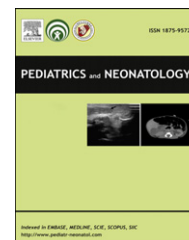


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ORIGINAL ARTICLE

The Macronutrients in Human Milk Change after Storage in Various Containers

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Received Sep 13, 2011; received in revised form Dec 27, 2011; accepted Jan 9, 2012

Key Wordshuman milk;
infrared analysis;
storage container

Background: The concentrations of macronutrients in human milk can be influenced by various processes, such as storage, freezing, and thawing, that are performed by lactating working mothers and breast milk banks. We evaluated the impact of various containers on the nutrient concentrations in human milk.

Methods: A total of 42 breast milk samples from 18 healthy lactating mothers were collected. A baseline macronutrient concentration was determined for each sample. Then, the breast milk samples were divided and stored in nine different commercial milk containers. After freezing at -20°C for 2 days, the milk samples were thawed and analyzed again. A midinfrared human milk analyzer (HMA) was used to measure the protein, fat, and carbohydrate contents.

Results: There was a significant decrease in the fat content following the storage, freezing, and thawing processes, ranging from 0.27–0.30 g/dL ($p = 0.02$), but no significant decrease in energy content ($p = 0.069$) was noted in the nine different containers. There were statistically significant increases in protein and carbohydrate concentrations in all containers ($p = 0.021$ and 0.001 , respectively), however there were no significant differences between the containers in terms of fat, protein, carbohydrate, or energy contents.

Conclusion: Human milk, when subjected to storage, freezing, and thawing processes, demonstrated a significant decrease in fat content (up to 9% reduction) in various containers. It is better for infants to receive milk directly from the mother via breastfeeding. More studies are warranted to evaluate the effects of milk storage on infant growth and development.

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1. Introduction

Human milk is the best food for infants.^{1,2} The World Health Organization recommends human milk as the best way to provide optimal nutrition to full-term infants for at least 6 months.³ Breastfeeding rates in Taiwan for 1- and 6-month-old infants were 85.4% and 46.0%, respectively, in 2010, but the rate has increased each year.⁴ When the mother is separated from her infant due to illness or work, she can still secrete and store her milk for caregivers to feed to the infant.⁵ However, the storage container, the temperature of storage, and the heating process may influence the nutritional components of human milk.^{6,7}

Previous studies report that proteolysis and lipolysis occur in human milk at different temperatures.⁶ Denaturation of proteins has been noted after freezing (-20°C) and thawing.⁷ Lactose has been found to be stable after pasteurization and freezing.⁸ The storage of human milk in polyethylene bags results in reduced fat content due to adherence to the inside surface of the bag.⁹ Similarly, sterilization of human milk causes a decrease in the fat percentage by enhancing fat adherence to the container surface.¹⁰ Some lipid-soluble nutrients in human milk demonstrate a similar propensity to adhere to the surfaces of containers made of glass and polypropylene.^{11,12} Little is known about the effects of the sequential processes of storage, freezing, and heating to the macronutrients in human milk. Furthermore, there is little data on the effects of different storage containers on the macronutrient composition of human milk.

Infrared spectroscopy has become a widely used technique for the determination of the macronutrient content of human milk.^{13–17} Fat, protein, and carbohydrate concentrations can be accurately calculated by measuring the absorption at specific wavebands. Measurements can be performed quickly, and only small amounts of milk are required (2–3 mL for each measurement). Positive correlations have been found between data obtained using an infrared analyzer and results obtained using conventional laboratory methods,^{16,17} demonstrating that the infrared technique is reliable.

For this study, we studied the effects of different containers on the contents of human milk under normal storage conditions using infrared analysis.

2. Material and Methods

The study was conducted at Taichung Veteran General Hospital, Taichung, Taiwan, between November 2010 and January 2011. The trial was approved by the institutional review board of Taichung Veteran General Hospital, and informed consent was obtained from all participants before initiating the study.

2.1. Samples

Forty-two fresh human milk samples were collected from 18 healthy lactating mothers. All infants were full term, with ages ranging from 1–23 months. Milk was obtained by hand or breast pump from either the left or right breast and immediately stored in glass containers in a refrigerator for

no more than 3 days before analysis. The total volume of each stored sample was 280 mL and was obtained from 1–2 donors.

2.2. Procedures

2.2.1. Homogenization, division, freezing, and thawing

Fresh breast human milk was stored in a glass container and homogenized using a homogenizer (ultrasonic vibrator VCX 130; Sonics & Material, Newtown, Connecticut, USA) for a duration of 1.5 seconds per milliliter of milk. A 10-mL sample was taken as the baseline for analysis, and then the remaining milk was divided into nine different containers (Table 1). Each container contained 30 mL of milk and was stored at -20°C for 48 hours. The containers were then put into a refrigerator at 4°C for 12 hours to thaw. After removal from the refrigerator, the samples were subjected to the same homogenization and analysis process described above.

2.3. Analysis

We used a midinfrared (MIR) human milk analyzer (HMA) that was developed by Miris AB (Uppsala, Sweden) to measure the macronutrient components of the human milk samples. Miris HMA is certified by International Organization for Standardization (ISO) 9622:1999, the Association of Official Analytical Chemists, and the International Dairy Federation. It has different filters for specific milk components and uses four different wavebands to measure functional carbonyl groups (5.7 μm) and carbon-hydrogen groups (3.5 μm) for fat determination, amide groups (6.5 μm) for protein determination, and hydroxyl groups (9.6 μm) for lactose determination.¹⁶ The machine calculates energy using the equation: Energy Kcal/100ml = (9.25 Kcal/g × fat g/100ml) + (4.40 Kcal/g × protein g/100ml) + (3.95 Kcal/g × lactose g/100ml). A

Table 1 Descriptions of the 9 containers that were investigated.

Container	Material	Form	Notes
1	Outer layer: Nylon Inner layer: PE	bag	
2	PE	bag	
3	PE	bag	
4	PP	bag	Cloudy with semi-opaque color
5	Outer layer: polyester Inner layer: PE	bag	
6	Lid: PP Bottle: PC	bottle	
7	Lid: PP Bottle: PP	bottle	Cloudy with semi-opaque color
8	Lid: PP Bottle: PES	bottle	Light brown color
9	Lid: PP Bottle: Glass	bottle	

PE = polyethylene; PP = polypropylene; PC = polycarbonate; PES = polyethersulfone.

3-mL volume of breast milk was heated, and the homogenized human milk in each container was analyzed. Each analysis was repeated three times, and the average value was used for further analysis.

2.4. Statistical analysis

Data were analyzed using SPSS 10.0 (SPSS Inc., Chicago, IL, USA), specifically using the Student *t* test and one-way analysis of variance (ANOVA). Significant differences are defined as $p < 0.05$.

3. Results

3.1. Influence of storage process on the macronutrients of human milk

There were significant reductions ($p = 0.02$) in the percentages of fat in each of the nine containers, ranging from -0.26 g/dL to -0.30 g/dL (8.2% to 9.4% decrease). The total protein and carbohydrate concentrations increased significantly ($p = 0.021$ and 0.001 respectively), ranging from 0.04 g/dL to 0.06 g/dL (4.4% to 7.7% increase) and 0.06 g/dL to 0.1 g/dL (0.8% to 1.4% increase), respectively. The energy levels decreased in all containers, but this did not reach a statistical level of significance ($p = 0.069$; Figure 1).

3.2. Influence of different storage containers on macronutrient contents

Although container 8 (see Table 1) demonstrated the least fat and energy loss (8.2% and 2.9%, respectively), and container

5 (see Table 1) demonstrated the most fat and energy loss (9.4% and 3.6%, respectively), there were no statistical differences in terms of the percentages of fat, protein, carbohydrate, and energy among the nine containers ($p = 0.993$, 0.167 , 0.837 , and 0.947 , respectively).

4. Discussion

Fat accounts for approximately 50% of the nonprotein energy in human milk and facilitates the absorption, delivery, and transport of fat-soluble vitamins.¹⁸ This study demonstrates a statistically significant reduction in the fat content in each of the containers that were analyzed, although no significance difference in terms of the energy density was found.

The loss of fat is most likely due to the adherence of breast milk to the container wall, lipolysis, or lipid peroxidation. The greatest loss, which was measured in container 5, was approximately 2.7 kcal/dL, which accounts for 4% of the total calories found in human milk. However, the clinical impact of this loss on an infant's growth and development needs to be further studied.

The adherence of fat to the tubing system and walls of a bag has been reported in several studies and appears to be unavoidable.^{9,12,19,20} Ultrasonic homogenization can reduce fat loss during tube feeding²¹ and increase fat absorption in very low birth weight infants^{22,23} by decreasing clumping and reducing the size of the fat globules. Therefore, we can reasonably expect that the loss of fat content may be greater during regular processing at home where homogenization is not performed.

Lipolysis with fatty acid formation has been observed during storage at 4°C for 96 hours, demonstrating a 3-fold

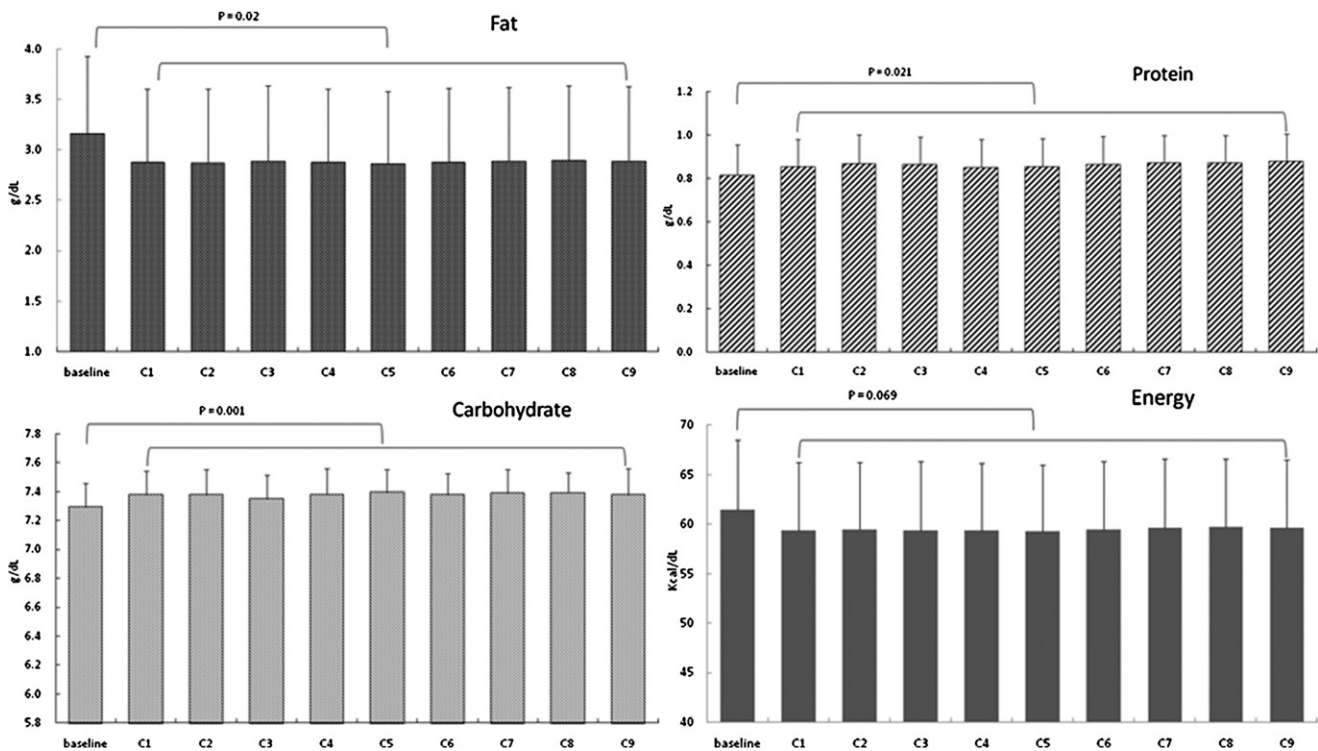


Figure 1 Comparison of macronutrient and energy changes before and after storage in various containers. C = container.

increase in the concentration of fatty acids.²⁴ Studies by Marget et al reported lipolysis at 15°C, 25°C, and 38°C after storage for 24 hours.⁶ They also reported that lipolysis occurred rapidly during the first hours of storage and was most evident at 25°C, demonstrating 5–6-fold increases compared with fresh breast milk at 24 hours. Another study showed that the human milk creatinocrit level remains stable after freezing at -20°C for 28 days, but decreases after two cycles of freezing and thawing,²⁵ and lipolysis could occur after freezing for long periods.⁷ The abovementioned studies indicate that the lipolysis observed in our study may have been induced by the thawing and warming procedures, although the extent to which they contributed to lipolysis could not be determined. However, the fatty acids produced by lipolysis might prevent the growth of microorganisms, as these fatty acids have anti-infective properties against bacteria, viruses, and protozoan.^{26–29}

Lipid peroxidation is the oxidative degradation of lipids, which leads to free radical formation and impairment of the antioxidant defense system. Previous studies have shown that little oxidation occurs in stored frozen breast milk (-20°C), but oxidation does take place in breast milk stored in a refrigerator (4°C).^{30,31} Our study investigated both forms of storage, so lipid peroxidation could have played a role in the loss of fat observed in this study.

The increased concentrations of protein and carbohydrate may be due to water evaporation (volatilization), sublimation, and the increased infrared absorbance of protein at the 5.7- μm waveband over time.^{32,33} Although protein may denaturalize when thawed,⁷ and some protein breakdown has been observed after storage at 38°C for 24 hours,⁶ we did not find a significant decrease in the protein content as a result of these procedures. Lactose, the main carbohydrate of human milk, has been noted as stable after pasteurization, freezing, and thawing in some studies,^{7,8} which is consistent with our results. In addition, thawed frozen breast milk could cause the aggregation of casein micelles, resulting in variations in the protein content.¹⁷ The increased concentrations of protein and carbohydrate in our study was minimal and had little impact on the energy content.

One of the limitations of this study was that the human milk analyzer (HMA) we used could only measure macronutrient components. It was not capable of measuring the immunological and biochemical properties of human milk, which may also change after storage and processing. Another limitation is that we only studied mature human milk from the mothers of full-term infants. The results may be different for the human milk of the mothers of preterm infants. However, one study showed no difference in fat content between human milk obtained from the mothers of full-term and preterm infants (30–37 weeks of gestation), but there was a significant difference in comparison with milk obtained from the mothers of very premature infants (< 30 weeks of gestation).³⁴ Our results, therefore, could be applied to human milk obtained from the mothers of preterm infants (30–37 weeks of gestation) in the hospital.

In conclusion, the freezing and warming of stored human milk is a common practice of mothers who are separated from their babies due to work or illness. Our study reveals that fat loss occurred in all of the breast milk samples that were stored in the nine containers that were investigated. We found that the loss of fat was as high as 9% in one

polyethylene bag, though this decrease did not reach a statistical level of significance compared with the reductions that were observed in the breast milk that was stored in the other containers, and the resultant energy difference was not significant. The effects of the loss of milk fat on the growth and development of full-term/preterm infants needs to be further investigated. We encourage mothers to directly breastfeed their infants, which may avoid the loss of fat and related nutrients that result from storage and processing. To the best of our knowledge, this is the first study to evaluate the effects of different containers on macronutritional changes in human milk after storage and processing. Further studies on the effects of storage and processing on the various bioactive components (e.g., cells, enzymes, immunoglobulins, hormones) in human milk are needed.

Acknowledgments

The authors would like to thank all the mothers who kindly donated their milk for analysis in this study.

References

1. American Academy of Pediatrics. Nutrition Committee of the Canadian Paediatric Society and the Committee on Nutrition of the American Academy of Pediatrics. Breast-feeding: a commentary in celebration of the International Year of the Child, 1979. *Pediatrics* 1978;**62**:591–601.
2. Gartner LM, Morton J, Lawrence RA, et al. Breastfeeding and the use of human milk. *Pediatrics* 2005;**115**:496–506.
3. World Health Organization. WHO statement 2011;1. Available at: http://www.who.int/mediacentre/news/statements/2011/breastfeeding_20110115/en/index.html [Date accessed: October 9, 2011].
4. Bureau of Health Promotion, Department of Health, Taiwan. The current breastfeeding situations in Taiwan, 2010. Available at: http://www.bhp.doh.gov.tw/breastfeeding/02qna_01.htm [Date accessed: October 9, 2011].
5. Ogbuanu C, Glover S, Probst J, Hussey J, Liu J. Balancing work and family: effect of employment characteristics on breastfeeding. *J Hum Lact* 2011;**27**:225–38.
6. Hamosh M, Ellis LA, Pollock DR, Henderson TR, Hamosh P. Breastfeeding and the working mother: effect of time and temperature of short-term storage on proteolysis, lipolysis, and bacterial growth in milk. *Pediatrics* 1996;**97**:492–8.
7. Lawrence RA. Storage of human milk and the influence of procedures on immunological components of human milk. *Acta Paediatr Suppl* 1999;**88**:14–8.
8. Vieira AA, Soares FV, Pimenta HP, Abranches AD, Moreira ME. Analysis of the influence of pasteurization, freezing/thawing, and offer processes on human milk's macronutrient concentrations. *Early Hum Dev* 2011;**87**:577–80.
9. Arnold LD. Storage containers for human milk: an issue revisited. *J Hum Lact* 1995;**11**:325–8.
10. Fidler N, Sauerwald TU, Koletzko B, Demmelmair H. Effects of human milk pasteurization and sterilization on available fat content and fatty acid composition. *J Pediatr Gastroenterol Nutr* 1998;**27**:317–22.
11. Eglash A. ABM clinical protocol #8: human milk storage information for home use for full-term infants (original protocol: March 2004; revision #1: March 2010. *Breastfeed Med* 2010;**5**:127–30.

12. Garza C, Johnson CA, Harrist R, Nichols BL. Effects of methods of collection and storage on nutrients in human milk. *Early Hum Dev* 1982;**6**:295–303.
13. Michaelsen KF, Pedersen SB, Skaftø L, Jaeger P, Peitersen B. Infrared analysis for determining macronutrients in human milk. *J Pediatr Gastroenterol Nutr* 1988;**7**:229–35.
14. Wojcik KY, Rechtman DJ, Lee ML, Montoya A, Medo ET. Macronutrient analysis of a nationwide sample of donor breast milk. *J Am Diet Assoc* 2009;**109**:137–40.
15. Corvaglia L, Battistini B, Paoletti V, Aceti A, Capretti MG, Faldella G. Near-infrared reflectance analysis to evaluate the nitrogen and fat content of human milk in neonatal intensive care units. *Arch Dis Child Fetal Neonatal Ed* 2008;**93**:F372–5.
16. Casadio YS, Williams TM, Lai CT, Olsson SE, Hepworth AR, Hartmann PE. Evaluation of a mid-infrared analyzer for the determination of the macronutrient composition of human milk. *J Hum Lact* 2010;**26**:376–83.
17. Menjo A, Mizuno K, Murase M, et al. Bedside analysis of human milk for adjustable nutrition strategy. *Acta Paediatr* 2009;**98**:380–4.
18. Kleinman RE. *Pediatric nutrition handbook*. 6th ed. USA: American Academy of Pediatrics; 2008, p. 357–79.
19. Narayanan I, Singh B, Harvey D. Fat loss during feeding of human milk. *Arch Dis Child* 1984;**59**:475–7.
20. Stocks RJ, Davies DP, Allen F, Sewell D. Loss of breast milk nutrients during tube feeding. *Arch Dis Child* 1985;**60**:164–6.
21. Martinez FE, Desai ID, Davidson AG, Nakai S, Radcliffe A. Ultrasonic homogenization of expressed human milk to prevent fat loss during tube feeding. *J Pediatr Gastroenterol Nutr* 1987;**6**:593–7.
22. Thomaz A, Goncalves A, Martinez F. Effects of human milk homogenization on fat absorption in very low birth weight infants. *Nutr Res* 1999;**19**:483–92.
23. Rayol MR, Martinez FE, Jorge SM, Goncalves AL, Desai ID. Feeding premature infants banked human milk homogenized by ultrasonic treatment. *J Pediatr* 1993;**123**:985–8.
24. Slutzah M, Codipilly CN, Potak D, Clark RM, Schanler RJ. Refrigerator storage of expressed human milk in the neonatal intensive care unit. *J Pediatr* 2010;**156**:26–8.
25. Silprasert A, Dejsarai W, Keawvichit R, Amatayakul K. Effect of storage on the creatinocrit and total energy content of human milk. *Hum Nutr Clin Nutr* 1987;**41**:31–6.
26. Hernel O, Ward H, Blackberg L, Pereira ME. Killing of *Giardia lamblia* by human milk lipases: an effect mediated by lipolysis of milk lipids. *J Infect Dis* 1986;**153**:715–20.
27. Estados U. Products of lipolysis in human milk destroy parasites. *Nutr Rev* 1987;**45**:236–8.
28. Isaacs CE, Thormar H. The role of milk-derived antimicrobial lipids as antiviral and antibacterial agents. *Adv Exp Med Biol* 1991;**310**:159–65.
29. Thormar H, Isaacs CE, Kim KS, Brown HR. Inactivation of visna virus and other enveloped viruses by free fatty acids and monoglycerides. *Ann N Y Acad Sci* 1994;**724**:465–71.
30. Miranda M, Muriach M, Almansa I, et al. Oxidative status of human milk and its variations during cold storage. *Biofactors* 2004;**20**:129–37.
31. Turolí D, Testolin G, Zanini R, Bellu R. Determination of oxidative status in breast and formula milk. *Acta Paediatr* 2004;**93**:1569–74.
32. Kaylegian KE, Lynch JM, Fleming JR, Barbano DM. Lipolysis and proteolysis of modified and producer milks used for calibration of mid-infrared milk analyzers. *J Dairy Sci* 2007;**90**:602–15.
33. Sjaunja LO. Studies on milk analyses of individual cow milk samples, III: the effect of different treatments on infrared analyses. *Acta Agric Scand* 1984;**34**:273–85.
34. Molto-Puigmarti C, Castellote AI, Carbonell-Estrany X, Lopez-Sabater MC. Differences in fat content and fatty acid proportions among colostrum, transitional, and mature milk from women delivering very preterm, preterm, and term infants. *Clin Nutr* 2011;**30**:116–23.