. Fortunately, the resulting variation did not increase but was smaller than that of routinely fortified milk. Once set up to provide target fortification as a clinical routine, the milk analyzer and osmometer could be located in the NICU as a point of care device similar to a blood gas analyzer. NICU staff could be trained to perform measurements of milk composition and osmolality, and the fortification of human milk would move toward an algorithm enabling real-time, same-day fortification of each sample without delay. As a consequence, there would be minimal variation of macronutrient content between fortified breast milk (Figure 2). Based on our experience, we estimate an additional work load of approximately 5-10 minutes per milk batch to process target fortification in NICU.

Because optimized fortification impacts final osmolality of feeds, we measured the osmolality in the fortified breast milk samples for additional safety. For enteral feeding of neonates, an upper limit for osmolality of 450 mOsmol/kg has been recommended.25 Although unfortified breast milk has an average osmolality of 300 mOsmol/kg, addition of routine fortifier increases the osmolality by 115 mOsmol/kg resulting in 415 mOsmol/kg. With further addition of target fortification, there is a risk that the osmolality could reach or exceed the upper limit of the recommended range. Also, accidental over-fortification of breast milk might exceed the recommended range of osmolality with the risk of feeding intolerance or even necrotizing enterocolitis. Thus, osmolality measurements should be used for safety.

The low enrollment rate could raise concerns about the generalizability of this trial. Three reasons contributed to this. The target fortification was not yet an established routine procedure. Recruitment and consenting took place only during working days for a highly selected population and, of course, there were parents that did not consent. Secondly, the McMaster NICU was traditionally slow in introducing and advancing enteral feeds as indicated by the late entry point for target fortification at a median age of 30 days. This practice reduced the number of eligible infants that might complete 3 weeks before being discharged or transferred. Many NICUs and McMaster as of 2012 now advance feeds faster. There is evidence and our own experience that early feeding and faster advancement is possible and will improve outcome.10,26,27 Faster enteral feeding advancement will allow an earlier entry point by 2 weeks of life. Finally, at McMaster NICU 40% of VLBW infants are transferred after initial stabilization to a regional L2 nursery from where they are discharged home. Age at transfer of these infants was around 32 weeks, reducing the number of eligible infants for the trial.

A finding of our study was that, despite their better weight gain, infants on target fortification received a 7%-10% lower feeding volume (147 ± 5 mL/kg/d) than expected (155-160 mL/kg/d), and 8.4 ± 6.5 mL/kg/d less than infants in the matched pair group. Apparently, bedside staff decreased feeding volumes because of “unusual” high weight gain after introduction of target fortification. The effects on weight gain by target fortification might have been greater if feeding volumes and calories had been provided as per protocol.28

Weight gain at rates of 21 g/kg/d and above could reflect proper growth with desirable tissue accretion in healthy preterm infant but must be differentiated from fluid accumulation with its risk for pulmonary compromise that is sometimes observed in sick preterm infants.

In summary, target fortification of breast milk for VLBW infants with daily measurements and adjustments of fat, protein, and carbohydrate was feasible, and safety measurements were within normal ranges. This study supports the need for a randomized controlled clinical trial to evaluate effects on short- and long-term growth, body composition, and neurodevelopment.

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