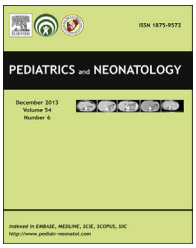
Pediatrics and Neonatology (2013) 54, 360–366

Available online at www.sciencedirect.com

SciVerse ScienceDirect

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



ORIGINAL ARTICLE

Influence of Prolonged Storage Process, Pasteurization, and Heat Treatment on Biologically-active Human Milk Proteins

Jih-Chin Chang [a], Chao-Huei Chen [a,*], Li-Jung Fang [b], Chi-Ren Tsai [a], Yu-Chuan Chang [c], Teh-Ming Wang [a]

[a] *Division of Neonatology, Department of Pediatrics, Taichung Veterans General Hospital, Taichung, Taiwan*
[b] *Department of Pediatrics, Taipei City Hospital, Heping Fuyou Branch, Taipei, Taiwan*
[c] *Department of Pediatrics, Chang Bing Show Chwan Memorial Hospital, Changhua, Taiwan*

Received Oct 1, 2012; received in revised form Jan 9, 2013; accepted Apr 16, 2013

Key Words
human milk;
lactoferrin;
leptin;
lysozyme;
secretory immunoglobulin A

Objectives: The bioactive proteins in human milk may be influenced by prolonged storage process, pasteurization, and heat treatment. This study was conducted to evaluate the effects of these procedures.
Materials and methods: Three forms of human milk — freshly expressed, frozen at −20°C for a prolonged duration, and pasteurized milk — were collected from 14 healthy lactating mothers and a milk bank. The concentrations of major bioactive proteins (secretory immunoglobulin A, lactoferrin, lysozyme, and leptin) were quantified using enzyme-linked immunosorbent assay kits. Changes in these proteins by heat treatment at 40°C or 60°C for 30 minutes were further evaluated.
Results: The mean concentrations of lactoferrin and secretory immunoglobulin A were significantly reduced by 66% and 25.9%, respectively, in pasteurized milk compared with those in freshly-expressed milk. Heat treatment at 40°C or 60°C did not cause significant changes in lactoferrin and secretory immunoglobulin A, but there was an apparent increase in lysozyme ($p = 0.016$). There were no significant differences in leptin level among these three forms of milk prior to ($p = 0.153$) or after heat treatment ($p = 0.053$).
Conclusion: Various freezing/heating/pasteurization processes applied to human milk prior to delivery to neonates could affect the concentration of immunomodulatory proteins, especially lactoferrin, secretory immunoglobulin A, and lysozyme. Leptin was unaffected by the various

* Corresponding author. Division of Neonatology, Department of Pediatrics, Taichung Veterans General Hospital, Number 160, Section 3, Chung-Kang Road, Taichung 407, Taiwan.
E-mail address: joy1477@gmail.com (C.-H. Chen).

http://dx.doi.org/10.1016/j.pedneo.2013.03.018

handling processes tested. Fresh milk was found to be the best food for neonates. Further studies are warranted to evaluate the functional activity of these proteins and their effects on infants' immunological status.

1. Introduction

Human milk is the best food for neonates and is recommended by the American Academy of Pediatrics and the World Health Organization.[1,2] Extensive research has documented the diverse and compelling short- and long-term benefits of breastfeeding in comparison with cows' milk-based formulas. It is an invaluable food source for neonates as it contains not only numerous nutrients but also biologically-active proteins and immune factors[3] that are not present in other forms of milk and are particularly beneficial for very low birth-weight neonates.[4] Secretory immunoglobulin A (sIgA), lysozyme, lactoferrin, and leptin, for instance, have anti-infective, immune, or neuroendocrine properties.[3,5] Several studies have shown that these bioactive proteins are influenced by numerous factors, including gestational age,[3,6] lactation stage,[7,8] the mother's body mass index,[6] and the mother's nutrition.[9]

Breastfeeding is the optimal way to preserve all of these biologically-active proteins. However, if a baby is sick or a mother returns to work, breastfeeding is not an option. Mothers can express milk and refrigerate it for use later after gentle warming. Frozen stored milk can also be used after thawing and heating at a temperature of around 40—60°C. When the mother's own milk is unavailable or in short supply, donor milk that has undergone Holder pasteurization at a milk bank can also be used.

Handling of expressed milk mainly comprises cooling, freezing, heating, and pasteurization. Studies over decades have revealed that some of the above mentioned procedures including storage,[10] pasteurization,[11,12] sonification,[13] and heat treatment,[14] may have an effect on the immunologic components contained therein.

Little is known about the effect of frozen storage, pasteurization, and heat treatment of human milk on the level of leptin it contains. Moreover, there is no recommendation for "sub-pasteurization" heat treatment (4—60°C) regarding these proteins in clinical practice. Thus, there were two main objectives in the present study: (1) to compare the concentrations of sIgA, lysozyme, lactoferrin, and leptin in the different storage forms of human milk; and (2) to further analyze the changes of these proteins after two different temperature heat treatments (40°C and 60°C) used in daily practice.

2. Materials and Methods

This study was approved by the Institutional Review Board of Taichung Veterans General Hospital and Taipei City Hospital. Informed consent was obtained from all participants prior to the study. The study was conducted at Taichung Veterans General Hospital, Taichung, Taiwan, between October 2011 and January 2012.

2.1. Acquisition of milk samples

The three forms of human milk used in this study were as follows: freshly expressed milk ("fresh milk" group) obtained from 14 healthy lactating mothers through the Division of Neonatology, Department of Pediatrics, Taichung Veterans General Hospital; mothers' milk stored at −20°C for at least four weeks ("frozen milk" group), which was intended for donation to the Mothers' Milk Bank of Taipei City Hospital; and pasteurized milk ("pasteurized milk" group) obtained from the Mothers' Milk Bank of Taipei City Hospital. Milk in the frozen and pasteurized groups was surplus milk obtained via random sampling for donor milk quality control and was collected from 15 mothers through the branch for women and children of Taipei City Hospital. The clinical data (gestational age, birth body weight, parity, and lactation date) and demographic data (maternal age, maternal body weight, and maternal body height) of the enrolled mothers were collected and informed consent was obtained from all milk donors.

Milk specimens were handled as follows: fresh milk specimens were stored at 4°C from the time of collection and processed within 24 hours; frozen or pasteurized milk specimens were thawed at 4°C for 24 hours prior to processing as described below (see Figure 1).

2.2. Heat treatment and skimmed milk preparation

We used a water bath for heat treatment. A volume of 10 mL of thawed frozen milk was bathed in plastic tubes at 40°C and 60°C for 30 minutes, respectively, and was collected for further analysis. Other specimens were analyzed at 4°C. The 10 mL human milk sample was centrifuged at 1500*g* for 20 minutes at 4°C to separate the fat layer, which was subsequently discarded. These defatted milk specimens were centrifuged again at 10,000*g* for 10 minutes at 4°C, and the supernatants (whey fraction) were isolated. All collected samples were stored in 1-mL aliquots and frozen at −70°C until further assay.

2.3. Analytical methods

Each specimen was assayed in duplicate and measurements were carried out according to the manufacturer's directions. The reported results represent the mean of these two measurements. The samples were diluted if necessary. After appropriate preparation, enzyme-linked immunosorbent assay (ELISA) or the following enzyme immunoassay

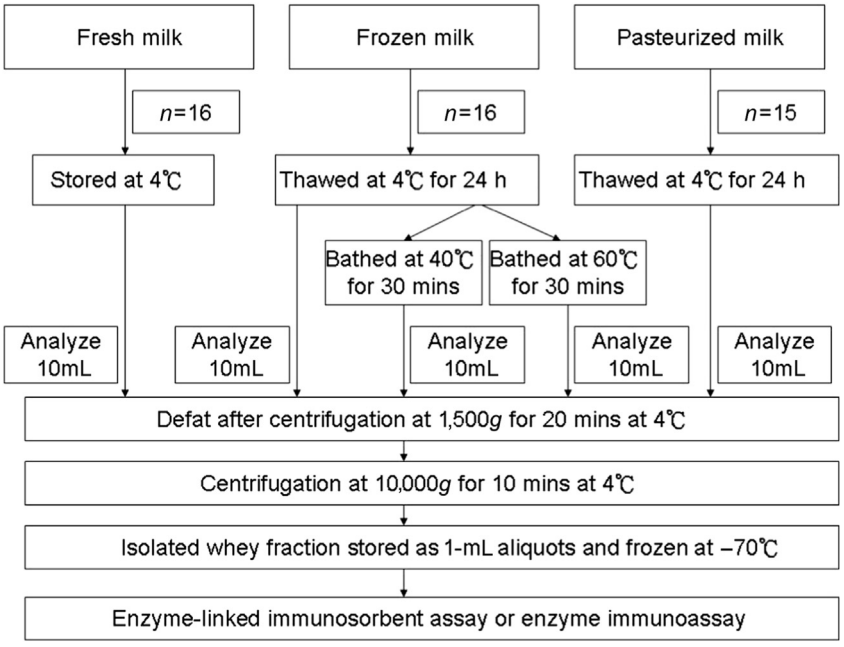


Figure 1 Description of the study design.

kits were used to quantify the levels of sIgA, lysozyme, lactoferrin, and leptin of each milk sample: (1) sIgA ELISA kit (K8870; Immundiagnostik AG, Bensheim, Germany) with a sensitivity of 13.4 ng/mL, intra- and interassay coefficients of variation of 5—9% and 7.4—8.0%, respectively; (2) lysozyme enzyme immunoassay kit (Biomedical Technologies Inc., Stoughton, MA, USA) with a sensitivity of 0.78 ng/mL, intra- and interassay coefficients of variation of 5.3% and 7%, respectively; (3) lactoferrin enzyme immunoassay kit (Oxis International, Burlingame, CA, USA) with a sensitivity of 1.0 ng/mL, intra- and interassay coefficients of variation of 5.0% and 9.6%, respectively; (4) leptin ELISA kit (Millipore Corporation, Billerica, MA, USA) with a sensitivity of 0.5 ng/mL, intra- and interassay coefficients of variation of 2.6—4.6% and 2.6—6.2%, respectively.

2.4. Statistical analysis

Data were analyzed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA), the Kruskal-Wallis test, and the Mann-Whitney U test. Significant differences were defined as $p < 0.05$.

3. Results

3.1. Demographic characteristics of milk donors

A total of 47 milk specimens were used in the study: 16 fresh milk, 16 frozen milk, and 15 pasteurized milk samples. Frozen and pasteurized specimens were provided by the Mothers' Milk Bank of Taipei City Hospital from 15 donors, and the fresh milk samples were collected from 14 other lactating mothers. For pasteurized milk, each specimen had been subjected to Holder pasteurization (62.5°C for 30 minutes, conducted by the Mothers' Milk Bank) and refreezing at −20°C until further analysis. Among these mothers, one of the 14 fresh milk donors had a late preterm twin delivery, and three of the 15 frozen/pasteurized milk donors comprised two mothers with a late preterm delivery and one with early preterm delivery (data not shown). Perinatal screening examination data were not available. The postnatal ages of these infants on the day of lactation ranged from 2 weeks to 8 months. The demographic characteristics of the donors are compared in

Table 1 Demographic characteristics of milk donors.

	Fresh milk ($n = 14$)	Frozen/pasteurized milk ($n = 15$)	p
Age, y	31.43 ± 2.85	32.67 ± 4.08	0.533
Body weight, kg	55.75 ± 9.06	56.67 ± 9.12	0.983
Body height, m	1.60 ± 0.06	1.61 ± 0.05	0.591
Body mass index	21.72 ± 3.09	21.87 ± 3.41	0.813
Gestational age, wk	39.06 ± 1.26	37.74 ± 4.27	0.591
Parity	1.64 ± 0.63	1.60 ± 0.91	0.591
Day of lactation	148.86 ± 78.12	128.67 ± 70.07	0.505
Birth body weight, g	3157.60 ± 408.23	3074.40 ± 797.21	0.533

Data are presented as mean ± SD.

Table 1. There were no significant differences between the two groups of mothers in age, body weight, body height, body mass index, parity, day of lactation or birth body weight of infants.

3.2. Concentrations of bioactive proteins in different forms of milk

A comparison of the bioactive protein analyzed in the three different forms of human milk is shown in Figure 2. The mean concentrations of lactoferrin in pasteurized milk and frozen milk were 66% and 11.5% lower, respectively, compared with that in fresh milk ($p < 0.0001$ and $p = 0.445$). Compared with fresh milk, the concentration of lysozyme in frozen milk was 39.8% lower ($p < 0.0001$). The concentration of lysozyme was slightly lower in pasteurized milk, but much lower in frozen milk in comparison with that in fresh milk ($p = 0.078$ and $p < 0.0001$, respectively). The mean concentrations of sIgA were 8.2% and 25.9% lower in frozen milk and pasteurized milk, respectively ($p = 0.171$ and $p = 0.0004$), compared with that in fresh milk. In comparison with fresh milk, sIgA in frozen milk was lower but not significantly so ($p = 0.171$). No differences in concentrations of leptin were found among the three forms of human milk ($p = 0.153$).

3.3. Change in bioactive proteins after heat treatment

A comparison of the bioactive proteins after two different temperature heat treatments is shown in Figure 3. Compared with the 4°C group, the concentration of lactoferrin was not significantly affected at 40°C or 60°C ($p = 0.317$). Similarly, heat treatment did not cause significant changes in the concentration of sIgA ($p = 0.589$). The mean concentrations of lysozyme increased significantly, however, with heat treatment: by 53.2% and 56.3% at 40°C and 60°C, respectively ($p = 0.032$ and $p = 0.0039$). No significant differences were noted in leptin level between each of the heat treatment groups and that of the 4°C group, but there was a slight difference between the 40°C and 60°C groups ($p = 0.021$).

4. Discussion

Neonates and preterm infants are especially prone to a variety of infectious pathogens as their immune system is immature. Passive immunity provides sufficient protection against infections via maternal immunoglobulin G antibodies transported transplacentally during the last trimester of pregnancy and via sIgA antibodies in breast

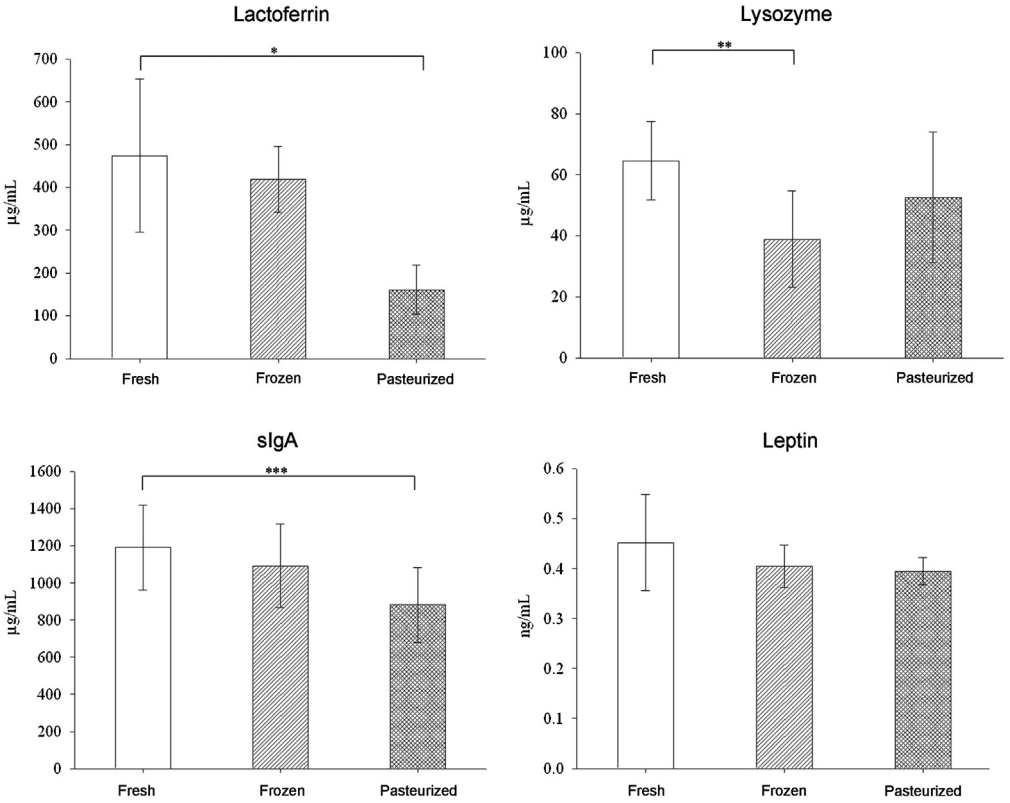


Figure 2 Concentrations of bioactive proteins in different forms of human milk. The concentrations of lactoferrin, lysozyme, secretory immunoglobulin A (sIgA), and leptin, in whey fractions isolated from freshly expressed human milk (fresh, $n = 16$), prolonged frozen human milk (frozen, $n = 16$) or pasteurized human milk (pasteurized, $n = 15$) were analyzed as described in the Materials and methods section. *$p < 0.0001$ for fresh or frozen *versus* pasteurized milk; **$p < 0.0001$ for fresh *versus* frozen milk; ***$p < 0.05$ for fresh or frozen *versus* pasteurized milk.

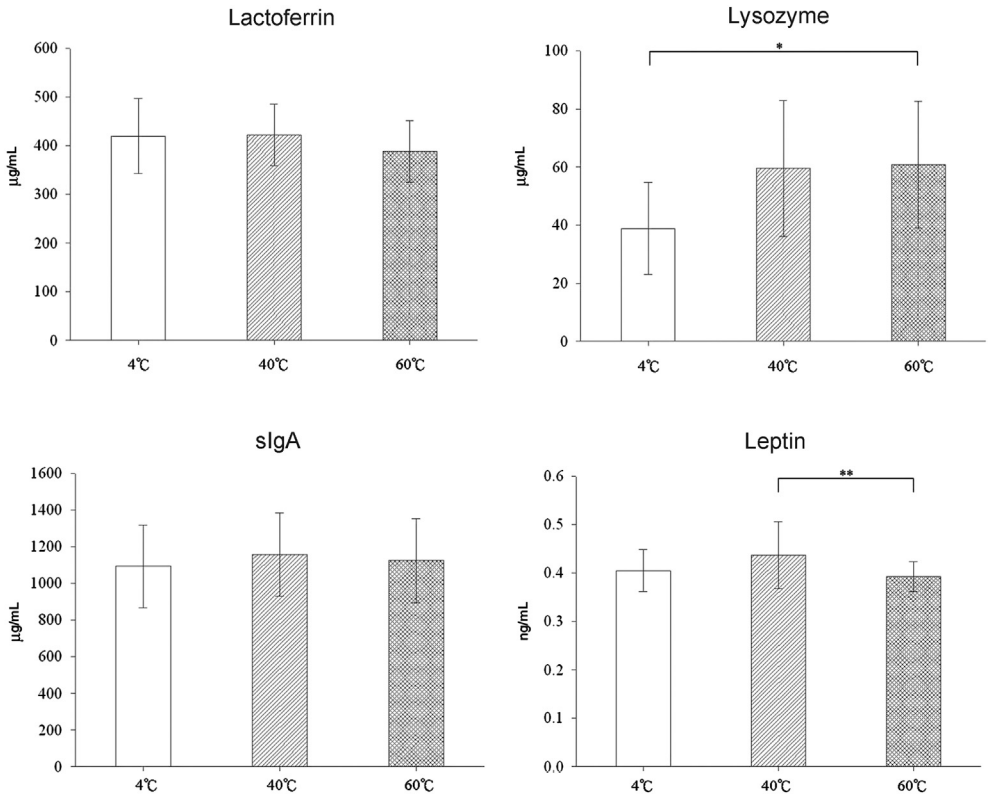


Figure 3 Change in bioactive proteins after heat treatment. The concentrations of lactoferrin, lysozyme, secretory immunoglobulin A (sIgA), and leptin in whey fraction isolated from prolonged frozen human milk at 4°C, 40°C or 60°C were assessed as described in the Materials and methods section. *$p < 0.05$ for 4°C *versus* 40°C or 60°C; **$p < 0.05$ for 40°C *versus* 60°C.

milk.[15,16] Besides sIgA, there are plenty of bioactive proteins in human milk (such as lactoferrin, lysozyme, and leptin) that serve as immunomodulatory factors. These precious factors are produced by abundant cells in human milk.[17] Studies have revealed a number of their functions: (1) sIgA has specific antigen-targeted anti-infective action; (2) lactoferrin plays important roles in immunomodulation, iron chelation and antimicrobial action, and exhibits the anti-adhesive and trophic properties necessary for intestinal growth; (3) lysozyme is required for bacterial lysis and immunomodulation[18,19]; and (4) leptin regulates the neuroendocrine system and hemopoetic cells,[3] and correlates with neonatal weight gain[20] as well as subsequent development of obesity.[21]

These proteins are dynamic and most of them change with lactation stage, but they remain at a relatively constant level in mature milk.[22,23]

When freshly-expressed milk is unavailable, frozen stored milk or pasteurized donor milk can be used after appropriate handling. However, routine handling methods in daily practice can induce changes in the valuable nutrients contained in human milk. In the present study, concentrations of lactoferrin, lysozyme, and sIgA were reduced by varying amounts in the frozen and pasteurized human milk, but not by heat treatment at 40°C or 60°C. By contrast, lysozyme was found to be present in greater concentrations in sub-pasteurization heat-treated milk. The concentration of leptin, however, remained relatively unchanged in all forms of human milk tested as well in the different heat treatment groups.

Expressed human milk is often refrigerated for days and frozen for months if not used immediately. Earlier studies by Bjorksten et al and Evans et al reported a minimal effect on human milk by freezing[24] and found no significant changes in lactoferrin, lysozyme, or IgA after freezing for 3 months.[25] A recent study by Akinbi and associates suggested that frozen storage resulted in a lower reduction in sIgA, lactoferrin, and lysozyme in comparison with pasteurization.[12] Similarly, our study revealed a non-significant decrease in most of these proteins, except for lysozyme, in frozen milk. With respect to the retention of bioactive proteins, our data support the use of frozen milk as an alternative to fresh milk in daily practice.

Holder pasteurization (62.5°C for 30 minutes) arose from the need to eliminate pathogen transmission via raw donor milk and is now required by the Human Milk Banking Association of North America and many other national milk banking guidelines.[26] However, pasteurized milk from milk banks undergoes complex handling after collection, including two cycles of freezing and thawing interspaced by heating using the Holder method prior to delivery to neonates. Several studies have shown significant attenuation of macro- and micronutrients after pasteurization.[12,26,27] The findings of the present study support previously reported results showing a substantial decrease in sIgA and lactoferrin in pasteurized milk. The minimal effect on lysozyme by the Holder method in this study is compatible with

findings of earlier research.[14,28] This study added that leptin seemed more stable to pasteurization in comparison with other bioactive proteins. This finding is somewhat different from the report by Resto et al, who suggested that leptin diminished after pasteurization.[29] This difference may be due to the different pasteurization methods used in our study and theirs. Although the correlation between diminutions of these proteins and clinical outcome is not yet well clarified, faster and significantly increased bacterial proliferation in pasteurized donor milk was observed.[12,24] Later contamination of pasteurized milk during shipment should be carefully avoided due to the compromised innate bacteriostatic properties. For better preservation of these proteins, different types of pasteurization method were developed,[11,30,31] but the Holder method has remained the standard and is the most widely accepted.

Generally proteins denature more rapidly with increased temperature. In the 1970s several experts reported on human milk content that was subjected to heat treatments of graded severity.[14,25,28] In Figure 3 we present the changes in these protein levels after subpasteurization heat treatments at 40°C or 60°C. Little effect on lactoferrin, sIgA, and leptin was noted, whereas lysozyme concentration was significantly increased by heat treatment, which is consistent with previous reports that suggested this component was not only thermally stable but also largely sequestered in human milk.[25,30,32] Sensitivity of lactoferrin, sIgA and lysozyme to heat treatments of graded severity has been illustrated in different reports,[11,14,25,28,30,32] and we may find a small consistency as heat treatments below 62.5°C (the heat treatment temperature in the Holder method) have a minimal impact on lactoferrin, sIgA, and lysozyme, whereas heat treatments above 62.5°C make inactivate these proteins more rapidly. When heating up to 70—73°C, few proteins survive.[24] However, many heat treatment-associated factors, such as heating time, heat treatment design, milk volume processed, total milk proteins, and analytical method, may also influence the result and should be taken into consideration when being applied to daily practice.

Freshly-expressed human milk remains the optimal food for infants. Our data again support this opinion and indicate that the handling process can have a large or small influence on the nutrient contents of human milk. Lactoferrin, sIgA, and lysozyme, the most frequently studied immunoactive proteins in human milk, are susceptible to complex handling but are mostly preserved under freezing or heat treatments below 60°C. Leptin has the greatest stability to freezing, heating, or pasteurization. It is novel and of interest because preterm or low-birth-weight infants are often fed with frozen or pasteurized milk, which has as much leptin as fresh milk. Further studies about the effects on neonatal weight gain and neuroendocrine system maturation by leptin in processed milk are warranted. There are still some limitations in this study: we quantified these proteins but their qualities and activities were not measured. Correlations between diminished immunomodulatory proteins in human milk and neonatal immunological outcome are also needed for clarification.

In conclusion, biologically-active proteins in human milk are susceptible to variable handling, such as heating and pasteurization. A mother's own freshly expressed milk remains the best food for neonates as it contains the most unaffected, valuable nutrients. Frozen milk may be suggested as an alternative choice if fresh milk is unavailable and low-grade heat treatments (below 60°C) are permissible.

Acknowledgments

The authors are grateful to the Biostatistics Task Force of Taichung Veterans General Hospital, Taichung, Taiwan, ROC for help with statistical analysis and the Mothers' Milk Bank of Taipei City Hospital for help with milk specimens collection.

References

1. World Health Organization. *Exclusive breastfeeding*, WHO. Available at: http://www.who.int/nutrition/topics/exclusive_breastfeeding/en/ Accessed April 25, 2013.
2. Section on Breastfeeding. Breastfeeding and the use of human milk. *Pediatrics* 2012;**129**:e827—41.
3. Mehta R, Petrova A. Biologically active breast milk proteins in association with very preterm delivery and stage of lactation. *J Perinatol* 2011;**31**:58—62.
4. Labbok MH, Clark D, Goldman AS. Breastfeeding: maintaining an irreplaceable immunological resource. *Nat Rev Immunol* 2004;**4**:565—72.
5. Bertino E, Giuliani F, Occhi L, Coscia A, Tonetto P, Marchino F, et al. Benefits of donor human milk for preterm infants: current evidence. *Early Hum Dev* 2009;**85**(10 Suppl):S9—10.
6. Eilers E, Ziska T, Harder T, Plagemann A, Obladen M, Loui A. Leptin determination in colostrum and early human milk from mothers of preterm and term infants. *Early Hum Dev* 2011;**87**:415—9.
7. Weaver LT, Arthur HM, Bunn JE, Thomas JE. Human milk IgA concentrations during the first year of lactation. *Arch Dis Child* 1998;**78**:235—9.
8. Bronsky J, Mitrova K, Karpisek M, Mazoch J, Durilova M, Fisarkova B, et al. Adiponectin, AFABP, and leptin in human breast milk during 12 months of lactation. *J Pediatr Gastroenterol Nutr* 2011;**52**:474—7.
9. Bronsky J, Karpísek M, Bronská E, Pechová M, Jancíková B, Kotolová H, et al. Adiponectin, adipocyte fatty acid binding protein, and epidermal fatty acid binding protein: proteins newly identified in human breast milk. *Clin Chem* 2006;**52**:1763—70.
10. Ogundele MO. Techniques for the storage of human breast milk: implications for anti-microbial functions and safety of stored milk. *Eur J Pediatr* 2000;**159**:793—7.
11. Czank C, Prime DK, Hartmann B, Simmer K, Hartmann PE. Retention of the immunological proteins of pasteurized human milk in relation to pasteurizer design and practice. *Pediatr Res* 2009;**66**:374—9.
12. Akinbi H, Meinzen-Derr J, Auer C, Ma Y, Pullum D, Kusano R, et al. Alterations in the host defense properties of human milk following prolonged storage or pasteurization. *J Pediatr Gastroenterol Nutr* 2010;**51**:347—52.
13. Lawrence RA. Milk banking: the influence of storage procedures and subsequent processing on immunologic components of human milk. *Adv Nutr Res* 2001;**10**:389—404.
14. Ford JE, Law BA, Marshall VM, Reiter B. Influence of the heat treatment of human milk on some of its protective constituents. *J Pediatr* 1977;**90**:29—35.

15. Niers L, Stasse-Wolthuis M, Rombouts FM, Rijkers GT. Nutritional support for the infant's immune system. *Nutr Rev* 2007; **65**:347—60.
16. Kleinman RE, Walker WA. The enteromammary immune system: an important new concept in breast milk host defense. *Dig Dis Sci* 1979;**24**:876—82.
17. Lawrence RA, Lawrence RM. *Breastfeeding*. 6th ed. St. Louis: Mosby; 2005.
18. Hamosh M. Bioactive factors in human milk. *Pediatr Clin North Am* 2001;**48**:69—86.
19. American Academy of Pediatrics and The American College of Obstetricians and Gynecologists. *Breastfeeding handbook for physicians*. Elk Grove Village, IL: Washington, DC: American Academy of Pediatrics; American College of Obstetricians and Gynecology American Academy of Pediatrics; 2006.
20. Doneray H, Orbak Z, Yildiz L. The relationship between breast milk leptin and neonatal weight gain. *Acta Paediatr* 2009;**98**: 643—7.
21. Savino F, Fissore MF, Liguori SA, Oggero R. Can hormones contained in mothers' milk account for the beneficial effect of breast-feeding on obesity in children? *Clin Endocrinol (Oxf)* 2009;**71**:757—65.
22. Goldman AS, Garza C, Nichols BL, Goldblum RM. Immunologic factors in human milk during the first year of lactation. *J Pediatr* 1982;**100**:563—7.
23. Lewis-Jones DI, Lewis-Jones MS, Connolly RC, Lloyd DC, West CR. Sequential changes in the antimicrobial protein concentrations in human milk during lactation and its relevance to banked human milk. *Pediatr Res* 1985;**19**:561—5.
24. Björkstén B, Burman LG, De Château P, Fredrikzon B, Gothefors L, Hernell O. Collecting and banking human milk: to heat or not to heat? *Br Med J* 1980;**281**:765—9.
25. Evans TJ, Ryley HC, Neale LM, Dodge JA, Lewarne VM. Effect of storage and heat on antimicrobial proteins in human milk. *Arch Dis Child* 1978;**53**:239—41.
26. Tully DB, Jones F, Tully MR. Donor milk: what's in it and what's not. *J Hum Lact* 2001;**17**:152—5.
27. 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.
28. Eyres R, Elliott RB, Howie RN, Farmer K. Low-temperature pasteurisation of human milk. *N Z Med J* 1978;**87**:134—5.
29. Resto M, O'Connor D, Leef K, Funanage V, Spear M, Locke R. Leptin levels in preterm human breast milk and infant formula. *Pediatrics* 2001;**108**:E15.
30. Wills ME, Han VE, Harris DA, Baum JD. Short-time low-temperature pasteurisation of human milk. *Early Hum Dev* 1982;**7**:71—80.
31. Baro C, Giribaldi M, Arslanoglu S, Giuffrida MG, Dellavalle G, Conti A, et al. Effect of two pasteurization methods on the protein content of human milk. *Front Biosci (Elite Ed)* 2011;**3**:818—29.
32. Goldblum RM, Dill CW, Albrecht TB, Alford ES, Garza C, Goldman AS. Rapid high-temperature treatment of human milk. *J Pediatr* 1984;**104**:380—5.