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