J Pediatr (Rio J). 2014;**90(4)**:384−388

Jornal de
Pediatria
www.jped.com.br

SOCIEDADE BRASILEIRA DE PEDIATRIA

ORIGINAL ARTICLE

Freezing and thawing effects on fat, protein, and lactose levels of human natural milk administered by gavage and continuous infusion☆

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Received 12 July 2013; accepted 11 November 2013
Available online 29 March 2014

KEYWORDS
Human milk;
Nutrition;
Newborn

Abstract
Objectives: to analyze the changes in human milk macronutrients: fat, protein, and lactose in natural human milk (raw), frozen and thawed, after administration simulation by gavage and continuous infusion.
Method: an experimental study was performed with 34 human milk samples. The infrared spectrophotometry using the infrared analysis equipment MilkoScan Minor® (Foss, Denmark) equipment was used to analyze the macronutrients in human milk during the study phases. The analyses were performed in natural (raw) samples and after freezing and fast thawing following two steps: gavage and continuous infusion. The non-parametric Wilcoxon test for paired samples was used for the statistical analysis.
Results: the fat content was significantly reduced after administration by continuous infusion ($p < 0.001$) during administration of both raw and thawed samples. No changes in protein and lactose content were observed between the two forms of infusion. However, the thawing process significantly increased the levels of lactose and milk protein.
Conclusion: the route of administration by continuous infusion showed the greatest influence on fat loss among all the processes required for human milk administration.

☆ Please cite this article as: Abranches AD, Soares FV, Junior SC, Moreira ME. Freezing and thawing effects on fat, protein, and lactose levels of human natural milk administered by gavage and continuous infusion. J Pediatr (Rio J). 2014;90:384−8.
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http://dx.doi.org/10.1016/j.jped.2013.11.001
0021-7557/

PALAVRAS-CHAVE
Leite humano;
Nutrição;
Recém-nascido

Efeito do congelamento e descongelamento nos níveis de gordura, proteína e lactose do leite humano natural administrados por gavagem e infusão contínua

Resumo

Objetivo: analisar as alterações dos macronutrientes gordura, proteína e lactose no leite humano natural, congelado e descongelado, após a simulação da administração da dieta por gavagem e infusão contínua.

Método: foi conduzido um estudo experimental com 34 amostras de leite humano. Foi utilizada a técnica da espectofotometria infravermelha (Milko Scan Minor®) para analisar os macronutrientes do leite humano nas etapas do estudo. As amostras foram analisadas na forma natural (crua) e após congelamento e descongelamento rápido nas duas formas de infusão: gavagem e infusão contínua. Foi usado o teste não paramétrico de Wilcoxon para amostras pareadas na análise estatística.

Resultado: a gordura apresentou redução significativa após administração por infusão contínua ($p < 0,001$), tanto durante administração na forma natural quanto na forma descongelada. Não houve alteração da proteína e lactose segundo forma de infusão no leite descongelado e no leite in natura. O processo de descongelamento aumentou significativamente os níveis de lactose e de proteína do leite.

Conclusão: a via de administração por infusão contínua foi o procedimento que mais influenciou na perda de gordura, dentre todos os processos necessários para administração do leite humano.

Introduction

Breast milk is the ideal food to newborns born at term and preterm, facilitating cognitive development.[1,2] At a gestational age of less than 34 weeks, newborns are still unable to suck, swallow, and breathe properly and coordinately. In such cases, the oral diet is administered through a feeding tube, which implies collecting, handling, storing, and administering human milk.[3] These procedures may compromise the nutritional quality of breast milk, depriving preterm infants from a significant portion of calories from fat.[4–6]

Vieira et al.[7] observed a significant reduction in fat between natural donated breast milk (raw) milk and the milk that is offered. Among the processes related to supply of human milk studied, the greatest reduction occurred after the simulation of milk supply by continuous infusion.

The process of freezing and thawing can change the physicochemical properties of breast milk and, therefore, the losses during continuous infusion could be affected by these changes. The freezing and thawing processes favor the formation of micelles, which can adhere to plastic, facilitating the loss of fat.[4,7] Therefore, it became necessary to clarify whether this increased loss with continuous infusion might be caused by the thawing process or whether the administration route (gavage or continuous infusion) would be the main responsible factor.

The aim of this study was to analyze changes in the following macronutrients: fat, protein, and lactose in natural human milk, frozen and thawed, after administration simulation by gavage and continuous infusion.

Method

An experimental study was conducted with human milk samples from volunteer donors of the Human Milk Bank of the Instituto Nacional em Saúde da Mulher, da Criança e do Adolescente Fernandes Figueira, Rio de Janeiro, RJ, Brazil. All donors were mothers of newborns born at term, and the milk was collected in the morning.

The milk was extracted by manual expression or electric pump and stored in glass vials. Of the total collected volume, 50 mL were used, which were divided into three aliquots of 10 mL and one aliquot of 20 mL. The latter was frozen at -20 °C for 24 hours and thawed in a microwave for 45 seconds.[6]

The analysis of natural human milk was performed immediately after the extraction. Of the three 10-mL aliquots, one was identified as reference (not subjected to any process), the other was assigned for administration simulation by gavage, and the last was assigned for administration simulation by continuous infusion.

The administration by gavage was performed with a 10 mL-syringe and disposable #4 siliconized tube; the content was gravity-fed.

The administration by continuous infusion was performed with a 10 mL-syringe, a disposable #4 siliconized tube, a 120 cm perfusor, and a Samtronic ST6000® infusion pump (São Paulo, Brazil). The time set for infusion was 1 hour. All materials and techniques used followed the routine of the Neonatal Unit of the Instituto Fernandes Figueira/Fiocruz, Brazil.

The amount of fat, protein, and lactose in human milk was measured by infrared spectrophotometry, using the infrared analysis equipment MilkoScan Minor (Foss, Denmark), previously validated for human milk.[7]

Sample size calculation was performed considering the magnitude of the difference found between measurements of fat in the two forms of administration (gavage and continuous infusion) in the study by Vieira et al.,[7] power of 90%, and significance of 95%. In this study, the magnitude of the difference was 0.94 g/100 mL.

Table 1 Median, minimum, and maximum values of macronutrients and total calories per 100 mL of human milk according to the studied processes. Rio de Janeiro, 2013.

Route of administration	Fat[a]	p	Protein[a]	p	Lactose[a]	p	Total calories[b]	p
Natural	2.9 (1.1-5.8)	–	1.1 (0.5-2.6)	–	6.4 (4.9-6.7)	–	54.9 (37.4-87.5)	–
Natural gavage	3.0 (1.1-5.8)	0.054	1.2 (0.6-2.6)	0.060	6.4 (4.9-6.8)	0.110	55.4 (37.7-88.0)	0.052
Natural	2.9 (1.1-5.8)	–	1.1 (0.5-2.6)	–	6.4 (4.9-6.7)	–	54.9 (37.4-87.5)	–
Natural continuous infusion	2.7 (1.0-5.9)	0.000[c]	1.2 (0.4-2.7)	0.308	6.4 (4.9-6.8)	0.190	53.1 (36.4-84.0)	0.001[c]
Natural	2.9 (1.1-5.8)	–	1.1 (0.5-2.6)	–	6.4 (4.9-6.7)	–	54.9 (37.4-87.5)	–
Thawed gavage	2.8 (1.3-5.8)	0.335	1.3 (0.2-2.5)	0.046[c]	6.5 (5.1-7.2)	0.000[c]	54.3 (37.7-83.6)	0.966
Natural	2.9 (1.1-5.8)	–	1.1 (0.5-2.6)	–	6.4 (4.9-6.7)	–	54.9 (37.4-87.5)	–
Thawed continuous infusion	2.4 (1.0-5.1)	0.000[c]	1.3 (0.2-2.5)	0.007[c]	6.5 (5.0-7.0)	0.096	53.2 (35.3-78.2)	0.185
Natural gavage	3.0 (1.1-5.8)	–	1.2 (0.6-2.6)	–	6.4 (4.9-6.8)	–	55.4 (37.7-88.0)	–
Thawed gavage	2.8 (1.3-5.8)	0.108	1.3 (0.2-2.5)	0.014[c]	6.5 (5.1-7.2)	0.000[c]	54.3 (37.7-83.6)	0.726
Natural continuous infusion	2.7 (1.0-5.9)	–	1.2 (0.4-2.7)	–	6.4 (4.9-6.8)	–	53.1 (36.4-84.0)	–
Thawed continuous infusion	2.4 (1.0-5.1)	0.091	1.3 (0.2-2.5)	0.017[c]	6.5 (5.0-7.0)	0.001[c]	53.2 (35.3-78.2)	0.871
Natural gavage	3.0 (1.1-5.8)	–	1.2 (0.6-2.6)	–	6.4 (4.9-6.8)	–	55.4 (37.7-88.0)	–
Natural continuous infusion	2.7 (1.0-5.9)	0.000[c]	1.2 (0.4-2.7)	0.812	6.4 (4.9-6.8)	0.123	53.1 (36.4-84.0)	0.000[c]
Thawed gavage	2.8 (1.3-5.8)	–	1.3 (0.2-2.5)	–	6.5 (5.1-7.2)	–	54.3 (37.7-83.6)	–
Thawed continuous infusion	2.4 (1.0-5.1)	0.000[c]	1.3 (0.2-2.5)	0.147	6.5 (5.0-7.0)	0.060	53.2 (35.3-78.2)	0.040[c]

Wilcoxon Test.
[a] g/100 mL.
[b] Kcal/100 mL.
[c] Statistically significant value ($p < 0.05$).

Considering these parameters, the initial sample size consisted of 16 samples, which was doubled due to variability of fat content in milk samples.[8,9]

The measurements of macronutrients and total calories in human milk samples were compared at each phase using the Wilcoxon test for paired samples. The SPSS software, version 20.0 (IBM Corp, USA), was used for the statistical analysis.

This study was approved by the Research Ethics Committee of the Instituto Nacional da Saúde da Mulher, Criança e Adolescentes Fernandes Figueira and an informed consent was obtained from all participants.

Results

A total of 34 human milk samples were analyzed. There was a variation in macronutrients between donated samples of 19% for fat, 1.9% for protein, and 1.6% for lactose. No samples of pooled human milk were analyzed.

The mean content of macronutrients in g/100 mL in natural milk was 3.05 ± 1.18 for fat, 1.22 ± 0.50 for protein, and 6.09 ± 0.55 for lactose. The mean of total calories was 56.66 ± 11.76 Kcal/100 mL.

Milk administration by continuous infusion significantly altered the levels of fat when compared to gavage, both during the infusion of natural and thawed milk (Table 1).

A significant increase of protein in thawed milk was also observed when compared to natural milk. However, no significant difference was observed in the amounts of protein in thawed milk offered either by gavage or continuous infusion. (Table 1)

The use of gavage did not result in loss of macronutrients in both natural and thawed milk (Table 1).

The mean difference between fat content in natural milk administered by gavage and by continuous infusion was 0.24 ± 0.31 (median = 0.18); in thawed milk offered by gavage and continuous infusion, this difference was 0.26 ± 0.17 (median = 0.17).

Fat loss caused by thawing was similar for both routes of administration ($p = 0.853$). The difference in fat content between natural and thawed milk was 0.3 g/100 mL for continuous infusion and 0.2 g/100 mL for gavage.

Discussion

The analysis of the influence of human milk handling on macronutrients, from its expression to the final offer to the newborn, is of great importance when considering the effects of proper nutrition on growth and development of preterm newborns.[2] This study demonstrated that the choice of administration by continuous infusion significantly impairs the concentration of fat, both in natural and thawed human milk.

Fat loss is generally attributed to its adherence to the container, to lipolysis, or to lipid peroxidation.[10] The reduction of fat content in thawed human milk has also been observed in other studies,[11,12] and it has been suggested that lipolysis would still occur in frozen milk.[13,14] When at rest, the fat easily separates and adheres to the container, tubes, and syringes, which reduces its supply to the newborn. Although the effect of freezing/thawing was not statistically significant in the two forms of infusion, the association between thawing and continuous infusion resulted in a loss of 0.5 g of fat per 100 mL of milk, implying a reduction of approximately 18% of the fat content of the milk, which may cause important clinical and nutritional consequences for preterm infants.[1] One way to reduce these losses is by homogenizing milk before offering it to the newborn.[15]

One question raised in this study was the lower concentrations of fat and total calories in human milk than those reported in other international studies.[8,9,16] Other studies performed in Brazil have also observed lower fat content values, even though different techniques were used.[17,18]

With regard to protein and lactose, it was observed that their values had an unexpected and significant increase after thawing. This fact may be related to the loss of water during the freezing and thawing process (volatilization), and sublimation, with increased infrared absorbance of protein at wavelength 5.7 μm, which was also observed in other studies and attributed to these properties.[10,19] Furthermore, thawing of human milk may cause aggregation of the protein micelles, resulting in a variation of the protein content.[20]

In relation to energy content, there was a significant variation (50.1 Kcal/100 mL) between the studied samples of natural milk, demonstrating the importance of control related to the nutritional content of donated human milk in human milk banks. The energy content of the milk is mainly related to overall fat content, as the energy density of this macronutrient is responsible for most of the calories in human milk.[7,8] In this study, the energy values were lower in samples where the fat content was lower.

Therefore, the processes used from human milk extraction until its offer imply in important changes in its macronutrient contents, which have been observed by several authors.[4,7,16,19–24] Changes found due to the milk infusion route were also observed in the studies by Vieira et al.[7] and by Stoks et al.[25] The milk infusion process by gavage did not result in significant fat loss, probably because there was less loss related to fat adhesion to plastic, as the probe is much smaller than the perfusor used for continuous infusion. The time spent during infusion for the two modalities may also have influenced fat loss.[25]

The limitations of this study include the fact that it analyzed only macronutrients and used only the fast thawing method in the microwave. Excessive heating can destroy the immunological factors in human milk, but not necessarily the nutritional components that were evaluated in this study.[26] The Brazilian National Health Surveillance Agency (Agência Nacional de Vigilância Sanitária - ANVISA) and the Brazilian Human Milk Bank Network mention this practice in their instructional manuals.[6] Another result found in this study was a smaller magnitude of the differences in the amounts of fat according to the infusion route when compared to the study by Vieira et al.,[7] which would indicate larger sample sizes in future studies.

Human milk remains the best food to be offered to newborns, including preterm, but the nutritional fat losses related to continuous infusion should be considered when choosing the route of administration.

Funding

FAPERJ (Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro).

Conflicts of interest

Maria Elisabeth L Moreira was a lecturer at Mead Johnson, Nestlé, and ABBOTT in 2012.

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