Original articles

Osmolality analysis of human milk and an infant formula with modified viscosity for use in infants with dysphagia

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ABSTRACT

Purpose: to evaluate the effects of a thickening agent on the osmolality of human milk and on an infant formula, with respect to concentration and time.

Methods: six trials were performed to evaluate the osmolality of a natural and thickened infant formula, raw human milk, and pasteurized human milk. Rice cereal was used as a thickening agent (at concentrations of 2%, 3%, 5%, and 7%). Osmolality was measured using the *Advanced Micro Osmometer Model 3300* after sample preparation periods of 0–60 minutes. Statistical evaluations were performed using ANOVA.

Results: pasteurized human milk exhibited time- and concentration-dependent variation in osmolality. The osmolality of raw human milk differed among time points and between the samples with 5% and 7%, when compared to the non-thickened milk. The infant formula did not show differences in osmolality with respect to time. At time zero, there were differences in osmolality between the infant formula samples with 2%, 3%, and 5% thickener. At other time points, there were differences in osmolality between the sample with a 5% thickener and the non-thickened formula.

Conclusion: the osmolality of diets varied over time and according to the concentration of thickener in human milk and the infant formula. However, the observed variation remained within the recommended parameters, indicating that rice cereal is a safe thickener for the feeding of infants presented with mild or moderate oropharyngeal dysphagia.

Keywords: Milk, Human; Deglutition Disorders; Thickeners; Osmolar Concentration; Viscosity

INTRODUCTION

Dysphagia is a clinical condition affecting many newborns and infants with neurological, cardiorespiratory, digestive,and congenital anomalies, as well as premature infants. It is characterized by difficulty coordinating between sucking, breathing, and swallowing. It is not considered a disease, but a symptom or manifestation of a variety of congenital or structural abnormalities and/or clinical conditions or dysfunctions of the centers responsible for the neurophysiological control of the related structures, i.e., the oral cavity, pharynx, larynx, and esophagus[1-3].

Assistance in dysphagia requires an individualized approach according to the clinical conditions. Speech-language therapy intervention involves oral sensory motor stimulation, postural organization during feeding, oral support and suction rhythm techniques, handling and management of breastfeeding, adaptation of feeding utensils, and, when necessary, changes in milk consistency[4-6].

Human milk is species-specific and product-specific and is the first choice for feeding infants. It provides a unique combination of proteins, lipids, carbohydrates, minerals, vitamins, enzymes, and living cells and has firmly established nutritional, immunological, psychological, and economic benefits[7,8]. When an infant is not able to maintain clinical stability or is unable to swallow safely during feeding, after the exhaustion of oral stimulation techniques, the consistency of milk must be adjusted[5, 9-14].

Modified diets, including the use of thickening agents, should be monitored with respect to viscosity and osmolality when provided to infants with dysphagia. In particular, it is necessary to examine modified diets for potential hyperosmolality. Hyperosmolar formulas can cause abdominal discomfort, delayed gastric emptying, cramps, diarrhea, and dehydration, and can promote the development of necrotizing enterocolitis and damage to the kidneys and the brain in infants[15].

Osmolality is a measure of the quantity of osmotically active particles in a solution, expressed as the number of milliosmoles of solute per kilogram of solvent (mOsm/kg)[16]. The osmolality of human milk varies from 277 to 303 mOsm/L[15], and the recommended osmolality of infant dairy food is less than 450 mOsm/kg (or 400 mOsm/L)[17], although these parameters are currently debated in the scientific literature[18].

Studies of the osmolality of human milk have typically focused in its fortification and nutritional aspects. However, little is known about the osmolality of diets that are thickened for infants with the goal of improving the coordination of suction, breathing, and swallowing. Thus, the objective of this study was to evaluate the osmolality of human milk and infant formula modified using rice cereal at various concentrations (2%, 3%, 5%, and 7%) at 37°C over a period of 60 minutes.

METHODS

This experimental study was performed at the Quality Control Laboratory of the Human Milk Bank (BLH–IFF), with the approval of the Committee of Ethics in Human Research of IFF/Fiocruz under CAAE (14607113.4.0000.5269). All of the human milk samples were obtained from donors enrolled in the BLH-IFF from December 2013 to November 2017 in according with the provisions of the legislation that regulates the implantation and operation of human milk banks[19]. Samples of human milk selected for the study were stored at -20°C with an acidity of between 1 and 4ºD and 500–650 kcal/L.

Samples ofraw human milk andpasteurized human milk were used.During hospitalization in the neonatal intensive care unit, pasteurized human milk from the BLH-IFF is thickened and, after hospital discharge, if there is still indication, the mother may continue to thicken raw human milk at home.

Pasteurization is a thermal treatment applied to raw human milk aimed at inactivating 100% of pathogenic microorganisms as well as saprophytic bacteria or the normal microbiota. The process is performed at 62.5°C after preheating, and the duration of pasteurization depends on the volume of milk, as recommended by Board of director's resolution (BDR) 171[19].

The samples of human milk were thawed and maintained in a water bath at 37 ± 2°C. The infant formula was prepared according to the manufacturer's recommendations to obtain a volume of 30mL and was maintained in a water bath at 37 ± 2°C. The analysis of each concentration was performed separately.

The thickening agent was rice cereal mucilage, which does not contain traces of milk in its composition (Vitalon; WOW Nutrition, São Paulo, Brazil). For each concentration, the thickening agent was weighed using the Marte A500 digital scale and subsequently added to milk according to the manufacturer's recommendation. The samples were shaken manually during the preparation for 30 seconds to avoid excessive air entrainment and have better solubilization. A calibrated thermometer was used to verify thermal stability. Each

trial was performed in triplicate; 32 human milk samples of 30 mL were used,for a total of 128 measurements, and 12 infant formula samples of 30 mL were used, for a total of 48 measurements.

The osmolality of infant formula without thickening and with thickening of2%, 3%, and 5% thickening agent and raw and pasteurized human milk without thickening and with thickening of 2%, 3%, 5%, and 7% thickening agent were determined over a 60-minute period at intervals of 20 minutes at 37°C, comprising 6 studies. Osmolality was measured based on freezing point depression using the Advanced Micro Osmometer Model 3300 (Advanced Instruments, Inc., Norwood, MA, USA). The equipment was calibrated with quality control calibration standards (*Advanced Instruments Inc.*) after every 10 measurements.

Descriptive analyses were performed using means and standard deviation. Normality was verified by the *Kolmogorov–Smirnov* (KS) and *Shapiro–Wilk* tests. Analysis of variance (ANOVA) was used to verify significant differences in parameters with respect to the degree of thickening. A *post-hoc* Tukey test was used to evaluate pairwise differences. All analyses were implemented in SPSS 21, adopting a significant level of 0.05.

RESULTS

Without the addition of a thickener, the mean osmolality was similar for raw human milk (281 ±2.23mOsm/kg), pasteurized human milk (285 ± 1.28mOsm/kg), and an infant formula (287 ±15.43mOsm/kg) after 1 hour at 37± 2°C (Table 1).

Milk samples thickened with rice cereal exhibited a mean osmolality of 341 ±16.2mOsm/kg.

Table 1. Mean osmolality of various milk samples incubated at 37°C

Type of milk	Mean O (mOsm/kg)	Standard deviation	Minimum O (mOsm/kg)	Maximum O (mOsm/kg)
Raw human milk	281	±2.23	278	283
Pasteurized human milk	285	±1.28	283	286
Infant formula	287	±15.43	264	300
Raw human milk, 2% thickener	320	±9.87	306	328
Raw human milk, 3% thickener	325	±12.44	308	336
Raw human milk, 5% thickener	347	±23.16	313	362
Raw human milk, 7% thickener	353	±22.15	324	375
Pasteurized human milk, 2% thickener	325	±9.08	314	335
Pasteurized human milk, 3% thickener	309	±19.28	281	323
Pasteurized Human milk, 5% thickener	368	±26.39	332	391
Pasteurized human milk, 7% thickener	379	±34.97	332	408
Infant formula, 2% thickener	338	±6.88	329	346
Infant formula, 3% thickener	331	±10.02	320	343
Infant formula, 5% thickener	362	±3.79	357	365

O = osmolality (mOsm/kg)

In the infant formula trial, there were no differences in osmolality among time periods for all concentrations (p>0.05). Considering the concentrations, at time zero, we observed differences in osmolality between the formula without a thickening agent and those with various concentrations of the thickening agent (p<0.05). At all other time points, there was a difference between formula prepared without a thickening agent and with 5% thickener (p = 0.009, 0.010, and 0.015, respectively, at 20, 40, and 60 minutes). There were no significant differences in osmolality among the other concentrations (2%, 3%, and 5%) (Table 2).

Table 2. Effect of time on the osmolality of an infant formula with and without thickener during incubation at 37°C

Time (minutes)	O (mOsm/kg) without thickener	O (mOsm/kg) at 2%	O (mOsm/kg) at 3%	O (mOsm/kg) at 5%
0	264.33 ± 19.35	329.33 ± 23.71	320.33 ± 26.50	356.67 ± 5.86
20	289.67 ± 22.19	337 ± 27.22	326.33 ± 20.60	364.67 ± 7.51
40	292 ± 22.72	339.33 ± 26.27	334.67 ± 16.50	364.67 ± 12.70
60	300 ± 23.52	346 ± 20.81	343.33 ± 18.34	361.33 ± 5.51
p-value	0.293	0.870	0.581	0.628

O = osmolality (mOsm/kg) Statistical analysis: Anova test
0% vs. 2%, p = 0.000   2% vs. 3%, p = 0.827
0% vs. 3%, p = 0.000   2% vs. 5%, p = 0.020
0% vs. 5%, p = 0.000   3% vs. 5%, p = 0.002

In the trial with pasteurized human milk, we observed that there was no variation among time points in the osmolality of unmodified milk samples (p > 0.05). For samples with 2% thickener, the osmolality differed between samples obtained at times 0 and 60 minutes (p <0.05), and there were no differences between other time points. For samples with 3% thickener, we detected differences between 0 and 20 minutes, 0 and 40 minutes, and 0 and 60 minutes (p < 0.001), but no differences between the other time points. The 5 and 7% concentrations showed the same behavior in relation to time, i.e., there were no differences only between 40 and 60 minutes (p =0.125 and 0.920, respectively), indicating stabilization.

Regarding the concentrations, we also observed a difference in unmodified pasteurized human milk among the 2%, 3%, 5% and 7% concentrations, except at times zero and 20 for the 3% concentration (p = 0.976). In samples with modified viscosity, there were differences between samples with 2% and 3%, 3% and 5%, and 3% and 7% thickener at time zero (p < 0.05). At the other times, there were no differences between 2% and 3% (p = 0.932, 0.873, and 0.547) and 5% and 7% (p = 0.940, 0.224, and 0.286), respectively, at 20, 40, and 60 minutes (Table 3).

Table 3. Effect of time on the osmolality of pasteurized human milk with and without a thickening agent, incubated at 37°C

Time (minutes)	O (mOsm/kg) without thickener	O (mOsm/kg) at 2%	O (mOsm/kg) at 3%	O (mOsm/kg) at 5%	O (mOsm/kg) at 7%
0	285.5 ± 17.75	314.33 ± 5.69	281 ± 5.20	331.67 ± 1.53	332 ± 6.08
20	283.25 ± 19.14	321.33 ± 4.62	313 ± 4.58	364.67 ± 4.62	372.67 ± 18.23
40	284.75 ± 16.92	328.67 ± 8.50	320.33 ± 7.57	382.67 ± 3.06	402.67 ± 8.39
60	286.25 ± 15.69	335.33 ± 9.07	322.67 ± 2.08	391.33 ± 6.03	408.33 ± 7.02
p-value	0.995	0.035	0.000	0.000	0.000

O = osmolality (mOsm/kg) Statistical analysis: Anova test
0% vs. 2%, p = 0.000   2% vs. 5%, p = 0.000
0% vs. 3%, p = 0.032   2% vs. 7%, p = 0.000
0% vs. 5%, p = 0.000   3% vs. 5%, p = 0.000
0% vs. 7%, p = 0.000   3% vs. 7%, p = 0.000
2% vs. 3%, p = 0.383   5% vs. 7%, p = 0.690

In an analysis of the osmolality of raw human milk with respect to time, we observed that milk without thickening and with thickening of 2% and 7% thickener did not vary over time (p>0.05). When we compared the samples with 3% and 5% thickener, we observed differences between 0 and 40 minutes (p = 0.023 and 0.029, respectively) and between 0 and 60 minutes (p = 0.010 and 0.030, respectively). Considering the concentrations, at time zero, we detected a difference between samples without thickening and with thickening of 7% thickener (p = 0.012). At the other time points, we observed a difference between samples with 5% and 7% thickener (p <0.05). Samples with other concentrations did not differ with respect to osmolality (p>0.05) (Table 4).

Table 4. Effect of time on the osmolality of raw human milk with and without the addition of a thickener, incubated at 37°C

Time (minutes)	O (mOsm/kg) without thickener	O (mOsm/kg) at at 2%	O (mOsm/kg) at 3%	O (mOsm/kg) at 5%	O (mOsm/kg) at 7%
0	279.25 ± 18.39	306.33 ± 0.58	308 ± 1.00	313.33 ± 11.24	323.67 ± 21.50
20	280 ± 15.47	320.67 ± 24.79	322.67 ± 12.50	351 ± 15.72	348.33 ± 25.11
40	282.25 ± 22.20	326 ± 19.47	332 ± 6.24	362.33 ± 23.29	363.33 ± 27.43
60	292.25 ± 29.96	328.33 ± 24.11	336 ± 7.00	362 ± 13.89	375 ± 25.24
p-value	0.829	0.556	0.010	0.021	0.146

O = osmolality (mOsm/kg)
0% vs. 2%, p = 0.000
0% vs. 3%, p = 0.000
0% vs. 5%, p = 0.000
0% vs. 7%, p = 0.000
2% vs. 3%, p = 0.989

Statistical analysis: Anova test
2% vs. 5%, p = 0.033
2% vs. 7%, p = 0.006
3% vs. 5%, p = 0.104
3% vs. 7%, p = 0.024
5% vs. 7%, p = 0.974

DISCUSSION

In this study, the impact of rice cereal, which is used as a thickener for infants with dysphagia, on the osmolality of human milk and milk formula was systematically evaluated with respect to concentration and time.The time variable was an important determinant of variation in osmolality among human milk samples. The infant formula showed increased stability over time. The variation in osmolality among samples with different concentrations of thickener was greater for pasteurized human milk than for raw human milk and an infant formula, which only exhibited differences between the thickened and non-thickened diet.

The pasteurization did not alter the osmolality of the milk samples analyzed in this study, consistent with the findings of Braga and Palhares[20] who also did not observe significant differences in this parameter. Thus, variations observed in human milk may be associated with the presence of the amylase enzyme. Amylase in human milk can cause the hydrolysis of starch and consequently can result in a concentration- and time-dependent increase in osmolality[21]. The differences observed among the infant formula samples were associated with the concentration of thickener, because the differences began to be noticed when the concentration of the thickener varied.

Srinivasan et al.[22] suggested that hyperosmolar diets are associated with necrotizing enterocolitis. An understanding of the factors influencing osmolality has important clinical implications, i.e., for understanding the diffusion of substances through cell membranes and the pressure exerted by solutes, i.e., to analyze body water balance[23].

Choi et al.[18] analyzed the effect of time on the osmolality of fortified breast milk and carbohydrate supplementation for a period of 24 hours at 4°C and verified that increased osmolality can be attributed to amylase enzyme activity.

However, despite substantial variation, the osmolality of human milk remained within the recommended values reported in the literature[15,17,18,21] for safe use, suggesting that rice cereal supplementation is a potential therapeutic feeding strategy for infants with mild or moderate oropharyngeal dysphagia. Human milk is a protective factor for infants with dysphagia because until approximately 6 months of age, the

amylase enzyme involved in the hydrolysis of the starch is lacking or present in small quantities; this enzyme is obtained via human milk which aids in the digestion of the starch[24].

The use of thickening agents should be widely discussed among the care team to assess their need after the exhaustion of therapeutic techniques for the stimulation of oral and feeding functions. Immediate use after the addition of the thickener, with feeding equipment that enables measurements, can minimize negative effects and preserve the consistency and osmolality.

Evaluations of osmolality and a previous study of viscosity[25] were performed by the National Institute of Women, Children, and Adolescents Health Fernandes Figueira (IFF), the maternal and child unit of Oswaldo Cruz Foundation (FIOCRUZ), which is certified as a "Baby Friendly Hospital" (BFH)[26], which encourages breastfeeding and also has a Human Milk Bank. It is a reference center of the Global Network of Human Milk Banks in Brazil (nHMB), operating in Latin America, Central America, the Iberian Peninsula, and Africa. It improves the services of a maternal-infant referral hospital, with full support for breastfeeding, regardless of the pathophysiological conditions of its patients, a factor that made this study feasible.

A major motivation for this work was the fact that the thickening agent standardized by IFF apparently has a viscosity and osmolality that do not change over time. Most previous studies, based on a literature search, have focused on human milk fortification and nutritional aspects, and not on analyses of the osmolality of thickened diets for infants with respect to time, temperature, and concentration, despite the important implications for the coordination of sucking, breathing, and swallowing. The results of this study demonstrated that human milk and a thickened infant formula can be considered safe from the point of view of osmolality.

Human milk is a species-specific product, and its composition varies throughout breastfeeding and depending on the maternal characteristics, gestational period, and bioactive compounds[27] capable of reacting with the thickening agent. These biological variations may interfere in the variability of the results. One way to minimize this variation is to increase the sample size. However, the complexity of controlling all variables that interfere in its characteristics would require an analysis of a huge amount of milk samples, with no guarantee of reproducibility of results.

CONCLUSION

The osmolality of the diets analyzed in this study varied according to the time and the concentrations for human milk and the infant formula. However, these variations remained within the recommended parameters in the literature. Thus, the diets are safe for the feeding of newborns and infants with mild or moderate oropharyngeal dysphagia.

Thickeners should be used with caution, with a focus on the minimal acceptable parameters, to ensure safety. Viscosity and osmolality are directly related, accordingly, the greater the viscosity, the greater the osmolality and the greater the adverse effects.

ACKNOWLEDGMENTS

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