Effect of Pooling Practices and Time Postpartum of Milk Donations on the Energy, Macronutrient, and Zinc Concentrations of Resultant Donor Human Milk Pools

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Objective To characterize the macronutrient, energy, and zinc composition of pasteurized donor human milk pools and evaluate how composition varies based on pooling practices and "time postpartum" (ie, elapsed time from parturition to expression date) of individual milk donations.

Study design The Mothers' Milk Bank (Arvada, Colorado) donated 128 donor human milk pools. Caloric density was assessed via mid-infrared spectroscopy, and zinc concentration was measured by atomic absorption spectroscopy. Pool time postpartum was calculated as the unweighted average of the time postpartum of all milk donations included in any given pool.

Results Time postpartum of donor human milk pools ranged from 3 days to 9.8 months. The majority (91%) of donor human milk pools included milk from either 1 donor or 2 donors. Pool energy density ranged from 14.7 to 23.1 kcal/oz, and protein ranged from 0.52 to 1.43 g/dL. Milk zinc concentrations were higher in preterm pools and were negatively correlated with pool time postpartum. We present an equation that estimates donor human milk pool zinc content based on time postpartum and explains 49% of the variability in zinc concentrations ($P < .0001$). Including more donors in donor human milk pools decreased the variability in protein, but not zinc, concentrations.

Conclusions Donor human milk pools were lower in calories than is normally assumed in standard human milk fortification practices. Zinc concentrations were related to donor human milk time postpartum and were on average insufficient to meet preterm and term infants' needs without fortification or supplementation. *(J Pediatr 2019;214:54-9)*.

The American Academy of Pediatrics recommends pasteurized donor human milk as the best alternative to feed a premature infant when the mother's own milk is unavailable.[1] The Human Milk Banking Association of North America supplied more than 5.75 million ounces of pasteurized donor human milk to recipient infants in 2017.[2] The majority of this milk was provided to premature infants in neonatal intensive care units (NICUs), due to the protective effects of donor human milk against necrotizing enterocolitis.[3,4]

Human milk composition is dynamic, changing over the course of a feed, over the course of a day, and over the course of lactation. For example, milk fat concentrations increase over the course of a feed and over the course of a day,[5,6] and maternal dietary and lifestyle factors impact the nutritional and hormone profile of milk.[7-13] For these reasons, donor human milk banks routinely pool milk donations from multiple donors to limit extreme variation.[14] This results in decreased variability in donor human milk pool macronutrient content,[15] but it is not known whether the time postpartum in which donated milk is expressed is associated with donor human milk macronutrient and caloric composition. This concept is not well understood in the context of milk banking. Understanding the variability in donor human milk macronutrient content has particular relevance to premature infants, who are the primary recipients of donor human milk and who have elevated nutritional needs.

In addition to macronutrients, of the essential micronutrients in human milk, zinc concentrations exhibit the sharpest physiological decline as lactation progresses,[16] with large variations between individuals at every stage of lactation from 0 to 7 months.[17] Human milk composition after 7 months is less well understood, but the time trend for decreasing zinc concentrations is less

NICU Neonatal intensive care unit

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Portions of this study were presented as on oral presentation at the Human Milk Banking Association of North America annual meeting, April 16-18, 2018, Denver, Colorado; as an oral presentation at the Western Medical Research Conference, January 25-27, 2018, Carmel, California; and as a poster presentation at the Pediatric Academic Societies annual meeting, May 5-8, 2018, Toronto, Ontario, Canada.

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severe, and variability between individuals remains high.[18] Given their critical roles in infant growth, we sought to characterize the macronutrient content and zinc concentrations in donor human milk pools and to investigate relationships with both donor human milk time postpartum and pool size (ie, number of donors included in the pool). These results are relevant to NICU human milk fortification practices, as well as to the growing trend of providing unfortified donor human milk to term infants.

Methods

Postpasteurization samples from 138 donor human milk pools were obtained from the Colorado Mothers' Milk Bank (Rocky Mountain Children's Health Foundation, Arvada, Colorado). These pools represent all of the pools generated by the milk bank on 2 separate sets of consecutive days in May and June 2017.

At this milk bank, individual frozen milk bags that arrive in a single donation batch are assigned the earliest expression date as long as the individual expression dates do not span >4 months. In that case, individual bags of milk are separated into smaller batches to minimize the span in expression dates. The vast majority of donations to this milk bank contain individual bags of milk that span <2 months in expression dates. The expiration date of donated milk is defined as 12 months after the batch expression date. "Time postpartum" is defined as the postpartum age at the expression date.

This milk bank selects individual donations to pool based on the expiration date of donated milk, independent of time postpartum. Pool size was defined as the number of milk donors represented in each pool. Once pooled, donor human milk pools are aliquoted into glass bottles, pasteurized via Holder pasteurization (ie, heated to 62.5°C for 30 minutes, followed by rapid cooling), and then frozen for distribution.

The specialty milk pools included in this present analysis include "preterm" and "dairy-free" milk. Preterm milk was defined as human milk expressed by a mother who delivered at ≤36 weeks gestation within the first 4 weeks postpartum or up until her infant's corrected age is 40 weeks (whichever is shorter). Dairy-free milk was defined as milk expressed by a mother who had maintained a dairy-free diet for at least 14 days before expression. Both preterm and dairy-free milk pools contained only individual donations adhering to these criteria. Term donor human milk pools included milk expressed by mothers who gave birth after 36 weeks or before 36 weeks but after 4 weeks postpartum.

Each sample was subjected to 2 freeze-thaw cycles before macronutrient analysis, first to generate the pool at the milk bank and then at the time of macronutrient analysis. Zinc aliquots were subjected to an additional freeze-thaw cycle.

This study was deemed not human subject research by the Colorado Multiple Institutional Review Board.

Milk Analyses

Macronutrient and caloric profiles were assessed in postpasteurization samples via mid-infrared spectroscopy (Human Milk Analyzer; Miris, Uppsala, Sweden). True protein values are reported. Samples were analyzed in duplicate, and the average of the results was recorded. The milk analyzer was flushed with warmed deionized water between each sample. The analyzer was calibrated against a control sample of known composition on every day of use and also after every 50 experimental samples in a single day when relevant. A check solution (Miris) was run on the machine after every 10 samples.

Mid-infrared spectroscopy is an accepted method of macronutrient measurements. Although measurement errors can be exaggerated in samples with elevated fat content,[19] this methodology can provide an accurate and practical estimate of milk macronutrients.[19-21] The Miris analyzer is currently the only human milk analyzer approved by the US Food and Drug Administration for measuring human milk macronutrient composition in clinical settings for infants at risk of growth faltering.[22]

Zinc concentrations were measured by atomic absorption spectroscopy in post-pasteurization whole milk samples,[16] because milk zinc concentrations are not altered by pasteurization.[23] Milk samples and internal controls were analyzed in duplicate from an initial processing step, and the average of the results was recorded. Samples with a final zinc measurement outside the linear range of the spectrophotometer were diluted and reanalyzed until values fell within the linear range. Precautions were taken throughout sample handling to avoid zinc contamination.

Statistical Analyses and Calculations

The pool time postpartum of the donor human milk pools was calculated as the unweighted average of the individual donations' time postpartum and also categorized as ≤1 month, 1-3 months, 3-6 months, and >6 months postpartum. Comparisons of milk composition between preterm and term donor human milk pools and comparisons of donor human milk pool characteristics by number of donors were conducted using nonparametric tests. Simple linear regression was used to determine whether pool time postpartum was related to milk composition, and whether any individual milk components were intercorrelated. Normality of the regression residuals was established to ensure that model assumptions were satisfied. The O'Brien test was performed to assess whether variation in donor human milk composition varied by pool size. Testing for variation in protein or zinc content by pool size was done using the residuals of the variation in donor human milk content, after controlling for time postpartum.

Unless stated otherwise, data are reported as mean ± SD. Analyses were performed using JMP Pro 13 (SAS Institute, Cary, North Carolina).

Results

Characteristics of Pools

Among the 138 donor human milk pools, 6 (4.3%) were preterm and 5 (3.6%) were dairy-free. All dairy-free pools were composed of term donations. Data regarding the number of donors in each pool, pool volume, and pool time postpartum were available for 128 of the 138 pools. These 128 milk pools represented donations from 212 individual donors. There were 38 individual preterm donations (ranging from 3 to 14.5 days postpartum) and 174 individual term donations (ranging from 5 days to 18.3 months postpartum).

Figure 1 shows the number of donors in each milk pool and average pool volume of all 128 pools. For example, among the term and non–dairy-free pools (92% of all donor human milk pools), 41% included milk from 1 donor, 50% included milk from 2 donors, and 9% included milk from 3 or 4 donors (Figure 1, A). However, because pools originating from a single donor had the lowest volume ($P < .0001$), only 13% of the donor human milk volume dispensed originated from single-donor pools (Figure 1, B). Two dairy-free pools were composed of donations from 2 donors, and 2 were composed of donations from a single donor. (Donor information was unavailable for 1 pool.)

Table I shows the average time postpartum of the resultant milk pools. Of the 128 pools, 25 (accounting for 8.8% of the total dispensed donor human milk volume) represented milk with a time postpartum of ≤1 month, 32 (accounting for 23.0% of total dispensed donor human milk volume) represented milk with a time postpartum of 1-3 months, 43 (accounting for 42.4% of total dispensed donor human milk volume) represented milk with a time postpartum of 3-6 months, and 28 (accounting for 25.8% of total dispensed donor human milk volume) represented milk with a time postpartum >6 months.

Table I. Individual donor human milk pool, time postpartum*

Pool type	n	Mean ± SD	Minimum	Maximum
All pools	128	3.78 ± 2.6 mo	3 d	9.8 mo
Preterm	5	8.3 ± 4.6 d†	3 d	14.5 d
Term	123	3.8 ± 2.6 mo	7 d	9.8 mo
Dairy-free‡	4	4.6 ± 1.0 mo	3.7 mo	6.0 mo

*Time postpartum is calculated as the unweighted mean postpartum age (time elapsed between parturition and milk expression) of individual milk donations included in the donor human milk pool.
†Time postpartum of preterm pools is significantly less than term pools ($P = .0004$).
‡Dairy-free pools are all from term donations and are included in the term averages.

Pool Composition: Macronutrients

Milk pool energy density ranged from 14.7 to 23.1 kcal/oz. The macronutrient composition of pools is presented for term and preterm pools separately in Table II. Dairy-free milk pools (n = 5) are included in the term pools because all dairy-free pools were composed of term donations, and the average macronutrient composition of the dairy-free pools did not differ from that of the other term pools ($P > .25$).

The mean ± SD of the coefficient of variation for the macronutrient replicate measurements were 1.2 ± 0.9% for fat, 5.1 ± 7.2% for protein, 0.7 ± 0.8% for carbohydrate, and 1.0 ± 0.8% for calories. Donor human milk pool protein content was negatively associated with pool time postpartum ($P < .0001$; $R^2 = 0.20$; n = 128). Fat and carbohydrate were not associated with pool time postpartum. Pool macronutrient concentrations and energy density did not differ by

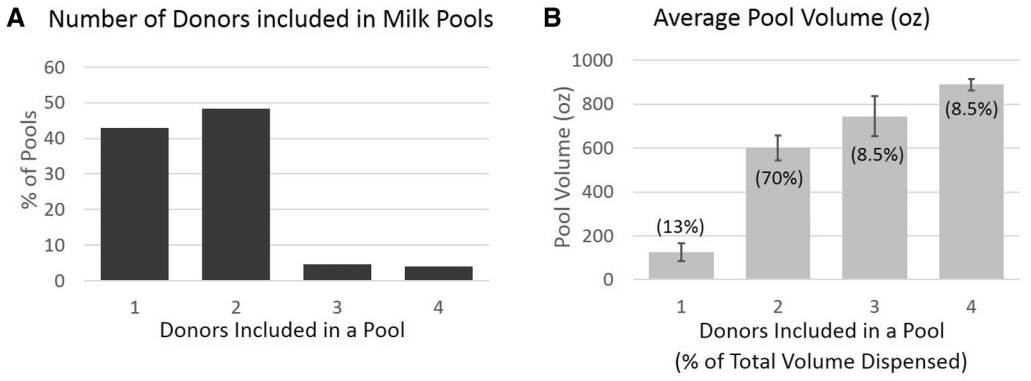

Figure 1. Characteristics of donor human milk pools characterized by the number of donors in each pool. **A**, the percentage of pools resulting from 1 to 4 donors. **B**, the average volume of pools generated that contained milk from 1 to 4 donors. Pools resulting from 1 donor had the lowest volume ($P < .0001$, Wilcoxon rank-sum test). Percentages at the top of each bar (**B**) indicate the percent of total donor human milk dispensed that originated from that classification; 87% of the total volume of donor human milk dispensed came from a pool with multiple donors.

Table II. Nutrient composition of preterm and term donor human milk pools

Variable	Preterm (n = 5), mean ± SD	Term (n = 123), mean ± SD	*P* value*
Kcal/oz	18.9 ± 1.2	17.6 ± 1.7	.043
Fat, g/dL	3.10 ± 0.36	2.94 ± 0.63	.313
Protein, g/dL	1.09 ± 0.11	0.74 ± 0.14	.0004
Carbohydrate, g/dL	7.30 ± 0.07	7.00 ± 0.20	.0005
Zinc, μg/mL	3.83 ± 0.84 (n = 6)	1.41 ± 0.73 (n = 132)	<.0001

*For nonparametric comparison (Wilcoxon) of preterm vs term.

pool size. The variation in donor human milk protein content decreased significantly as pool size increased from 1 to 2 to ≥3 donors ($P = .014$). The variation in donor human milk fat and carbohydrate content did not differ by pool size.

Pool Composition: Zinc

Three of the 138 samples were of insufficient volume to allow for zinc analysis, and thus the final sample size for zinc analysis was 135. The mean ± SD of the % coefficient of variation for the zinc replicate measurements was 3.3 ± 6.1%. Pool composition data were available for 125 of these samples.

As expected, given the earlier time postpartum, zinc concentrations were higher in preterm donor human milk pools (Table II). The mean ± SD of zinc concentrations was 3.83 ± 0.84 μg/mL in preterm pools (n = 6) vs 1.41 ± 0.73 μg/mL in term pools (n = 132; $P < .0001$). For samples with corresponding caloric data, the mean ± SD of zinc concentrations was 0.64 ± 0.12 mg/100 kcal in preterm pools (n = 5), compared with 0.24 ± 0.12 mg/100 kcal in term pools (n = 123; $P = .001$).

The log of donor human milk pool zinc concentrations were negatively associated with pool time postpartum ($P < .0001$; $R^2 = 0.49$; n = 125), such that:Ln (donor human milk [Zn] μg/mL) = 0.819-0.1536 (pool time postpartum in months) (Figure 2).

Donor human milk pool zinc concentrations were also positively associated with milk protein ($P < .0001$; $R^2 = 0.29$; n = 125) and milk lactose concentrations ($P < .0001$; $R^2 = 0.13$; n = 125). Neither donor human milk zinc concentrations nor the variation in zinc concentration differed significantly by pool size.

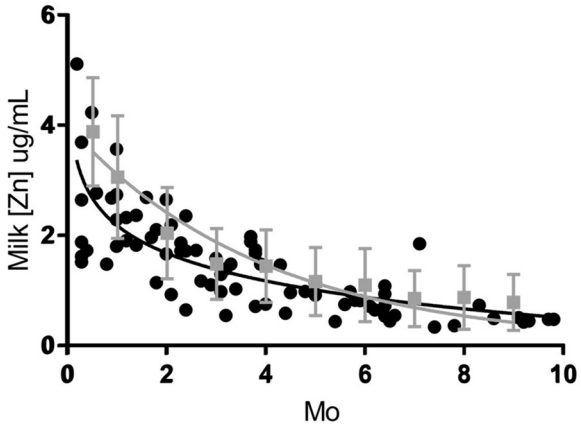

Figure 2. Donor human milk zinc concentration by pool time postpartum. Black dots represent the time postpartum and donor human milk zinc concentration of 125 donor human milk pools (including term and preterm donor human milk pools). The black line represents the regression line (see Results for regression equation). Transposed in gray squares and line is the mean ± SD deviation and analogous negative relationship between the log of human milk zinc vs time postpartum in a cohort of 71 mothers followed prospectively as lactation progressed. (Reproduced with permission from Krebs et al[16]).

Discussion

This study demonstrates that there was significant variability in donor human milk energy density (range, 15-23 kcal/oz), and that the average caloric and protein concentrations (18 kcal/oz and 0.74 g/dL in term donor human milk, respectively) were below the values normally assumed for human milk. In addition, donor human milk zinc concentrations were relatively low and strongly associated with the time postpartum of the donations included in a given pool. These data corroborate that exclusive and unfortified donor human milk feeding is inadequate to meet the nutrient needs of preterm infants. Fortification of donor human milk may address these gaps for preterm infants, but given the sharp physiological decline in zinc concentrations, unfortified donor human milk is also unlikely to provide adequate zinc intake for exclusively donor human milk–fed term newborns.

The caloric density of donor human milk pools in this study was relatively low, as has also been observed in other studies.[24] These relatively low caloric density and protein concentrations need to be considered when providing donor human milk to low birth weight and preterm infants. Specifically, standard donor human milk fortification generally presumes a starting point of 20 kcal/oz. Fortifying the average term donor human milk from this study with a standard commercial human milk fortifier concentrate (Abbot Nutrition, Lake Forest, Illinois[25]) as instructed to reach a presumed 24 kcal/oz would result in a feed consisting of 21.5 kcal/oz and 1.96 g/dL of protein. This is in contrast to a standard inpatient high-protein preterm formula that provides 24 kcal/oz and 2.7 g of protein/dL.[26] Similar reports indicate that 75% of mature donor human milk samples would not meet protein targets after standard fortification practices without exceeding intakes of 160 mL/kg/day.[15] Thus, the use of term donor human milk pools as the basis for feeding preterm infants may result in an underestimate of infant protein and caloric intake.

Fortification is absolutely necessary to meet preterm infants' zinc needs. Fortification of term donor human milk from this study to a presumed 24 kcal/oz would result in zinc concentrations of 11.2 μg/mL zinc. At this concentration, infants meeting target full feeding volumes of

150-160 ml/kg/day will meet the zinc intake target for preterm infants of 1.1 mg/kg body weight/day.[27]

There is a growing trend toward providing donor human milk to healthy term infants, with an estimated 18% of level 1 nurseries routinely using donor human milk.[28] Our data suggest that the average term donor human milk zinc concentration of 1.41 μg/mL is equivalent to milk produced for a healthy exclusively breastfed 3-month old infant.[16] Because infant dietary recommendations are based on human milk composition, unfortified donor human milk may contain insufficient zinc to meet the needs of infants aged <3 months. The Dietary Reference Intake (an adequate intake) for zinc for infants aged 0-6 months is 2 mg/day.[29] Assuming an intake of 750 mL/day for a healthy term infant, the average term donor human milk pool would provide only one-half this amount (1.06 mg zinc/day). Only 6% of term donor human milk pools (representing 2.0% of term donor human milk volume dispensed) had a sufficiently high zinc concentration to meet the infant zinc dietary reference intake. As an additional reference, the Food and Drug Administration has set a minimum zinc concentration of 3.38 μg/mL for standard infant formula, and assuming a lower bioavailability, standard term infant formulas provide between 5 and 7 μg zinc/mL of formula. This provides 3.75-5.25 mg/day of zinc at an intake of 750 mL/day. These observations should be considered when making assumptions about the nutrient intake of healthy term infants consuming unfortified term donor human milk.

Although it is well established that zinc concentration in breast milk decreases over time, it is not feasible to measure the zinc concentrations of every donor human milk pool. However, it is noteworthy that donor human milk pool time postpartum explained roughly 50% of the variation in zinc concentrations, even though time postpartum was a rough estimate of the postpartum age of the milk pool. The similarities in the regression curves produced by these data and the data collected prospectively from lactating individuals (shown in **Figure 2**) are striking and suggest the potential for donor human milk banks to use time postpartum to provide an estimate of whether resultant donor human milk pools will have high or low zinc concentrations. In the future, this estimation of pool time postpartum could potentially be used to provide more individually tailored fortification procedures in the NICU and potentially for term-born infants as well. Before attempts are made to test this estimation clinically, the investigations reported here should be replicated with several milk banks and with a more regimented and precise assignment of time postpartum to individual milk donations included in a single donor batch. Milk banks often need to function with limited donation availability, which limits the criteria on which they can select individual donations for any given pool. For example, only one-third of the volume in our study was from a time postpartum of <3 months, suggesting a supply constraint within milk banking for creating early time postpartum pools. As such, validating a cost-effective estimation of zinc concentrations would allow for targeted fortification without wasting milk donations or adding unnecessary burden on the milk banks.

A strength of our study is the use of donor human milk pools produced by a Human Milk Banking Association of North America milk bank. Another strength is the large sample size, which allowed for an accurate estimate of the variation in pool characteristics. Our ability to calculate a rough estimate of pool time postpartum provides a novel way to characterize donor human milk pools. Our inability to link donor human milk pools to outcomes of recipient infants, or to characteristics of individual donors, may be considered a weakness and represents a promising area for future research. Furthermore, our limited number of preterm donor human milk pools, dairy-free pools, and pools originating from more than 2 donors limited our ability to detect differences in composition in these specific subtypes of donor human milk.

We have shown that the majority of donor human milk does not meet estimated preterm infant protein and energy intake needs but does meet estimated zinc needs with standard fortification procedures. Almost all (94%) of term donor human milk pools in this study would fail to meet the zinc dietary reference intake for a healthy term infant if fed exclusively without fortification. However, our use of a low-cost and simple pool time postpartum may provide a promising means of estimating the zinc concentration of individual donor human milk pools to guide and inform the need for fortification. Finally, there may be benefits to standardizing how donations are classified, as well as the criteria for selecting individual donations that are incorporated into donor human milk pools across independent milk banks. ■

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