

Fat Loss in Thawed Breast Milk: Comparison Between Refrigerator and Warm Water

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Objective: To compare the fat loss between refrigerator and warm water thawed breast milk.

Design: Experimental.

Setting: Tertiary-care pediatric university hospital.

Participants: Ninety samples of expressed breast milk were collected from mothers with singleton babies of a gestational age 32-42 weeks.

Main Outcome Measures: Fat content in fresh breast milk (FM); thawed breast milk by refrigeration (RM); and thawed breast milk by warm water (WM).

Results: The mean (SD) total fat content in FM, RM and WM were 2.98 (0.97), 2.76 (0.99) and 2.66 (0.88) g/100 mL, respectively. The mean difference (SD) of the total fat in FM declined significantly after the frozen milk was thawed by refrigeration or warm water at -0.22 (0.50) g/100 mL ($P=0.0001$) and -0.32 (0.45) g/100 mL ($P<0.0001$), respectively. The mean (SD) total fat loss of frozen breast milk thawed by refrigeration was less than thawing in warm water at 0.094 (0.38) g/100 mL ($P=0.02$).

Conclusion: The fat loss of thawed breast milk by refrigeration was significantly less than placing it in warm water.

Key words: Breast milk; Fat content; Refrigeration; Thawing.

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Exclusive breastfeeding is the reference or normative model against which all alternative feeding methods must be compared with regard to growth, health, the development of the babies, and all other short- and long-term outcomes [1]. As more women in developing countries join the workforce, the need to store breastmilk is becoming an increasing necessity.

Academy Breastfeeding Medicine protocols recommend thawing frozen milk by placing it in the refrigerator the night before using or gently rewarm by placing the container under warm running water or in a bowl of warm water [2], whereas the American Academy of Pediatrics recommends that frozen milk should be thawed rapidly, usually by holding the container under running tepid (not hot) water [3].

Energy variation in milk is mainly due to the variation in fat content because the energy contribution of protein and lactose in milk is relatively constant. The loss of fat is important because fats are the main energy source of breast milk, comprising of 45-55% of the total calories [4]. The fat content in breast milk varies widely due to the influence of several factors, such as gestational age, genetic characteristics, dietary habits of different

populations, the stage of lactation, diurnal rhythm, postpartum age, temperature of storage and the method of tube feeding [5-7]. Rapid thawing has the advantage over slower thawing due to less destruction of milk fat globule membranes [8,9].

The present study was, therefore, conducted to compare the fat concentration of thawed milk between placing the frozen milk in the refrigerator for 24 hours and in a bowl of warm water.

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METHODS

This trial was performed in laboratory conditions between 1 April, 2010 to 31 March, 2011. Expressed breast milk (EBM) was collected from exclusive breastfeeding mothers aged 20-40 years and having singleton babies 32-42 weeks gestation, delivered in Songklanagarind Hospital, the major tertiary care institution in southern Thailand. Breast milk was collected between 08.00 and 12.00 hours. Socio-demographic characteristics were collected using a questionnaire. We excluded the infants with a history of any disease in the past likely to affect their weight gain.

The mother-infant pairs meeting these criteria were recruited consecutively. The study was approved by the Ethics Committee Board of Prince of Songkla University, and informed consent was obtained from the mothers.

The mother would breastfeed her baby or express breast milk 2-3 hours before the breast milk was expressed for study collection. The breasts were cleaned with sterile water before expression and the hard polypropylene plastic containers (Medela, Switzerland) were sterilized. Breast milk was expressed by hospital grade, automatic cyclic electric double pumping (Medela, Lactina, Switzerland) for at least 15-20 minutes and collected in the same container before dividing the breastmilk. The 60 mL of milk sample was collected into hard polypropylene and medical graded plastic containers, physically well mixed and divided into 20 mL aliquots to measure the fat content (fresh breast milk: FM) immediately. Lipids levels were measured using the Gerber method [10] by weight. We divided the other 40 mL of fresh breast milk into two groups in hard polypropylene plastic containers equally. Both milk samples were kept frozen at -20°C (Sharp FC 20, Japan) for 30 days before analysis and labeled with the name of the mother and the date. The first group of frozen milk was thawed by placing the container in the refrigerator (Sharp refrigerator, Japan) at 4°C (frozen breast milk was thawed by refrigeration: RM) for 24 hours, and the other group of frozen milk was thawed by placing the container in a water-bath (Mettler GmbH+Co. KG., West Germany) with a constant temperature of 37°C (frozen breast milk was thawed by warm water: WM) for 30 minutes, and the fat content of the thawed breast milk was measured by using the Gerber method.

Statistical analysis: The sample size was calculated by the two-sample mean equation where $Z_{\alpha/2}$ was a standard score at a 0.05, the power was 80%, and σ^2 was a variance of a previous study, where $\sigma = 1.1$ [11]. The calculated mean fat of fresh and frozen EBM was 3.34 (μ_2) and 2.80 (μ_1) g/dL, respectively. The sample size was calculated as 90 samples. STATA version 10 was used to compare the fat loss of FM, RM and WM. Paired *t*-test was used to compare the continuous data of the fat content between the milk thawed by refrigerator and warm water. All tests were 2-sided with a statistical significance of $P < 0.05$.

RESULTS

Ninety samples of EBM from lactating mothers were obtained. The mean (SD) age of the mothers and infants' gestational age were 33.2 (3.8) years and 37.9 (1.3) weeks, respectively. Before pregnancy, the means (SD) of maternal weight and height were 54.5 (7.9) kg and 156.3 (4.7) cm, respectively. Sixteen mothers had an underlying

disease; 13 with gestational diabetes mellitus, 1 overt diabetes mellitus and 2 hepatitis B virus carriers. The modes of delivery were emergency cesarean section (46.7%), normal labor (25.6%), vacuum extraction (14.4%), forceps extraction (11.1%) and planned cesarean section (2.2%). The mean (SD) postpartum age of lactating mothers at the time of breast milk collection was 131.9 (69.3) days.

The mean (SD) total fat content in FM, RM and WM groups were 2.98 (0.97), 2.76 (0.99) and 2.66 (0.88)/100 mL, respectively. The mean differences (SD) of the total fat in FM declined significantly after the frozen milk was thawed by refrigeration or warm water at -0.22 (0.50) grams/100 mL ($P=0.0001$) and -0.32 (0.45) grams/100 mL ($P<0.0001$), respectively. The mean (SD) total fat loss of frozen breast milk thawed by refrigeration was significantly less than warm water at 0.094 (0.38) grams/100 mL ($P=0.02$). The percentage of mean fat loss of thawed milk by refrigerator or warm water from the initial values of fat in FM was 7.38% and 10.07%, respectively.

DISCUSSION

Effective use of expressed human milk in neonatal feeding requires proper handling, processing, storage, and administration in order to maintain its unique nutritional properties. One of the problems with expressed human milk is the separation of fat during thawing. The mean total fat content in immediate EBM in this study (2.98 grams/100 mL) was less than the average fat content of human milk. Fat increases from 3.16 grams/100mL in colostrum to 3.49 grams/100mL in transitional milk and to 4.14 grams/100 mL in mature milk [2]. A previous study from a rural northern Thai population, showed the mean (SD) fat content of breast milk was 3.23 (0.47) grams/100mL during 4-6 months of postpartum age of lactating mothers [12]. The lower fat content in this study may be from the maternal nutritional status and circadian variation because the mean fat concentrations increased from 08.00-12.00 hours to 12.00-16.00 hours and reached a peak between 16.00 and 20.00 hours [13].

The temperature of storage and thawing may influence the fat content in human milk, especially at higher temperatures. Excessive heat can destroy the beneficial components of breast milk for infants that were premature or sick. The lower temperature of storage can decrease fat loss from breast milk because lipolysis occurs at temperatures higher than 25°C rather than 15°C [14]. Milk storage at greater than -70°C contains higher concentrations of free fatty acids than freshly collected milk [15]. Moreover, bile salt-dependent lipase in human milk is very sensitive to heat (heatlabile). This enzyme is remarkably stable during 24 hours storage at

WHAT IS ALREADY KNOWN?

- Various protocols recommend thawing frozen milk by placing it in the refrigerator or rewarm by placing the container under warm running water or in a bowl of warm water, thawing rapidly, usually by holding the container under running tepid (not hot) water.

WHAT THIS STUDY ADDS?

- Fat loss of the frozen storage of human milk (-20 °C), thawed by refrigeration (4°C) is less than by warm water (37°C).

temperatures up to 38°C. Milk storage at 15 or 25°C maintains full digestive lipase activity, and even at 38°C only 10-20% of initial activity is lost [16]. Freezing for 28 days at -20°C and thawing samples twice significantly decrease creatinocrit values [17]. The study revealed no difference in the mean triglyceride concentration of 97% of lipid content between fresh breast milk (2.64 g/100 mL) and milk was stored at -18°C for 28 days and then thawed in the refrigerator (4°C) (2.60 g/100 mL) [18].

There are different recommendations and limited laboratory and clinical data about thawing temperatures affecting fat in breast milk. Heat treatment may induce many undesirable changes, such as the loss of water-soluble vitamins (C, folacin and B₆) and adversely affect immune properties [19]. Pasteurization (62.5°C for 30 min) does not alter the milk fatty acid composition [19, 20]. However, sterilization (120°C for 30 min) causes a marked decrease in the total available milk fat content by about 13% due to fat adhesion to the container surface, and a slight decrease in the percentage of linoleic acid (18:2 n-6) and arachidonic acid (20:4 n-6) [19].

The cause of fat loss of thawed frozen breast milk is unclear. The explanation about fat loss by thawed-frozen breast milk between the two different methods of thawing is as follows; the melting point of lipids is a temperature that can change solid fat to oil or liquid fat. The final melting point of cow milk's fat is at 37°C [21]. Therefore, lipids of milk thawed by warm water appear increased in oil form. Oil can adhere to the side of the container at 37°C more than at 4°C. Therefore, the WM group may have levels of fat or oil loss more than the RM. In addition, the former group had oil globules in higher amounts than RM by gross observation. In further study, milk fat globule membrane fractions were examined by electron microscopy for physicochemical analysis of shape, size and amount of oil globules in thawed milk to explain the hypothesis about the cause of fat loss.

A limitation of this study is that it does not provide sufficient data for other nutrients, especially essential fatty acids. Another area of study would be to follow the

long term outcomes of clinical nutrition in neonates who are fed breast milk thawed by different methods.

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