Pediatrics and Neonatology (2012) 53, 205—209

Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: http://www.pediatr-neonatol.com

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 Words
human 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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1875-9572/$36
doi:10.1016/j.pedneo.2012.04.009

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.