ARTIGO ORIGINAL

Ultrasound Technology as a Method for Homogenizing Human Milk

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ABSTRACT

Background: The lipid content of human milk is its most variable component and provides from 35 to 50% of the daily energy needs of newborns. Losses occur during the freezing and thawing processes due to the coalescence of the fat globules and their adherence to bottle walls. **Objectives:** The objective was to test two methods of homogenizing pasteurized human milk in human milk banks in order to reduce the nutritional losses that occur between storage and feeding to newborns. **Methods:** Human milk samples collected in duplicate were homogenized either by sonication (MIRIS, Sweden) or vortex tube agitation. A total of 941 milk samples of different lactation stages from the human milk bank in Blumenau (SC, Brazil) were analyzed. A human milk analyzer (MIRIS, Sweden) was used to determine lipid content after homogenization. The statistical significance adopted in this study was $\alpha = 5\%$. **Results:** A mean of 1.87 grams of lipids per deciliter (g/dL) was observed in vortex-homogenized milk, whereas ultrasound homogenization yielded a mean of 2.07 g/dL, $p < 0.01$. The mean energy value of vortex-homogenized milk was 33.36 Kcal/dL, compared to 35.81 Kcal/dL for ultrasound-homogenized milk, $p < 0.01$. **Conclusion:** This study demonstrates that there is energy loss when human milk is not properly homogenized before being fed to newborns; better homogenization techniques decrease the adherence of fat globules to the bottle walls.

Keywords: Breastfeeding, ultrasound, energy value, fat, losses.

BACKGROUND

Human milk is considered the gold standard of infant feeding. The newborn's immune system is immature, and the anti-infectious properties of human milk protect the infant. In addition to immunoglobulin, this milk contains proteins such as lactoferrin, lysozyme and casein, lipids, oligosaccharides, enzymes, growth factors, hormones, prostaglandins, and cells that work in various ways to prevent infections and modulate the immune system.[1] These special properties of human milk develop protection against diseases such as acute otitis media, asthma, lower respiratory tract infections, sudden infant death syndrome, non-infectious and non-specified gastroenteritis and colitis, and necrotizing enterocolitis.[2] Human milk feeding is also associated with long-term reductions in the risk of obesity, types 1 and 2 diabetes and leukemias.[3]

It is recommended that during the first six months of life the child's nutrition consist exclusively of human milk, whose lipids account for 40% to 60%

of its total energy content. The lipid types in human milk, such as essential fatty acids, phospholipids, and cholesterol, play specific roles in healthy development, particularly regarding the nervous and digestive systems.[4] Vegetable or animal-based formulas cannot replicate the complexity of the protein-carbohydrate-fat composition or the functionality of other bioactive factors found in human milk.5 Human milk contains higher cholesterol concentrations than infant formula and, in this phase of life, a higher bioavailability of cholesterol has beneficial effects on the developing brain.[6, 7]

Human milk banks (HMB) keep milk frozen in order to guarantee microbiological safety, slow down the occurrence of undesired enzymatic and chemical reactions such as lipid oxidation and inhibit the multiplication and activity of microorganisms.[8] Freezing human milk leads to a series of physical alterations in its main components, such as rupturing the membranes of fat globules, which causes their coalescence and results in larger molecules. Studies about the effects of time spent at -20°C on macronutrients (fat, protein, and lactose) and energy content are scarce.[9, 10, 11, 12] Scientific evidence about nutritional quality losses during this procedure is needed.[12, 13]

The availability of fat in human milk is influenced by the duration of pregnancy, the number of postpartum months, the volume of milk produced, the time of breastfeeding, the mother's diet, and the mother's weight gain during pregnancy.[14, 15] The fat that reaches the newborn also varies due to factors related to neonatal intensive care units and human milk banks, where fat and energy content are lost through refrigeration, freezing, and thawing processes, as well as storage time and container transfers. Such handling not only causes the adhesion of fat to bottle walls, but it also separates this fat and causes its rearrangement into larger globules, thus compromising fat digestion and absorption.[12, 16, 17] Furthermore, feces analysis indicates that such lipids are not completely absorbed by infants. The amount of fat eliminated in feces may reach approximately 10% in full-term babies, and vary from 10 to 30% in preterm newborns, reaching even higher levels when the children are fed infant formula.[4, 18] Such processing losses can be reduced through the use of ultrasound homogenization.[13, 19]

Ultrasound divides the fat globules into smaller particles, stabilizing the suspension.16 Ultrasound contributes to greater fat digestibility and absorption, since it fragments the fat globules and increases the exposure area of lipids to digestive enzymes. Appropriate homogenization leads to less fat retention in hospital infusion systems or on the walls of milk storage containers in HMBs, which also leads to an acute increase of nutrient and calorie sources for newborns, resulting in weight gain and better development. Several studies have mentioned the ultrasound method as a way to boost milk homogenization and avoid fat loss. This treatment was demonstrated to be an efficient method for reducing the size of fat globules.[12, 13, 16, 19] For this reason, its application is being recommended for the homogenization of human milk, since it also causes an increase in protein-binding sites in globule membranes.

Thus, the objectives of this study were to determine whether ultrasound homogenization of pasteurized human milk significantly increases fat incorporation better than vortex homogenization at different lactation stages.

METHODS

This study had prior approval from the Research Ethics Committee of the Universidade Regional de Blumenau.

The preparatory research was based on keyword searches in internet databases such as Bireme and PubMed related to breastfeeding, human milk banks, human milk bank storage issues, human milk analysis, and ultrasound homogenization technologies.

Processed, non-homogenized human milk samples were collected in duplicate from the Blumenau HMB in sterilized test tubes. After collection, pasteurization, and freezing at the HMB, the samples were sent in an isothermal box to the Microbiology Laboratory at the Universidade Regional de Blumenau (Campus III) for analysis.

A total of 941 samples were evaluated between November 2012 and June 2013. These samples were classified according to lactation stage, yielding the following totals: 36 samples of colostrum, 58 of transition milk, 733 of mature milk and 144 unclassified. The mature milk came from multiple collection rounds at different times of the day.

For each pair of duplicate samples, one part was homogenized by receiving 1 second of ultrasound energy with a sonicator, while the other received 5 seconds of agitation in a tube agitator (Vortex AP 56 – PHOENIX). After homogenization, the amount of fat in the colostrum, transition milk, mature milk, and unclassified samples was measured with infrared spectroscopy in a human milk analyzer.

For the human milk analyzer to function properly, all samples were preheated in a double-boiler to 40°C (104°F) before homogenization. After homogenization, 1 mL aliquots of each sample were separated in sterilized disposable syringes and placed in the human milk analyzer to determine the lipid and calorie content.

The data were processed in Microsoft Excel and Epi Info 7. A one-factor analysis of variance (ANOVA – One way) was performed, followed by a Tukey test and Student's t-test, which was used to compare the mean fat and energy values between methods. The results were presented descriptively as mean ± standard deviation. The statistical significance adopted in this study was $\alpha = 5\%$ ($p < 0.05$).

RESULTS

Significant differences were found in fat and energy values between the two homogenization methods. Although the fat varied from 0.3 to 5.5 g/dL in the samples overall, the mean after vortex homogenization was 1.87 g/dL SD ± 0.69 g/dL, whereas after ultrasound homogenization the mean was 2.07 g/dL SD ± 0.7 g/dL, $p<0.01$. The mean energy value was also higher for the ultrasound-homogenized samples, 35.81 Kcal/dL SD ± 9.95 Kcal/dL, compared to 33.36 Kcal/dL SD ± 10.49 Kcal/dL in vortex-homogenized milk, $p<0.01$. These data are presented in Tables 1, 2 and 3.

The homogenization results were also analyzed according to the lactation stages. Significant differences were found between the 827 samples classified as colostrum, transition milk or mature milk (Tukey test). Significantly higher values in fat percentage were found in transition milk samples (vortex: 2.49 g/dL SD ± 0.77 g/dL vs. ultrasound: 2.65 g/dL SD ± 0.67 g/dL) than in colostrum and mature milk samples. There were no significant differences between the latter two categories, as shown in Tables 4 and 5.

A significantly higher energy value in vortex-homogenized mature milk (32.46 Kcal/100 ml SD ± 10.12 Kcal/100 ml) was observed compared to vortex-homogenized colostrum (40.22 Kcal/100 ml SD ± 10.27 Kcal/100 ml) and transition milk (41.34 Kcal/100 ml SD ± 12.65 Kcal/100 ml), which is shown in Table 6.

There was also a significant difference between the energy value of ultrasound-homogenized mature milk and ultrasound-homogenized colostrum and transition milk. The mature milk mean was 35.08 Kcal/100 ml SD ± 9.8 Kcal/100 ml, whereas the colostrum was 40.03 Kcal/100 ml SD ± 9.89 Kcal/100 ml, and the transition milk was 43.02 Kcal/100 ml SD ± 10.34 Kcal/100 ml. There were no significant differences in energy value between ultrasound-homogenized colostrum and transition milk (Tukey test), which can be seen in Table 7.

DISCUSSION

The fat present in human milk is the main energy source for an infant's development. Its lipids provide essential liposoluble vitamins (A, D, E and K), polyunsaturated fatty acids (omega-6 and omega-3) and modulate both the flavor and sensation of food in the mouth and gastrointestinal physiology and motility.20

When the mother is not with the baby or when the baby is unable to breastfeed, human milk collection is encouraged. However, the exclusive use of human milk in the case of very low birthweight babies has been associated with inappropriate weight gain and nutritional deficit during hospitalization.[21] Several factors may be connected with this phenomenon,

mainly due to the great variability of lipid content and the energy losses that occur during milk collection, storage, and feeding.[2, 21] This corroborates the results of the present study, which demonstrate better fat incorporation through more efficient homogenization.

Human milk stored for a long time in an HMB and thawed for hospital use tends to degenerate, resulting in fat loss.

The most variable component of human milk is fat, which fluctuates from 3 to 4 g/dL, and it is the main source of energy for the nursing infant, providing from 35 to 50% of the daily energy needs.[6] It is secreted in the form of fat globules from the alveolar epithelial cells of the mammary glands of the breastfeeding mother. In humans, these globules have a typical diameter of three to nine micrometers, and consist of a hydrophobic nucleus that contains mainly triglycerides wrapped in a triple layer of amphipathic compounds such as phospholipids, proteins, including enzymes, and cholesterol.[4]

The process of collection, storage, freezing, thawing, pasteurization, refreezing and re-thawing to which the human milk is submitted before it is fed to newborns causes significant energy losses, particularly in the fat content. The nucleus of a fat globule resulting from lactogenesis is rich in triglycerides surrounded by a fat membrane, which is a source of phospholipids and cholesterol for the infant. This structure prevents the fat globules from undergoing coalescence and reorganizing themselves into larger globules during secretion.[20] The freezing and thawing process ruptures the fat globule membranes, facilitating their coalescence and adherence to the walls of storage containers and the accessories used to feed the newborn.[22, 23, 24] Losses of up to 34% of the fat content may occur during gavage feeding due to the positioning of infusion syringes and the non-homogenization of the milk.[24, 25]

In this study, identical samples of milk that had been stored and frozen in an HMB were then homogenized by two different methods; the results of which demonstrated that the ultrasound method caused an increase of fat incorporation and, consequently, reduced energy loss. Literature suggests that ultrasound helps incorporate the fat into the milk, stabilizing the solution and breaking the globules into smaller molecules, which also facilitates the binding of digestive proteins in the gastrointestinal tracts of infants.

Mature milk had lower mean fat and energy values in both the ultrasound method (2.03 g/dL of fat and 35.08 Kcal/dL) and the vortex method (1.82 g/dL of fat and 32.46 Kcal/dL), which is explained by its composition and production time during breastfeeding. It begins to be produced two weeks after the baby is born and is less concentrated than transition milk, presenting less nutritional density and maintaining itself in this state for the remainder of the breastfeeding period.[26]

Colostrum, which is produced during the first 48 hours after a full-term baby is delivered, is a fluid rich in immunoglobulins and immune system cells.[26] After vortex homogenization, its mean fat was 2.2 g/dL and mean energy value was 40.22 Kcal/dL; in the ultrasound method, the mean fat was 2.24 g/dL and mean energy value was 40.03 Kcal/dL.

Transition milk lasts from the third to the fourteenth day after full-term delivery, and is rich in fat, lactose, and vitamins; however, like colostrum, its duration may be altered in case of prematurity.[26] The mean fat content for this type was 2.49 g/dL in vortex method and 2.65 g/dL in the ultrasound method; the mean energy values were 41.34 Kcal/dL in the vortex method and 43.02 Kcal/dL in the ultrasound method. There were improvements of 10.69% and 7.34% in mean fat incorporation and mean caloric value, respectively, in samples homogenized with ultrasound. There were statistically significant differences between the lactation stages (Tables 4, 5, 6 and 7). Ultrasound homogenization resulted in mean fat increases of 1.81% in colostrum, 6.42% in transition milk, and 11.53% in mature milk. Improvement in caloric value was lower across the milk types because this value depends on protein and carbohydrate content as well as lipid content. The mean energy value of colostrum samples was the only result higher in vortex homogenization (0.47%). In transition milk and mature milk, the energy values were 4.06% and 8.07% higher, respectively, in ultrasound than in vortex homogenization.

CONCLUSION

Human milk homogenized with the ultrasound method had higher means than vortex-homogenized milk in virtually all evaluated criteria. There were higher fat incorporation and increased energy value for the samples homogenized by sonication, which underscores the fact that nutrients are lost on bottle walls when homogenization is improperly performed. For newborns, even a slight improvement in feeding may have a great impact on health and development. Thus, investing in ultrasound equipment for homogenizing human milk after it has been frozen and re-thawed may be an important step toward improving postnatal health care.

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CONFLICT OF INTEREST

There are no conflicts of interest.

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Table 1 - Fat means in g/dL and energy value means in Kcal/dL after homogenization using both methods.

Variable	Vortex			Ultrasound			T-test
	n	Amplitude	Mean ± SD	n	Amplitude	Mean ± SD	
Fat	941	(0.3 - 5.5)	(1.87 ± 0.69)	941	(0.4 - 5.5)	(2.07 ± 0.7)	P < 0.01
Energy	941	(6 - 66)	(33.36 ± 10.49)	941	(7 - 74)	(35.81 ± 9.95)	P < 0.01

Table 2 - Mean amount of fat in g/dL after homogenization and 95% confidence intervals.

Variables	n	Amplitude	Mean ± SD	CI (95%)
Fat – Vortex	941	(0.3 - 5.5)	(1.87 ± 0.69)	(1.83 - 1.92)
Fat – Ultrasound	941	(0.4 - 5.5)	(2.07 ± 0.7)	(2.03 - 2.12)

Table 3 - Mean of energy values in Kcal/dL and 95% confidence intervals.

Variables	n	Amplitude	Mean ± SD	CI (95%)
Energy value – Vortex	941	(6 - 66)	(33.36 ± 10.49)	(32.69 - 34.03)
Energy value – Ultrasound	941	(7 - 74)	(35.81 ± 9.95)	(35.17 - 36.44)

Table 4 - Mean fat in g/dL after vortex homogenization according to lactation stage.

Variable	Treatments	n	(Mean ± SD)	P
Vortex	Fat – Colostrum	36	(2.2 ± 0.57)a	<0.001
	Fat – Transition	58	(2.49 ± 0.77)b	
	Fat – Mature	733	(1.82 ± 0.67)a	

I – F-test (1-factor ANOVA).
II – Appended letters represent significant differences (Tukey test).

Table 5 - Mean fat in g/dL after ultrasonic homogenization according to lactation stage.

Variable	Treatments	n	(Mean ± SD)	P
Ultrasound	Fat – Colostrum	36	(2.24 ± 0.79)a	<0.001
	Fat – Transition	58	(2.65 ± 0.67)b	
	Fat – Mature	733	(2.03 ± 0.69)a	

I – F-test (1-factor ANOVA).
II – Appended letters represent significant differences (Tukey test).

Table 6 - Mean energy values after vortex homogenization according to lactation stage.

Variable	Treatments	n	(Mean ± SD)	P
Vortex	Energy value – Colostrum	36	(40.22 ± 10.27)a	<0.001
	Energy value – Transition	58	(41.34 ± 12.65)a	
	Energy value – Mature	733	(32.46 ± 10.12)b	

I – F-test (1-factor ANOVA).
II – Appended letters represent significant differences (Tukey test).

Table 7 - Mean energy values after ultrasound homogenization according to lactation stage.

Variable	Treatments	n	(Mean ± SD)	P
Ultrasound	Energy value – Colostrum	36	(40.03 ± 9.89)a	<0.001
	Energy value – Transition	58	(43.02 ± 10.34)a	
	Energy value – Mature	733	(35.08 ± 9.8)b	

I – F-test (1-factor ANOVA).
II – Appended letters represent significant differences (Tukey test).