




REGULAR ARTICLE

Unpasteurised maternal breast milk is positively associated with growth outcomes in extremely preterm infants

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Abstract

Aim: Extrauterine growth restriction is common among extremely preterm infants. We explored whether intake of unpasteurised maternal milk (MM) and pasteurised donor milk (DM) was associated with longitudinal growth outcomes and neonatal morbidities in extremely preterm infants.

Methods: Observational study of 90 preterm infants born between 2013 and 2015 in Gothenburg, Sweden. Data were prospectively collected on nutritional and breast milk intakes during the first 28 days.

Results: Ninety infants (39 girls and 51 boys) with a median gestational age of 25.3 (22.7-27.9) weeks were evaluated. MM intake (mL/kg/d) correlated positively with almost all z-scores for weight, length and head circumference at 28 postnatal days and at postmenstrual age (PMA) 32 and 36 weeks. After multivariable adjustment, MM intake and weight z-score at 28 postnatal days and at PMA 32 and 36 weeks remained significantly associated. Infants consuming $\geq 80\%$ MM had more favourable weight z-scores at PMA 32 and 36 weeks. Intake of DM did not correlate with any growth outcomes. Infants without retinopathy of prematurity had a significantly higher intake of MM (mL/kg/d).

Conclusion: Unpasteurised MM was positively associated with longitudinal growth outcomes. Motivating mothers to provide their infants with their own milk after preterm birth should be emphasised.

KEYWORDS

donor milk, growth, maternal milk, pasteurisation, preterm

1 | INTRODUCTION

Preterm infants are at increased risk of extrauterine growth restriction, which at least in part is caused by very high extrauterine nutritional needs.¹ In addition, the inability to assimilate sufficient

nutrition due to feeding intolerance secondary to immaturity and neonatal morbidities is especially common in extremely preterm infants. The result is significant energy and protein deficits, primarily in the early postnatal period.² Most extremely preterm neonates suffer from growth restriction that can persist into childhood.³

Abbreviations: PMA, postmenstrual age; ROP, retinopathy of prematurity; SD, standard deviations.

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Energy, protein and other macronutrient intakes relate independently and positively to weight, length and head circumference growth in extremely preterm infants.^{4,5} In addition to nutrient intake, the type of breast milk fed to preterm infants influences primarily weight growth in the neonatal period. Preterm infants fed predominantly unpasteurised maternal milk, as opposed to pasteurised donor milk, regain their birth weight earlier and gain weight faster in the postnatal period.⁶⁻¹¹

Apart from growth outcomes, intake of predominantly unpasteurised maternal milk has been shown to be protective against short-term and long-term morbidities. These include infection-related events and late-onset sepsis, necrotising enterocolitis and retinopathy of prematurity (ROP).^{11,12} Unpasteurised maternal milk also seems to protect against adverse neurodevelopmental outcomes at 1 and 2 years of corrected age.⁹ Preterm infants fed predominantly maternal milk, as opposed to donor milk and/or preterm formula, also have shorter neonatal hospital stays.^{8,12} Moreover, a study of 926 French preterm infants found that those who received fresh maternal milk, rather than pasteurised maternal milk, had a significantly lower risk of developing bronchopulmonary dysplasia.¹³

In Sweden, the number of extremely preterm infants fed only maternal milk at discharge from the neonatal unit has decreased from 55% in 2004 to 16% in 2013.¹⁴

We hypothesised that extremely preterm infants that received a higher proportion of unpasteurised maternal milk during the first four postnatal weeks would have a more favourable growth from birth until 36 weeks of postmenstrual age (PMA). We also explored the potential protective effects of maternal milk on ROP, bronchopulmonary dysplasia and sepsis.

2 | METHODS

2.1 | Study design

This observational study originates from a randomised controlled trial that compared two different parenteral lipid solutions administered to extremely preterm infants. The solutions differed in their contents of long-chain polyunsaturated fatty acids and the outcomes studied in the trial were ROP, other neonatal morbidities and growth. The trial was carried out in the neonatal intensive care unit at Sahlgrenska University Hospital in Gothenburg, Sweden.¹⁵

2.2 | Study population

Out of 138 eligible infants, the randomised controlled trial included 90 infants with a gestational age less than 28 weeks, born between April 2013 and September 2015. Parental consent was obtained prior to inclusion. The exclusion criterion was major congenital malformations. Ethical approval was issued by the Regional Ethical Review Board, Gothenburg (Clinical trial NCT02760472).

Key Notes

- Knowledge is lacking regarding the effects of unpasteurised maternal milk compared with pasteurised donor milk on the associations with postnatal growth.
- Intake of unpasteurised maternal milk was associated with longitudinal growth outcomes.
- Future research should focus on specific components in unpasteurised breast milk that could be of importance for postnatal growth.

2.3 | Data collection

Data regarding nutritional intake, growth and maternal and neonatal morbidities were prospectively collected during hospitalisation according to the study protocol and verified against patient charts. The full amount of enteral and parenteral nutrition received by the infants were registered daily for the first 28 days of life in Nutrium, a computer-aided neonatal nutrition calculation program (Nutrium AB).¹⁶ Registration of nutritional intakes in Nutrium included parenteral nutrition and other non-nutritional parenteral fluids, maternal and donated breast milk, human milk fortifiers, other nutritional products used for fortification of feeds, enteral and parenteral supplements of vitamins, minerals and trace elements, and transfusions of blood products. Potential nutrients from blood products were not included in the data analysis. Neither was the recorded nutrition from the day of birth since the infants had received nutrition for a variable number of hours during the first day of life. If a maternal breast milk analysis was unavailable, estimated macronutrient and energy values from early maternal breast milk produced less than 4 weeks postpartum, were used. The estimate was based on previous data for analysed maternal milk in a larger cohort of extremely preterm infants born in Sweden.¹⁷ For the current study, we defined full enteral feeds as an enteral intake of at least 150 mL/kg/d.

2.4 | Exposure variables

The primary exposure variable was intake of unpasteurised maternal milk. However, because most infants in Sweden receive both unpasteurised maternal milk and pasteurised donor milk the first four postnatal weeks, we used intake of pasteurised donor milk as a secondary exposure variable.

The exposure variables, for maternal and donor milk intake during postnatal days 2-28, 2-14 and 15-28, were explored in two ways in relation to outcome variables:

1. As mean intake (mL/kg/d) of unpasteurised maternal milk and pasteurised donor milk.
2. As proportional intake of maternal milk (<80% or ≥80%).

2.5 | Outcome variables

The primary outcome variables were z-scores for weight, length and head circumference at postnatal age 28 days and at PMA 32 and 36 weeks. Secondary outcome variables were the neonatal morbidities ROP, bronchopulmonary dysplasia and sepsis.

2.6 | Parenteral nutrition protocol

After birth, parenteral nutrition was initiated as early as possible, targeting a volume of 80-90 mL/kg/d during the first 24 hours. The parenteral solution was a combination of amino acids and glucose. Unless contraindicated, a lipid solution was initiated at 6-12 hours after birth. The parenteral nutrition protocol has been described in more detail elsewhere.¹⁵

2.7 | Enteral nutrition protocol

The local routine was to initiate minimal enteral feedings with 1-2 mL of human milk per feeding within three hours after birth. Enteral volumes were increased by 10-20 mL/kg/d depending on the infant's feeding tolerance until the goal volume of 160-180 mL/kg/d was reached. Nutritional targets were set at 4-4.5 g protein/kg/d and 115-135 kcal/kg/d.

Unpasteurised expressed maternal milk, either fresh or thawed after freezing, was the primary choice of enteral feeds. If maternal milk was unavailable or insufficient to meet the infant's volume needs, pasteurised donor milk was used as a supplement. Maternal milk was never pasteurised, and donor milk was always pasteurised. Donor milk was given until 34 weeks PMA after which it was replaced by preterm formula. The local routine was to fortify both maternal and donor milk with a bovine human milk fortifier (Nutriprem, Nutricia, France). If additional fortification was required fat emulsions and/or carbohydrates were used. Fortification of all breast milk, maternal and donated, was based on breast milk analysis and was initiated when the enteral intake reached 70 mL/kg/d. Maternal and donor milk was fortified as long as the infant received expressed breast milk.

The first maternal breast milk analysis was undertaken when 50% of the infant's enteral nutrition originated from maternal milk. Maternal milk was analysed, based on a 24-hour sample, once weekly until fortification was discontinued. Maternal milk and donor milk were analysed with a human milk analyser, MilkoScan Minor (FOSS), generating nutritional values of kilocalories (energy) and grams of protein, carbohydrates and fat per 100 mL of breast milk.

2.8 | Growth measurements and definitions of growth restriction

Infants were weighed daily, as long as clinically stable, and length and head circumference were measured once weekly. Nursing staff

performed all anthropometric measurements, and the same nurse always performed the weekly length and head circumference measurements. Weight was measured with an accuracy of ± 5 g. Length was measured with a measuring tape if the infant was less than 35 cm long, otherwise a length board was used. Length and head circumference were measured to the nearest centimetre and millimetre.

All anthropometrics collected throughout the hospital stay were registered prospectively in Nutrium and digitally transformed into z-scores according to the Niklasson growth reference for preterm infants.¹⁸ If infants had no recorded weight, length or head circumference, measurements ± 1 day were assumed equal to measurements on 28 postnatal days, and weight measurements ± 3 days as well as length and head circumference measurements ± 7 days were assumed equal to measurements at PMA 32 and 36 weeks.

Small for gestational age was defined as a birth weight z-score below -2 standard deviations (SD) of the gestational age-related mean of the population.

2.9 | Clinical variables and definition of morbidities

ROP was classified according to the International Classification of Retinopathy of Prematurity.¹⁹ Bronchopulmonary dysplasia was defined as the need for supplemental oxygen at 36 weeks PMA. Sepsis was divided into presence of early-onset sepsis, that is sepsis episodes that occurred from birth until postnatal day seven, and presence of late-onset sepsis, defined as sepsis episodes that occurred from postnatal day eight until discharge from the neonatal unit or mortality. Sepsis was defined as presence of clinical symptoms concomitant with a positive blood culture. If the blood culture showed a mixed bacterial flora or coagulase-negative staphylococci an elevated C-reactive protein (>20 mg/L) or interleukin-6 (>1000 ng/L) was also required for diagnosis. Steroid treatment during the first four postnatal weeks was summarised to an accumulated dose of hydrocortisone (mg/kg) and betamethasone (mg/kg), in which the betamethasone dosage was converted into hydrocortisone equivalents by the ratio 1:35.

2.10 | Statistical analysis

The data were analysed using SPSS version 24 (IBM Corp) and SAS software version 9.4 (SAS Institute Inc). For tests between two groups, the Mann-Whitney *U* test was applied for continuous variables, the chi-square test for non-ordered categorical variables and Fisher's exact test for dichotomous variables. The relation between two continuous variables was examined by the Spearman rank correlation coefficient. The analyses of receiver operating characteristic curve, with growth variables at 28 postnatal days and at 32 and 36 weeks PMA dichotomised according to -2 SD as outcome, were used in order to identify the cut-off of more or less than 80% of maternal milk intake. In addition, the cut-off was selected based on a previous study using the same cut-off value.⁶ The association between intake of maternal or donor milk and growth variables was investigated by using univariable and multivariable linear

regression models. The assumption of normality was checked and found satisfactory. Confounders and other variables with a possible relationship to growth were accounted for in the linear regressions: gestational age, birth weight z-score, accumulated steroid intake (mg/kg) and maternal preeclampsia. The total energy (kcal/kg/d) and protein (g/kg/d) intakes were identified as mediators for the main analysis and hence were not adjusted for. Their relation to growth variables was instead separately analysed in a similar way. The association between intake of maternal or donor milk and neonatal morbidities was investigated using univariable and multivariable logistic regression models. For all analyses, the tests were two-tailed and statistical significance was set at $P < .05$.

3 | RESULTS

The described results refer to 28 days of life and to 32 and 36 weeks of postmenstrual age. Neonatal characteristics according to proportional intake of maternal milk (<80% vs ≥80%) during days 2-28 are presented in Table 1.

3.1 | Amount of maternal or donor milk intake, total nutritional intake and growth

The relationships between amount of maternal milk intake (mL/kg/d) and z-scores for weight, length and head circumference are presented

in Table 2. The intake of maternal milk from day 2 to day 28 correlated positively with z-scores for weight and head circumference at postnatal age 28 days as well as with z-scores for weight, length and head circumference at 32 and 36 weeks (weight z-scores: $r_s = 0.32$, $P = .005$ at 32 weeks and $r_s = 0.32$, $P = .006$ at 36 weeks). We also found positive correlations between maternal milk intake and subsequent weight growth, during days 2-14 (weight z-score at 32 weeks: $r_s = 0.23$, $P = .041$) and days 15-28 (weight z-score at 32 weeks: $r_s = 0.30$, $P = .008$). The strongest correlations were present during days 15-28. The relationship between maternal milk intake and weight z-score at 28 days and at 32 and 36 weeks remained significant after adjustment for confounding factors in linear regression models (Table 3). For every 10 mL/kg/d increase in unpasteurised maternal milk intake, weight z-score would increase by 0.03 SD ($P = .034$) at 28 days and by 0.05 SD ($P = .018$) at 32 weeks and 0.06 SD ($P = .042$) at 36 weeks (Figure 1). Intake of donor milk (mL/kg/d) did not correlate with any postnatal growth outcomes.

The relationship between total energy and total protein intake vs weight z-score at 28 days and at 32 and 36 weeks remained significant after adjustment for confounding factors in separate linear regression models (Tables S1 and S2). Univariably, the amount of explained variation measured by R^2 ranged between 6% and 8% for maternal milk, 8% and 11% for total energy and 8% and 18% for total protein. In the multivariable models, birth weight z-score contributed most to the increase in R^2 . These results were similar for the models with maternal milk, total energy and total protein at the respective time points (Table 3; Tables S1 and S2).

TABLE 1 Neonatal characteristics of the study population ($n = 90$). Presented according to mean proportional intake of maternal milk <80% or ≥80% during postnatal days 2-28

	Maternal milk <80% ($n = 28$)	Maternal milk ≥ 80% ($n = 62$)	P value
Gestational age (wk)	25.3 (22.9-27.9)	25.3 (22.7-27.9)	0.656
Sex (female)	13 (46)	26 (42)	0.690
Birth weight (g)	688 (420-1255)	780 (415-1260)	0.097
Birth weight z-score	-0.9 (-6.9 to 0.6)	-0.3 (-5.2 to 1.3)	0.032
SGA	4 (14)	8 (13)	1.000
Maternal preeclampsia	4 (15)	6 (10)	0.484
APGAR < 7 at 5 min	17 (63)	32 (54)	0.448
Days to full enteral intake ^a	14 (8-63)	14 (8-40)	0.634
Steroid intake (mg/kg) ^b	1.7 (0-144.2)	1.7 (0-86.7)	0.644
Retinopathy of prematurity	18/22 ^c (82)	43/56 ^c (77)	0.766
Supplemental oxygen at 36 wk postmenstrual age	11/22 ^c (50)	28/56 ^c (50)	1.000
Early-onset sepsis ^d	2 (7)	4 (7)	1.000
Late-onset sepsis ^e	5 (18)	13 (21)	0.733
Deceased	6 (21)	6 (10)	0.180

Note: Values are presented as median (range) or numbers (%).

Abbreviation: SGA, small for gestational age.

^aDefined as first day of enteral intake of 150 mL/kg/d.

^bAccumulated intake of hydrocortisone (mg/kg) and betamethasone (mg/kg) during the first 28 postnatal days. Betamethasone was converted into hydrocortisone equivalents by the ratio 1:35.

^cSurviving infants.

^dEarly-onset sepsis was defined as sepsis occurring up to 7 d of age.

^eLate-onset sepsis was defined as sepsis occurring after 7 d of age until discharge or death.

	Maternal milk postnatal days 2-14		Maternal milk postnatal days 15-28		Maternal milk postnatal days 2-28	
	r	P value	r	P value	r	P value
Postnatal age 28 d						
Weight z-score (n = 78)	.20	.074	.31	.006	.32	.004
Length z-score (n = 39)	.34	.036	.25	.129	.29	.075
Head circumference z-score (n = 44)	.35	.019	.28	.065	.34	.025
PMA 32 wk						
Weight z-score (n = 77)	.23	.041	.30	.008	.32	.005
Length z-score (n = 76)	.25	.027	.22	.056	.23	.043
Head circumference z-score (n = 76)	.27	.017	.29	.012	.31	.007
PMA 36 wk						
Weight z-score (n = 72)	.22	.069	.31	.007	.32	.006
Length z-score (n = 70)	.23	.056	.34	.004	.31	.008
Head circumference z-score (n = 71)	.23	.057	.27	.023	.28	.020

TABLE 2 Relationships between mean maternal milk intake (mL/kg/d) during postnatal days 2-14, 15-28 and 2-28 and growth outcomes at postnatal age 28 d and postmenstrual age (PMA) 32 and 36 wk

3.2 | Proportional intake of maternal milk and growth

The distribution of the proportional intake of maternal milk among infants receiving less than 80% maternal milk during days 2-28 is shown in Figure 2. Figure 3A, B depicts longitudinal z-score growth for weight

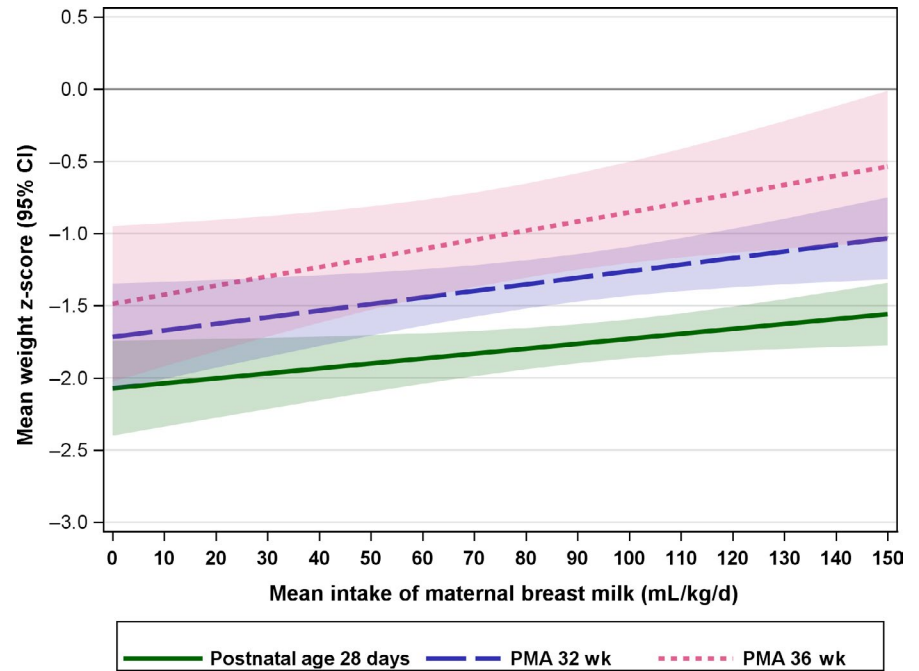
and head circumference according to proportional intake of maternal milk (<80% vs ≥80%) during days 2-28. Weight z-scores at 32 and 36 weeks differed significantly with respect to proportional intake of maternal milk, with infants receiving more than 80% maternal milk during the first 4 weeks of life having more favourable longitudinal weight z-scores. Statistical significance did not remain after adjustment for

TABLE 3 Contribution of mean intake of maternal milk (mL/kg/d) during postnatal days 2-28 to weight z-score at postnatal age 28 d and postmenstrual age (PMA) 32 and 36 wk in linear regression models

Variable	Univariable analysis			Multivariable analysis		
	Beta (95% CI)	P value	R ²	Beta (95% CI)	P value	R ² for the whole model
Postnatal age 28 d						
Maternal milk	0.007 (0.001-0.013)	.022	.067	0.003 (0.000-0.007)	.034	.797
Gestational age	0.081 (-0.107 to 0.270)	.394	.010	0.070 (-0.034 to 0.175)	.185	
Birth weight z-score	0.818 (0.713-0.923)	<.001	.759	0.853 (0.735-0.972)	<.001	
Accumulated steroid intake	-0.008 (-0.020 to 0.003)	0.136	0.029	0.000 (-0.006 to 0.006)	0.954	
Preeclampsia	-1.213 (-1.954 to -0.471)	0.002	0.124	0.357 (-0.086 to 0.800)	0.113	
PMA 32 wk						
Maternal milk	0.008 (0.002-0.014)	.015	.076	0.005 (0.001-0.008)	.018	.733
Gestational age	0.020 (-0.170 to 0.209)	.837	.001	-0.037 (-0.157 to 0.083)	.538	
Birth weight z-score	0.775 (0.658-0.893)	<.001	.698	0.763 (0.627-0.900)	<.001	
Accumulated steroid intake	-0.010 (-0.021 to 0.001)	.074	.043	-0.005 (-0.011 to 0.002)	.178	
Preeclampsia	-1.349 (-2.079 to -0.619)	<.001	.155	0.149 (-0.364 to 0.663)	.564	
PMA 36 wk						
Maternal milk	0.008 (0.000-0.015)	.038	.060	0.006 (0.000-0.012)	.042	.473
Gestational age	-0.012 (-0.236 to 0.212)	.917	.000	0.026 (-0.175 to 0.228)	.795	
Birth weight z-score	0.673 (0.484-0.861)	<.001	.420	0.684 (0.466-0.902)	<.001	
Accumulated steroid intake	0.000 (-0.013 to 0.013)	.960	.000	0.005 (-0.006 to 0.016)	.407	
Preeclampsia	-1.184 (-2.035 to -0.332)	.007	.100	0.123 (-0.690 to 0.935)	.764	

Abbreviation: CI, confidence interval.

FIGURE 1 Increase in mean weight z-score at postnatal age 28 d and postmenstrual age (PMA) 32 and 36 wk as an adjusted function of unpasteurised maternal milk intake during postnatal days 2-28



confounding factors in the linear regression models. The presence of extrauterine growth restriction, defined as a weight z-score below -2 SD at 36 weeks PMA, was higher among infants receiving less than 80% maternal milk during days 2-28: 36.4% vs 16% among infants receiving more than 80% maternal milk ($P = .070$).

3.3 | Proportional intake of maternal milk and nutrition

Total, enteral and parenteral energy and protein intakes according to proportion of maternal milk intake are presented in Table 4. Infants that received more than 80% unpasteurised maternal milk had significantly higher intakes of total energy and protein during days 2-28. In a subgroup analysis, this difference emerged only for days 2-14. Similarly, infants that received more than 80% unpasteurised maternal milk had significantly higher intakes of enteral energy and protein during days 2-14, whereas enteral energy and protein intake did not differ significantly during days 15-28 or 2-28. Parenteral intakes of energy and protein did not differ between the groups at any time period investigated.

3.4 | Breast milk and morbidities

The proportion of maternal milk intake was not associated with presence of ROP, bronchopulmonary dysplasia or early or late-onset sepsis (Table 1). Infants without ROP had a significantly higher mean intake of maternal milk (mL/kg/d) during days 15-28 than their counterparts who later developed ROP (148 vs 110 mL/kg/d, $P = .039$). There was also a trend towards this relationship for days 2-28 (105 vs 85 mL/kg/d, $P = .055$). After adjustment for either gestational age or birth weight z-score in logistic regression

models, these relationships no longer remained significant. Intake of maternal milk (mL/kg/d) was not associated with presence of bronchopulmonary dysplasia or sepsis. Amount of donor milk intake (mL/kg/d) was not associated with ROP, bronchopulmonary dysplasia or sepsis.

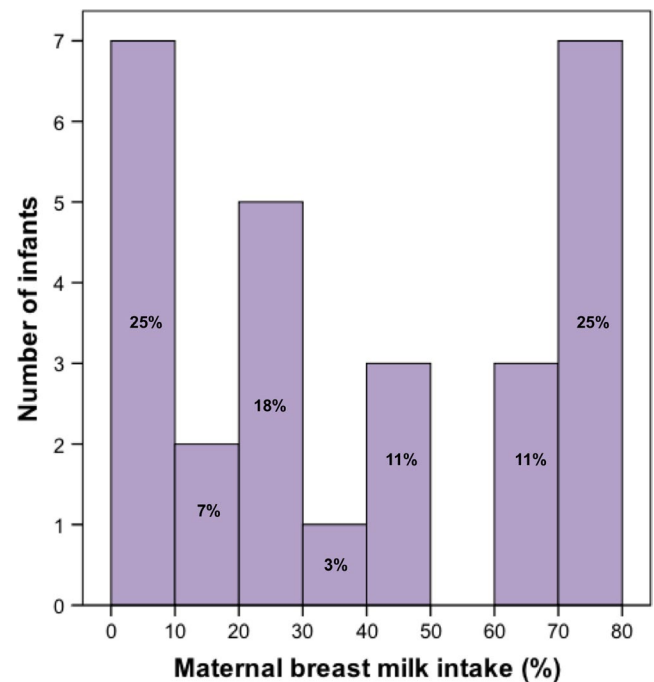


FIGURE 2 Distribution of proportional intake of maternal milk among infants receiving $<80\%$ maternal milk ($n = 28$) during postnatal days 2-28

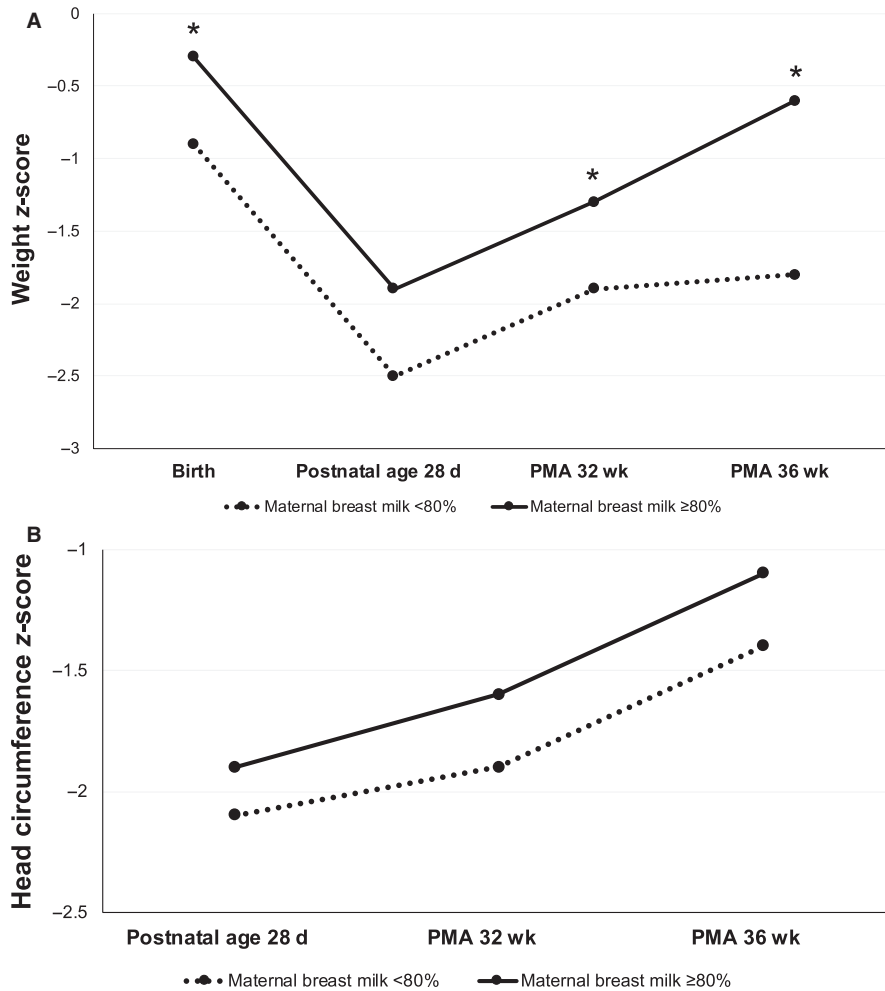


FIGURE 3 A, Weight z-score growth during hospital stay in the neonatal unit according to proportion of received maternal milk postnatal days 2-28.

*Indicates statistically significant difference between the groups ($P < .05$).

B, Head circumference z-score growth during hospital stay in the neonatal unit according to proportion of received maternal milk postnatal days 2-28

4 | DISCUSSION

Our main findings suggest that a higher intake of unpasteurised maternal milk during the first four postnatal weeks was associated with a more favourable weight growth. A higher intake of maternal milk was also univariably associated with less ROP. In contrast, pasteurised donor milk showed no association with any longitudinal growth outcomes or morbidities.

Human breast milk has a unique composition. It does not only supply infants with energy and essential nutrients, but also contains other important elements for infant development and well-being. Immunoprotective components, growth factors and hormones present in human breast milk promote infant growth. Moreover, these bioactive components may offer protection against morbidities such as sepsis, bronchopulmonary dysplasia and ROP.²⁰

In Sweden, donor milk is routinely pasteurised with the purpose of inactivating infectious agents. However, pasteurisation reduces the content and/or activity of various growth factors and immunoprotective components. Furthermore, pasteurisation decreases the antioxidant capacity of the breast milk, inactivates cellular elements and destroys almost all lipase present in the breast milk.²¹

Unpasteurised preterm maternal milk has a significantly higher content of macronutrients and energy compared with pasteurised donor milk.^{17,22} This may trace to the fact that most donor milk originate from mothers giving birth at term. In addition, the milk is usually donated after a period of breastfeeding. Compared with term human milk, preterm human milk contains more protein.¹⁷ However, as with term human milk, protein content in preterm human milk also decreases over time, although more slowly than in term human milk.²³

In our cohort, the mean intake of maternal milk (mL/kg/d) correlated positively with longitudinal weight, length and head circumference outcomes. We found the strongest correlations for maternal milk intake at postnatal days 15-28. After multivariable adjustment for confounding factors, these associations remained significant for weight z-scores at 28 postnatal days and at 32 and 36 weeks of postmenstrual age but not for length and head circumference z-scores.

Similar to intake of maternal milk, total energy (kcal/kg/d) and total protein (g/kg/d) intake displayed independent associations with growth after adjustment in multivariable models. Overall in these models, birth weight z-score contributed most to the explained variation. When analysed alone, maternal milk showed numerically somewhat lower explained variation compared with that

TABLE 4 Mean nutritional intake of the study population (n = 90) during postnatal days 2-14, 15-28 and 2-28 according to proportional intake of maternal milk

	Maternal milk postnatal days 2-14			Maternal milk postnatal days 15-28			Maternal milk postnatal days 2-28		
	<80% (n = 26)	≥80% (n = 64)	P value	<80% (n = 22)	≥80% (n = 62)	P value	<80% (n = 28)	≥80% (n = 62)	P value
Total energy (kcal/kg/d)	91 (8-120)	99 (59-123)	.002	128 (103-158)	132 (95-179)	.784	108 (8-140)	113 (59-149)	.023
Total protein (g/kg/d)	2.9 (0.3-3.9)	3.2 (1.8-4.5)	.002	4.2 (3.1-5.0)	4.1 (3.0-5.7)	.887	3.4 (0.3-4.3)	3.6 (1.8-5.0)	.020
Enteral energy (kcal/kg/d)	43 (0-108)	63 (7-108)	.026	122 (57-158)	115 (3-179)	.562	81 (0-134)	85 (13-136)	.150
Enteral protein (g/kg/d)	1.1 (0-3.4)	1.8 (0.2-3.6)	.019	3.7 (1.7-5.0)	3.7 (0.1-5.5)	.387	2.4 (0.0-4.1)	2.6 (0.3-4.5)	.197
Parenteral energy (kcal/kg/d)	38 (8-81)	38 (9-83)	.817	2 (0-47)	15 (0-96)	.136	28 (6-89)	27 (5-86)	.821
Parenteral protein (g/kg/d)	1.4 (0.3-3.0)	1.3 (0.3-3.0)	.950	0.1 (0-1.6)	0.5 (0-3.8)	.097	0.9 (0.2-3.4)	1.0 (0.1-3.3)	.676

Note: Values are presented as median (range).

of total energy and total protein. However, the full models for each of the three exposures provided a similarly large R^2 .

Infants that received at least 80% unpasteurised maternal milk had more favourable weight z-scores at 32 and 36 weeks. These results are in line with those of a prospective observational study by Montjau-Régis et al,⁶ who used a similar stratification for maternal milk intake. They found a positive association between proportion of maternal milk intake and weight gain.

In our study, the effect was most pronounced for maternal milk intake during days 15-28 (data not shown), a time point when the enteral intake of human breast milk had increased substantially. The difference in postnatal weight z-scores may be explained by the difference in birth weight z-scores: infants who later received at least 80% maternal milk also had significantly higher weight z-scores at birth. The presence of maternal preeclampsia did not differ between the groups and could thus not explain the difference in birth weight z-scores. However, the enteral intake of energy and protein did not differ significantly between the two groups during days 15-28. At this time, on group level, the infants were fully enterally fed. A probable explanation for this is the individualised targeted fortification of all breast milk that was practised within this cohort.

These findings of more beneficial growth outcomes for infants that received predominantly unpasteurised maternal milk suggest that other breast milk components, rather than energy and macronutrients alone, may affect infant growth. This is supported by other studies where infants fed unpasteurised as compared to pasteurised maternal milk had a faster weight gain (g/kg/d).^{13,24}

Few previous studies have efficiently assessed the effects of unpasteurised maternal milk compared with pasteurised donor milk on growth and other neonatal outcomes. Conducting a randomised controlled trial would be unethical given the proven benefits of maternal breast milk. A likely explanation for the scarcity of studies is that many neonatal units use preterm formula rather than donor milk as a supplement to maternal milk.²⁵ However, a previous randomised controlled trial provided evidence of more favourable weight growth outcomes for low-birth-weight infants that were fed unpasteurised preterm maternal milk. These infants had a faster growth velocity and regained their birth weight earlier than infants fed pasteurised pooled donor milk originating from mothers giving birth preterm. Of interest, the total energy content of the unpasteurised maternal milk was not significantly different from the pasteurised preterm donor milk.¹⁰ This finding further supports the possibility that not only the nutritional content, but also other factors present in unpasteurised human milk may be important for infant growth.

Some studies have found positive associations between unpasteurised maternal milk and longitudinal length and head circumference outcomes,^{7,8} whereas others have not.^{6,9,10} Schanler et al¹² found that infants fed predominantly maternal milk had significantly less length growth than infants fed donor milk or preterm formula. We found a greater incidence (36.4%) of extrauterine growth restriction among those infants that received less than 80% maternal milk. Similar to our findings, Colaizy et al²⁶ reported a greater presence of postnatal growth restriction in infants receiving predominantly

donor milk. Our study differed from the other studies in that our infants were extremely immature with a median gestational age of 25 weeks. In addition, we individually calculated nutritional intake and based the amount of breast milk fortification on prospective analysis of composition of maternal and donor milk. This approach ensured that all infants, whether they received maternal or donor milk, achieved similar nutrient intakes. In contrast, the other studies rather fortified maternal and donor milk interchangeably.

In our cohort, infants who did not develop ROP had a significantly higher mean intake of unpasteurised maternal milk during days 15–28. There was also a trend towards this relationship for days 2–28. However, after adjustment for either gestational age or birth weight z-score, the significance disappeared. Other studies have reported lower rates of any and severe ROP in infants that predominantly received maternal milk.^{8,12} Moreover, in our cohort total breast milk intake, that is maternal and donor milk combined, and total energy intake differed between infants with and without ROP. Total breast milk intake (mL/kg/d) and total energy intake (kcal/kg/d) were significantly higher for infants without any ROP during all time periods investigated (data not shown). Others have shown that human milk feeding is associated with a reduced odds ratio of ROP.²⁷ Furthermore, it has been reported that a low total energy intake during the first postnatal month is an independent risk factor for development of severe ROP.²⁸ In line with other studies,²⁹ those infants that did not develop ROP in our cohort exhibited a more favourable postnatal growth. They had greater z-scores at 28 days and at 32 and 36 weeks (data not shown).

A systematic review and meta-analysis published in 2018 found the evidence inconclusive for an effect of human milk pasteurisation on any and severe ROP.³⁰ However, the included studies did not necessarily compare unpasteurised maternal milk and pasteurised donor milk, some studies compared unpasteurised vs pasteurised maternal milk only.

The strengths of our study include the prospective detailed collection of breast milk and nutritional intakes. A limitation is that the stratification of infants into groups of maternal milk intake less than or more than 80% may not have been optimal. However, this approach allowed for an acceptable distribution of infants between the groups. Other weaknesses include the limited time period of complete nutritional and breast milk data. As a result, on group level, infants were supported by parenteral nutrition in addition to enteral breast milk intake during at least half of the analysed time period. Our rather small sample size could explain the lack of statistical significance for both growth outcomes and morbidities in the multivariable analysis. The study population was based on a randomised clinical trial, powered for a different outcome and potentially introducing a selection bias. Lastly, we cannot exclude that residual confounding may have occurred.

5 | CONCLUSION

In conclusion, we found that an increased intake of unpasteurised maternal milk was positively associated with longitudinal growth

outcomes from birth until 36 weeks postmenstrual age. Postnatal growth in extremely preterm infants is likely affected by a combination of various events where nutritional intake per se is an important contributor. We were not able to elucidate the causes of the growth differences derived from a predominant intake of either maternal milk or donor milk. These differences could perhaps be explained by the effects of pasteurisation, the handling of breast milk in the neonatal unit or differences in content of bioactive components in maternal and donor milk. Future research in this field should focus on specific components in unpasteurised breast milk important for postnatal growth.

In the meantime, attempts to motivate mothers to provide their infants with their own milk after preterm birth should be promoted.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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REFERENCES

- Ziegler EE. Meeting the nutritional needs of the low-birth-weight infant. *Ann Nutr Metab.* 2011;58(Suppl 1):8–18.
- Embleton NE, Pang N, Cooke RJ. Postnatal malnutrition and growth retardation: an inevitable consequence of current recommendations in preterm infants? *Pediatrics.* 2001;107(2):270–273.
- Farooqi A, Hagglof B, Sedin G, Gothefors L, Serenius F. Growth in 10- to 12-year-old children born at 23 to 25 weeks' gestation in the 1990s: a Swedish national prospective follow-up study. *Pediatrics.* 2006;118(5):e1452–e1465.
- Stoltz Sjöström E, Öhlund I, Ahlsson F, et al. Nutrient intakes independently affect growth in extremely preterm infants: results from a population-based study. *Acta Paediatr.* 2013;102(11):1067–1074.
- Henriksen C, Westerberg AC, Rønnestad A, et al. Growth and nutrient intake among very-low-birth-weight infants fed fortified human milk during hospitalisation. *Br J Nutr.* 2009;102(8):1179–1186.
- Montjoux-Regis N, Cristini C, Arnaud C, Glorieux I, Vanpee M, Casper C. Improved growth of preterm infants receiving mother's own raw milk compared with pasteurized donor milk. *Acta Paediatr.* 2011;100(12):1548–1554.
- Brownell EA, Matson AP, Smith KC, et al. Dose-response relationship between donor human milk, mother's own milk, preterm formula, and neonatal growth outcomes. *J Pediatr Gastroenterol Nutr.* 2018;67(1):90–96.
- Dritsakou K, Liosis G, Valsami G, Polychronopoulos E, Skouroliaou M. Improved outcomes of feeding low birth weight infants with predominantly raw human milk versus donor banked milk and formula. *J Matern Fetal Neonatal Med.* 2016;29(7):1131–1138.
- Madore LS, Bora S, Erdei C, Jumani T, Dengos AR, Sen S. Effects of donor breastmilk feeding on growth and early neurodevelopmental

- outcomes in preterm infants: an observational study. *Clin Ther*. 2017;39(6):1210-1220.
10. Stein H, Cohen D, Herman AAB, et al. Pooled pasteurized breast milk and untreated own mother's milk in the feeding of very low birth weight babies: a randomized controlled trial. *J Pediatr Gastroenterol Nutr*. 1986;5(2):242-247.
 11. Sun H, Han S, Cheng R, Hei M, Kakulas F, Lee SK. Testing the feasibility and safety of feeding preterm infants fresh mother's own milk in the NICU: a pilot study. *Sci Rep*. 2019;9(1):941.
 12. Schanler RJ, Lau C, Hurst NM, Smith EO. Randomized trial of donor human milk versus preterm formula as substitutes for mothers' own milk in the feeding of extremely premature infants. *Pediatrics*. 2005;116(2):400-406.
 13. Dicky O, Ehlinger V, Montjoux N, et al. Policy of feeding very preterm infants with their mother's own fresh expressed milk was associated with a reduced risk of bronchopulmonary dysplasia. *Acta Paediatr*. 2017;106(5):755-762.
 14. Ericson J, Flacking R, Hellstrom-Westas L, Eriksson M. Changes in the prevalence of breast feeding in preterm infants discharged from neonatal units: a register study over 10 years. *BMJ Open*. 2016;6(12):e012900.
 15. Najm S, Löfqvist C, Hellgren G, et al. Effects of a lipid emulsion containing fish oil on polyunsaturated fatty acid profiles, growth and morbidities in extremely premature infants: a randomized controlled trial. *Clin Nutr ESPEN*. 2017;20:17-23.
 16. Wackernagel D, Bruckner A, Ahlsson F. Computer-aided nutrition – effects on nutrition and growth in preterm infants <32 weeks of gestation. *Clin Nutr ESPEN*. 2015;10(6):e234-e241.
 17. Stoltz Sjostrom E, Ohlund I, Tornevi A, Domellof M. Intake and macronutrient content of human milk given to extremely preterm infants. *J Hum Lact*. 2014;30(4):442-449.
 18. Niklasson A, Albertsson-Wikland K. Continuous growth reference from 24th week of gestation to 24 months by gender. *BMC Pediatr*. 2008;8:8.
 19. International Committee for the Classification of Retinopathy of Prematurity. The International classification of retinopathy of prematurity revisited. *Arch Ophthalmol*. 2005;123(7):991-999.
 20. Hard AL, Nilsson AK, Lund AM, Hansen-Pupp I, Smith LEH, Hellstrom A. Review shows that donor milk does not promote the growth and development of preterm infants as well as maternal milk. *Acta Paediatr*. 2018; 108, pp. 998-1007.
 21. Arslanoglu S, Corpeleijn W, Moro G, et al. Donor human milk for preterm infants. *J Pediatr Gastroenterol Nutr*. 2013;57(4):535-542.
 22. de Halleux V, Pieltain C, Senterre T, Rigo J. Use of donor milk in the neonatal intensive care unit. *Semin Fetal Neonatal Med*. 2017;22(1):23-29.
 23. Dritsakou K, Liosis G, Valsami G, Polychronopoulos E, Skouroliakou M. The impact of maternal- and neonatal-associated factors on human milk's macronutrients and energy. *J Matern Fetal Neonatal Med*. 2017;30(11):1302-1308.
 24. de Halleux V, Pieltain C, Senterre T, et al. Growth benefits of own mother's milk in preterm infants fed daily individualized fortified human milk. *Nutrients*. 2019;11:4.
 25. Haiden N, Ziegler EE. Human milk banking. *Ann Nutr Metab*. 2016;69(Suppl 2):8-15.
 26. Colaizy TT, Carlson S, Saftlas AF, Morriss FH Jr. Growth in VLBW infants fed predominantly fortified maternal and donor human milk diets: a retrospective cohort study. *BMC Pediatr*. 2012;12:124.
 27. Ann HM, Strobino DM, Pezzullo JC, Ramasubbareddy D. Association of human milk feedings with a reduction in retinopathy of prematurity among very low birthweight infants. *J Perinatol*. 2001;21:356-362.
 28. Stoltz Sjostrom E, Lundgren P, Ohlund I, Holmstrom G, Hellstrom A, Domellof M. Low energy intake during the first 4 weeks of life increases the risk for severe retinopathy of prematurity in extremely preterm infants. *Arch Dis Child Fetal Neonatal Ed*. 2016;101(2):F108-F113.
 29. Hellström A, Ley D, Hansen-Pupp I, et al. New insights into the development of retinopathy of prematurity—importance of early weight gain. *Acta Paediatr*. 2010;99(4):502-508.
 30. Miller J, Tonkin E, Damarell R, et al. A systematic review and meta-analysis of human milk feeding and morbidity in very low birth weight infants. *Nutrients*. 2018;10:6.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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