Docosahexaenoic Acid and Amino Acid Contents in Pasteurized Donor Milk are Low for Preterm Infants

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Objective To evaluate whether pasteurized donor human milk meets the nutritional needs of preterm infants in terms of free fatty acid and amino acid contents.

Study design Milk samples were prospectively collected from 39 donors to the Mothers' Milk Bank of Ohio. The fatty acid and amino acid compositions in donor milk samples were measured before and after pasteurization, and values were compared with previously published findings and preterm infant nutrition guidelines. The nutritional adequacy of donor milk for preterm infants was based on estimated daily intake of 150 mL/kg. Statistical significance was adjusted to account for multiple comparisons.

Results Pasteurization did not appreciably affect donor milk composition. Docosahexaenoic acid level (0.1 mol wt %), and concentrations of glycine, aspartate, valine, phenylalanine, proline, lysine, arginine, serine, and histidine in donor milk were all significantly lower than previously reported concentrations in milk.

Conclusions Donor milk is not substantially affected by pasteurization, but has low concentrations of docosahexaenoic acid and amino acids. Targeted nutritional supplementation of human donor milk for feeding preterm infants might be warranted. *(J Pediatr 2010;157:906-10)*.

Despite aggressive postnatal nutritional therapy, preterm infants are deprived of nutritional support that otherwise would be provided by maternal sources during the last trimester of pregnancy.[1] Consequently, supplying adequate nutrition for appropriate growth is challenging.[2]

Fatty acids are essential components of membrane phospholipids and are important for the formation of lipid-signaling molecules, neurodevelopment, steroid synthesis, and innate immunity.[3-5] Free amino acids, required for protein synthesis, constitute 3% of the total nitrogen in human milk and are more abundant in colostrum than in transitional or mature milk.[6] Human milk provides an immediate source of both fatty acids and amino acids necessary for many physiological functions vital to the neonate.[7]

Preterm infants fed human milk, either fresh[8] or pasteurized donor milk,[9,10] have a reduced risk of developing necrotizing enterocolitis (NEC). Thus, human milk is the preferred nutritional source for preterm infants.[9] When mother's milk is not available, pasteurized donor milk is considered a viable alternative.[11,12] Despite the slower weight gain observed in preterm infants fed donor milk,[12,13] donor milk remains the preferred choice for reducing the likelihood of NEC. Data are sparse regarding the nutritional effects of pasteurization on fatty acid and amino acid content of donor milk. Given the current trend toward an increased use of donor milk in preterm infants, we investigated the suitability of donor milk composition to meet the nutritional needs of the preterm population. We examined the effects of pasteurization on the fatty acid and amino acid composition of donor human milk to test the hypothesis that pasteurization affects nutritional content. In addition, we compared the levels of fatty acids and amino acids in pooled donor milk from our midwestern population with previously reported levels in human milk.

Methods

The study was conducted at the Mother's Milk Bank of Ohio (Grant Medical Center, Columbus, Ohio) and the Center for Perinatal Research at The Research Institute at Nationwide Children's Hospital, Columbus, Ohio, with Institutional Review Board approval (IRB 0600532). After informed consent was obtained, 39 donor samples were collected from women ranging in age from 22-44 years, at lactational stage of 0-10 months, and with infants of gestational age of 24 weeks to term. All samples were collected from the donors in accordance with Human

DHA	Docosahexaenoic acid
NEC	Necrotizing enterocolitis

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Milk Banking Association of North America guidelines.[14] The states of origin for human milk included Ohio, Indiana, Kentucky, Pennsylvania, Wisconsin, and West Virginia.

Pasteurization Process

All mothers were screened for health and dietary habits before enrollment. Blood samples from donors were tested for the transmittable diseases human immunodeficiency virus, syphilis, hepatitis B and C, and human T-lymphotopic virus using standard techniques. Only mothers documented to be negative for these transmittable diseases were allowed to donate milk. Milk samples were thawed overnight in preparation for pasteurization (at 39°F or 4°C), creamotocrit levels were determined, and samples were pooled with the milk of 3 or 4 other mothers to achieve a minimum target of 20 calories/oz. The pooled milk was then placed in bottles designed for milk heating (Axifeed bottles, Richard Cassidy Limited, West Midlands, United Kingdom) and pasteurized by the Holder method at 62.5°C for 30 minutes using the ACE pasteurizer (ACE Intermed, Hants, United Kingdom). The milk was then cultured for bacteria and confirmed to be negative. The milk was stored in a -20°C freezer before being distributed to the institutions as needed.

Laboratory Analyses

Fatty acids were extracted from human milk samples collected both before and after pasteurization, and fatty acid methyl esters were measured by gas chromatography.[15] Free amino acids were analyzed using a Hitachi ion-exchange high-pressure liquid chromatography amino acid analyzer with lithium-based buffer chemistry (Hitachi High-Tech Trading Corp., Minato-ku, Japan). Aliquots of prepasteurization and postpasteurization milk samples were thawed, and whole proteins were precipitated with sulfosalicylic acid. After centrifugation, the samples were injected into a Hitachi amino acid analyzer for measurement of individual amino acids. At the Mothers' Milk Bank of Ohio, total protein content is measured using a MilkOScope instrument (Scope Electric, Razgrad, Bulgaria) calibrated using the traditional Kjeldahl process.[16] Calories are determined by creamatorcit of the pooled sample.[17]

Statistical Analysis

The effects of pasteurization were analyzed through a paired *t* test of matched samples collected before and after pasteurization. Donor milk composition was compared with previously published values using two-sample *t* tests with unequal variances. Adjustments for multiple comparisons were done using the Bonferroni correction. A *P* value $\leq$.0027 was considered significant.

Results

The median lactational stage of the donors was 3 months, and the median age was 27 years. The mean caloric content for pooled milk samples was 22 calories, and total protein average was 0.9 g/dL (data from the Mother's Milk Bank of Ohio). The prepasteurization and postpasteurization fatty acid levels of the pooled milk were not significantly different when expressed as mol wt % (Figure 1). Absolute fatty acid concentrations tended to be higher in the prepasteurization samples compared with the postpasteurization samples (linoleic acid, 125 ± 17 vs 118 ± 18 nmol/mL; linolenic acid, 8.8 ± 1.4 vs 8.3 ± 1.2 nmol/mL; arachidonic acid, 2.8 ± 0.4 vs 2.7 ± 0.4 nmol/mL; *P* <.027). However, no statistically significant differences were observed in docosahexaenoic acid (DHA) concentrations before and after pasteurization (0.7 ± 0.2 vs 0.7 ± 0.2 nmol/mL). Despite pooling, donor milk levels of DHA (0.1 mol wt %) were noticeably lower than mean levels reported by Jensen[18] for both Western (0.2 mol wt %) and non-Western diets (0.4 mol wt %), as well as the mean level recently reported by Brenna et al[19] for the United States (0.32 mol wt %). Furthermore using the expected fetal accretion levels as the most logical approach to feeding preterm infants,[20,21] donor milk provides substantially less DHA to the infants than they would have potentially received in utero[22] (Figure 2).

Free amino acid levels were measured in each pooled sample before and after pasteurization (Figure 3). Minor differences were found in arginine and leucine, which were higher in the postpasteurization samples (*P* <.0001), and aspartate, which was lower in the postpasteurization samples (*P* <.0027) (Figure 3). In addition, free amino acid levels from donor milk in the current study were compared with previously reported levels in human milk at the end of the first month of lactation.[23] Donor milk samples had significantly lower levels of glycine (*P* = .001), aspartate (*P* = .0005), histidine (*P* = .0008), valine, phenylalanine, proline, lysine, arginine, and serine (*P* <.0001), and higher

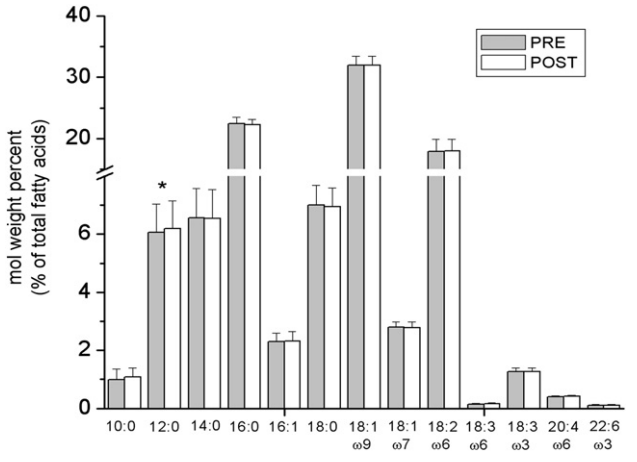

Figure 1. Effects of pasteurization on fatty acid content in pooled human donor milk. Fatty acid content was measured in donor breast milk samples before and after pasteurization, as described in Methods. Only one fatty acid was found to be different when expressed as mol wt %. Data were analyzed using the paired *t* test. *P* <.0027; n = 16.

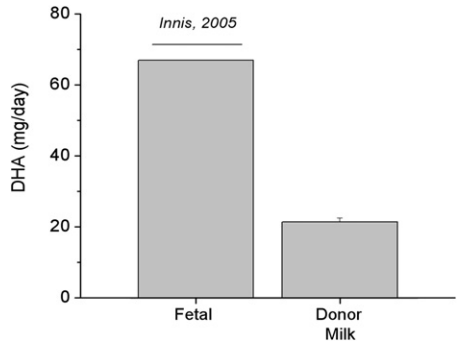

Figure 2. DHA in human milk compared with fetal accretion levels. Total DHA consumption was calculated by the methods described by Innis[1] using 150 mL/day as the amount of a full enteral feed. The total DHA levels in donor human milk were substantially lower than fetal accretion levels.

levels of tyrosine (P <.0001) compared with values reported by Agostoni et al[23] (Table).

Discussion

Human donor milk is routinely pooled to minimize variability and to decrease the influence of individual variation and stage of lactation.[14] Slow growth rates in preterm infants fed human donor milk have been documented,[24] but differences in the milk composition that might contribute to this growth failure have not been fully investigated. The total fat calories are adequate for feeding preterm infants when fortified with commercial fortifiers.[20-24] The total protein content of 0.9 g/dL may be a limitation, however, if a preterm infant is fed 150 mL/kg. With currently available fortifiers, the maximal protein intake would be 2.8 g/kg, below the recommended 3-4 g/kg/day.[25] Most convincingly, there is compelling evidence indicating improved preterm infant growth in parallel with increased protein nutrition.[26-30]

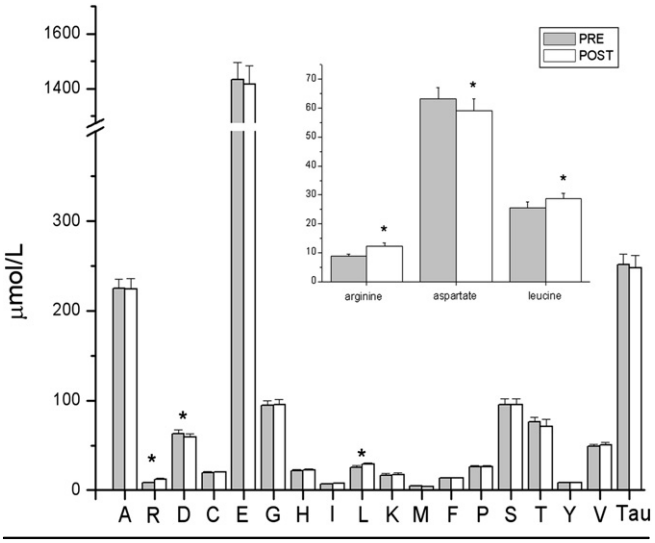

Figure 3. Effects of pasteurization on amino acid levels in pooled human donor milk. Amino acid content was measured in pooled donor breast milk samples before and after pasteurization, as described in Methods. Differences were observed in arginine, aspartate, and leucine, as shown in the inset. P <.0027; n = 16.

Table. Comparison of amino acid contents in pooled human donor milk and human milk at 1 month postpartum

	Donor milk (n = 16)		Healthy women (n =40)[19]		
Amino acid	Mean	SD	Mean	SD	*P* value
Glycine	95.5	23.18	124.6	39.6	.0014
Alanine	224.6	45.2	227.5	81.6	.8646
Valine	50.6	9.9	72.7	25.2	<.0001
Leucine	28.7	7.1	55.6	76.2	.0339
Isoleucine	7.4	2.4	33.4	71.1	.0262
Tyrosine	8.4	3.1	2.5	3.0	<.0001
Phenylalanine	13.5	2.5	23.6	9.1	<.0001
Aspartate	59.1	16.8	183.2	204.7	.0005
Glutamate	1415.4	268.6	1184.1	413.7	.0177
Proline	26.2	6.6	64.3	36.6	<.0001
Lysine	17.5	7.0	39.0	25.2	<.0001
Arginine	12.3	4.2	35.4	24.5	<.0001
Histidine	22.7	5.9	24.9	7.7	.0008
Methionine	4.4	1.6	8.8	11.5	.0221
Threonine	71.2	30.2	97.6	32.8	.0076
Taurine	248.1	54.7	301.1	116.4	.0248
Serine	95.4	27.7	273.7	138.1	<.0001

Data are reported as µmol/L. Amino acid content was measured in pooled human donor milk as described in Methods. The specific amino acid levels of breast milk collected at 1 month postpartum were compared with the levels measured in donor milk samples. Two-sample *t* tests for unequal variances with adjustment for multiple comparisons were performed; the critical α value was 0.0027.

Previous studies have evaluated the effect of Holder pasteurization (62.5°C for 30 minutes) on fatty acid content. Wardle et al[31] reported a 22% decrease in α-linolenic acid with a reasonably small sample size. Henderson et al[32] and Fidler et al[33] reported no significant decrease in fatty acid content due to pasteurization. In the present study, we sought to ascertain whether current clinical practices, including transportation of donor milk from depots to Milk Bank Processing Centers as well as courier handling and delivery, would affect the integrity of the fatty acids within the milk. We found no significant differences in total fatty acid levels, but we did find slightly lower concentrations of linoleic acid, linolenic acid, and arachadonic acid in the postpasteurization samples.

Compared with intrauterine accretion, human donor milk has sufficient fatty acid content to provide adequate intake for preterm infants, with the single exception of DHA.[3] Given the recent attention to omega-3 fatty acids and their importance in neurologic development and immunity, the relatively low levels of DHA in the donor milk samples that we analyzed were striking. Comparisons with the values reported by Jensen[18] revealed that the human donor milk had less than half the DHA content of milk obtained from women eating a Western diet and approximately one-sixth of the DHA content of milk obtained from women eating a non-Western diet

(ie, more fish). Likewise, our donor population had a lower concentration of DHA in milk compared with a previous study that documented a DHA content of 0.45 mol wt % in a longitudinal study of mother's milk at 1 week postnatal age,[34] as well as another study showing DHA concentrations of 0.25 mol wt % in term milk and 0.26 mol wt % in preterm milk samples.[35] The potential deficit in DHA consumption of our population is of concern, especially for preterm infants, whose neurologic and immune development is less complete compared with term infants. Using the fetal daily DHA accretion levels reported by Innis,[1] we observed that the donor milk levels in our study population were far below the fetal accretion levels (17 mg/day vs 67 mg/day). Jensen[1] also reported decreasing DHA levels with lactational stage. Consequently, donor milk obtained from women of various lactational stages (usually in the later stages) is more likely to lack sufficient quantities of DHA for preterm infants. In our donors, the low DHA levels likely can be explained by lactational stage, even though the median postnatal age was only 3 months. Polyunsaturated fatty acids levels decline with stage of lactation even as early as 1 month.[35] Donor milk is often fortified with commercial fortifiers, but DHA supplementation is not commonly provided. Preterm infants may need DHA supplementation to optimize overall outcome.

In line with our observations regarding fatty acids, we found that pasteurization had only a very minor effect on the free amino acid levels in donor breast milk. The differences were small, and the biological significance is unknown. Of concern however, are the substantial differences in amino acid levels in donor milk compared with published levels.[23,36] Chuang et al[36] reported higher free amino acid levels in preterm human milk compared with term human milk at all stages of lactation. The disproportionately high levels of free amino acids found in preterm human milk colostrum indicates a biological need for these specific nutrients early in postnatal life. The lactational stage of our donor population ranged from 1 to 10 months, but the pool was dominated by later lactational stage term milk.[6] However, many of the essential amino acids were lower in our donor milk compared with the mature term and preterm human milk levels published by Chuang.[36] Although the total protein content is relatively constant at the Mother's Milk Bank of Ohio at 0.9 g/dL (internal quality assurance; unpublished data), there is a relative deficiency of free amino acids.

Nutrient sources of free amino acids are a significant source of nonprotein nitrogen and can be readily used for new protein synthesis.[23] The relative importance of free amino acids in early postnatal nutrition is supported by the distinctive free amino acid profiles found in the milk of every mammalian species.[37,38] The free amino acid content in milk is known to correlate with plasma levels of amino acids and thus may provide an immediate source for metabolic processes such as gluconeogenesis (alanine), innate immunity with jejunal mucosal concentrations of IgG and IgA (threonine), lymphocyte development, nitric oxide production (arginine), Krebs cycle substrates (α-ketoglutarate), intestinal cell integrity (glutamate), and as precursor to glutathione and protection against oxidant stress (cysteine), all of which are vital to healthy physiology. A previous evaluation of amino acid stability during manipulation of human breast milk found decreased concentrations of lysine, which is an important biological indicator of the nutritional value of milk.[39] No such differences were observed in the present study, however, and the previously reported values might reflect differences in handling and the use of a commercial pasteurizer specifically designed for human milk.

Slower growth has been observed in preterm infants fed donor breast milk,[24] which has raised concerns about the sufficiency of donor milk to meet the needs of this special population. The deficiencies in many of the amino acids essential for protein assembly and growth might explain the failure to thrive observed in preterm infants.

In conclusion, we found only a minor effect of pasteurization on fatty acid and free amino acid composition, which is reassuring. The levels of DHA and many of the free amino acids were strikingly low, however. Further studies are needed from other North American milk banks to evaluate whether our findings reflect variations in laboratory analysis, maternal diet, region of the country, or stage of lactation. In addition, further examination of nitrogen balance, fat absorption, and body composition of preterm infants receiving donor milk are needed to validate our assumptions. Targeted supplementation might be necessary to improve growth in preterm infants while maintaining the important immunologic advantages of human milk nutrition. ■

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