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IgA, IgG, IgM and Lactoferrin Contents of Human Milk During Early Lactation and the Effect of Processing and Storage

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ABSTRACT

The total IgA, IgG, IgM and lactoferrin concentrations in human milk from 89 donors were studied at three lactational stages: early transitional (3 to 8 d postpartum), transitional (10 to 14 d postpartum) and mature (30 to 47 d postpartum). The effects of processing and storage on these components in composite samples of mature human milk were determined. There were no significant diurnal variations in any of the four protective factors at either the transitional or mature stages. Concentrations of total IgA, IgM and lactoferrin decreased significantly as time postpartum increased, whereas the IgG content showed no significant changes. The total IgA, IgM and lactoferrin levels were significantly decreased by all heat treatments (62.5°C for 30 min, 72°C for 15 s, 88°C for 5 s, and 100°C for 5 min). Heating at 62.5°C for 30 min did not affect the IgG content; however, the other heat treatments significantly reduced IgG concentration. At the times and temperatures selected for this study, the two lower temperature treatments were less detrimental to the protective factors than the higher temperature treatments.

The practice of breast-feeding as a major method of infant feeding has decreased significantly in developed countries of the world (*12*). In the United States, however, there has been a reversal of that trend. A survey of infants one week of age showed that the percentage who were being exclusively breast-fed increased from 24.9% in 1970 to 49.7% in 1979 (*16*). Likewise, there has been renewed interest in milk banks to supply human milk to infants who might benefit from its consumption.

One of the primary reasons for the resurgence of interest is the protection against infection that has been associated with breast-feeding (*25*). Larsen and Homer (*13*) reported that, of 107 infants hospitalized with acute gastroenteritis, only one was being breast-fed. Fallot et al. (*8*) found that 11% of 136 infants 0 to 3 months of age that were hospitalized during a 1-year period were breast-fed. The expected frequency of exclusively breast-fed infants based on community feeding patterns was 25.2%. These authors also reported that none of those in breast-fed groups suffered bacterial infections, whereas 27 cases of bacterial infections were documented among 121 non-breast-fed infants. Fallot et al. (*8*) concluded that exclusive breast-feeding of infants less than 3 months of age significantly reduced the incidence of infections that would lead to hospitalization.

Among the components that are thought to contribute to the antibacterial properties of human milk are immunoglobulins IgA, IgG and IgM and lactoferrin (*18*). Although these proteins are not absorbed, they do provide passive immunity in the intestinal tract (*11*). Lactoferrin has been shown to inhibit the growth of *Escherichia coli (3), Staphylococcus albus, Staphylococcus aureus* and *Pseudomonas aeruginosa (17)*. IgA is quite resistant to proteolysis and pH change, and is thought to prevent infection by blocking adherence of pathogens to the intestinal mucosa (*26*). Many researchers have studied levels of these protective factors in human milk (*4,5,10,19-23,27,28*), yet most have utilized relatively few donors. One of the most definitive studies concerning the changes in immunoglobulin levels was done by Ogra and Ogra (*21*). In sequential testing from 1 to 180 d postpartum, these authors found that IgA and IgM levels declined as lactation progressed and that the IgG content remained constant.

Considering the beneficial effects of immunoglobulins and lactoferrin, retention in banked human milk is important. While some scientists feel that it is unnecessary to pasteurize donated milk (*2*), the American Academy of Pediatrics Committee on Nutrition (*1*) feels that with the possibilities for bacterial contamination, some form of heat treatment is necessary. There is concern that processing adequate from a microbiological standpoint may be too severe for some of the protective factors. Previous studies on the effect of processing human milk have looked primarily at long time heat treatments at temperatures ranging from 56 to 100°C (*6,7,9,*). These reports showed that, while keeping the heating time constant at either 15 or 30 min,

TABLE 1. *Comparison of morning and evening levels of total IgA, IgG, IgM and lactoferrin in transitional and mature milk.*[a]

Protective factor	Mean ± SD mg/100 ml			
	Transitional		Mature	
	A.M.	P.M.	A.M.	P.M.
Total IgA	30.5 ± 14.6	31.5 ± 13.6	25.8 ± 12.3	26.7 ± 11.7
IgG	2.1 ± 1.4	1.9 ± 1.4	2.2 ± 1.3	2.1 ± 1.2
IgM	5.8 ± 3.5	5.8 ± 3.3	3.7 ± 2.6	3.8 ± 3.8
Lactoferrin	459 ± 198	456 ± 204	299 ± 177	324 ± 201

[a]There was no significant difference (P>0.05) between the A.M. and P.M. samples for any of the four protective factors at the transitional and mature stages.

increasing the temperature resulted in increased inactivation of the protective factors. There is limited information, however, on the effects of high-temperature, short-time (HTST) processing.

This study was designed to determine changes in the lactoferrin, IgA, IgG and IgM concentrations of human milk during the first month postpartum, to determine diurnal effects on concentration, and to determine the effects of both low-temperature, long-time (LTLT) and HTST processing and frozen storage on these components.

MATERIALS AND METHODS

Donors

Donors were contacted 1 d postpartum at two local hospitals in Athens, GA. Over a 2.5-year period, a total of 89 woman who signed an informed consent form participated in the project. The women were of middle socioeconomic status and ranged in age from 16 to 38 years. Of the women who participated, 84 were Caucasian, 3 were black and 2 were Oriental. All donors were in good health and delivered healthy, full-term infants.

Collection of milk samples

Milk samples were collected by the donors using either manual expression or a Lopuco breast pump (Lopuco Ltd., Woodbine, MD). The milk was collected at the following three stages of lactation: early transitional (3 to 8 d postpartum), transitional (10 to 14 d postpartum) and mature (30 to 47 d postpartum). One sample was collected at the early transitional stage during the course of several late evening and early morning feedings. Two samples were collected at the transitional and mature stages. For these, donors were instructed to collect approximately equal volumes of milk from the beginning, middle and end of one late evening feeding and one early morning feeding. The samples were refrigerated immediately after collection, picked up after the morning collection and transported on ice to the laboratory.

Immunoglobulin assay

Immunoglobulin levels were quantitated using radial immunodiffusion (*15*). No attempt was made to differentiate between total and secretory IgA; however, Goldman et al. (*10*) recently reported that essentially all of the total IgA in human milk is secretory IgA. The plates and standard reagents for the determination of total IgA, IgG and IgM were obtained from Calbiochem-Behring Corp. San Diego, CA. IgG and IgM were assayed by adding 20 µl of either the milk samples or the standard reagent to each well. For total IgA, the samples were diluted 1:5 with distilled water before adding a 20µl aliquot. The plates were placed in the dark for 6 d to allow for diffusion, washed in 0.85% NaCl for 4 d and stained 10 min with 0.5% Coomassie blue in a solution of 95% ethanol, water and glacial acetic acid (9:9:2). The plates were then destained for at least 10 min in a 5:5:1 mixture of methanol, water and glacial acetic acid (5:5:1). Diameter of precipitation zones was measured and a standard curve was prepared by plotting width vs. standard concentration.

Lactoferrin assay

Lactoferrin was quantitated using the rocket electrophoresis method of Laurell (*14*). Lactoferrin antiserum (Calbiochem-Behring Corp.) was added to a 1% agarose solution. Ten ml were pipetted onto an 8 × 10 cm glass plate and allowed to solidify. Wells 3 mm in diameter were cut along one edge of the plate. Lactoferrin standards were prepared using lyophilized human lactoferrin (Calbiochem-Behring Corp.). Portions of samples and standards were added to the wells. The plates were placed in an electrophoresis chamber at 7°C for 16 h with a constant voltage of 120 mV/cm^2. Plates were washed with 0.85% NaCl for 24 h, allowed to air dry and stained 10 min with 0.5% Coomassie blue. The methanol, water and glacial acetic acid were used for destaining. Standard curves were prepared by plotting peak height vs. log of the concentration.

Processing and storage

The processing and storage study was done on composite samples of mature human milk from at least five different donors. All of the donors had previously participated in the project and had been lactating 2 to 6 months. Each composite sample was divided into five subsamples: one as the control and the other four for the heat treatments. The processing consisted of one low-temperature, long-time (LTLT) pasteurization treatment of 62.5°C for 30 min, two high-temperature, short-time (HTST) treatments of 72°C for 15 s and 88°C for 5 s and one sterilization treatment at 100°C for 5 min. The three lower temperature treatments represent those commonly used for bovine milk. The 100°C-treatment is currently used at the Wilmington, Delaware Mothers Milk Bank. LTLT pasteurization was accomplished by adding 5 ml portions to test tubes preheated in a 62.5°C water bath and holding for 30 min. HTST samples were pumped through a stainless steel capillary heat exchanger tube in either a 72 or 88°C constant temperature water bath and held for 15 and 5 s, respectively. Come-up and cool-down times, monitored by thermocouples inserted into the heat exchanger tube, were 2.5 s. Portions (5 ml) of milk to be sterilized were put in test tubes and heated in an autoclave at 100°C for 5 min. All heat processing and frozen storage trials were done on a minimum of three different composite samples.

RESULTS AND DISCUSSION

There were no significant diurnal variations in any of the four protective factors at either the transitional or mature stages (Table 1). For all further statistical analyses, the morning and evening results were combined. Concentrations of total IgA, IgM and lactoferrin decreased significantly as time postpartum increased, whereas the IgG conshowed no significant changes (Table 2). The mean total IgA values of 43.4, 31.0 and 26.2 mg/100 ml for the three successive stages are considerably lower than most previously reported means. These earlier values range from 100 mg/100 ml for milk collected 2 wk postpartum (*10*) to 490 mg/100 ml for 5-d milk (*19*). All reports which give values for more than one time postpartum show a decline in IgA concentration as lactation progresses (*5,10,19,21,22*). Mean concentrations from 43 to 102 mg/100 ml for 30-d milk from women of different ethnic and socioeconomic groups were recently reported by Cruz et al. (*5*). Their use of the modified enzyme-linked immunosorbent assay may

TABLE 2. *Total IgA, IgG, IgM and lactoferrin contents of early transitional, transitional and mature milk.*

Stage of lactation	Protective factor (mg/100 ml) Mean ± SD			
	Total IgA	IgG	IgM	Lactoferrin
Early transitional[a,b,c]	43.4 ± 16.4x	1.9 ± 1.5x	7.5 ± 4.3x	551 ± 186x
	(4.5-77.0)	(0-7.7)	(0-19.7)	(150-1069)
Transitional[d]	31.0 ± 14.0y	2.0 ± 1.4x	5.8 ± 3.4y	457 ± 201y
	(0-92.5)	(0-6.2)	(0-19.2)	(43-898)
Mature[e]	26.2 ± 11.9z	2.1 ± 1.3x	3.8 ± 3.3z	311 ± 189z
	(4.5-71.5)	(0-6.1)	(0-26.8)	(26-782)

[a]Means in each column followed by different postscripts are significantly different at $p<0.05$ as determined by Duncan's multiple range test.
[b]The range of values is given in parentheses below each mean.
[c]The number of samples is 60 for total IgA, 85 for IgG, 89 for IgM and 85 for lactoferrin.
[d]The number of samples is 95 for total IgA, 133 for IgG, 139 for IgM and 150 for lactoferrin.
[e]The number of samples is 92 for total IgA, 129 for IgG, 124 for IgM and 133 for lactoferrin.

TABLE 3. *Total IgA, IgG, IgM and lactoferrin concentrations in heat-treated and frozen human milk.*

Treatment	Protective factor (mg/100 ml) Mean ± SD			
	Total IgA	IgG	IgM	Lactoferrin
Initial[a,b]	30.6 ± 1.4v	1.2 ± 0.6v	1.0 ± 1.3v	342 ± 102v
62.5°C, 30 min	20.2 ± 0.8w	1.0 ± 0.6vw	0.0w	122 ± 63w
	(66)	(86)	(0)	(36)
72°C, 15 s	19.5 ± 1.7w	0.7 ± 0.4w	0.0w	90 ± 43wx
	(64)	(58)	(0)	(27)
88°C, 5 s	0.0x	0.1 ± 0.3x	0.0w	51 ± 25xy
	(0)	(10)	(0)	(15)
100°C, 5 min	0.0x	0.2 ± 0.3x	0.0w	0y
	(0)	(13)	(0)	(0)
-20°C, 4 wk	27.1 ± 2.3y	1.5 ± 0.5v	0.5 ± 0.9vw	239 ± 38z
	(89)	(123)	(51)	(70)

[a]Means followed by different postscripts are significantly different at $p<0.05$ as determined by Duncan's multiple range test.
[b]Percent retention is given in parentheses below each mean.

explain the higher values they obtained. Carlsson et al. (*4*) indicated that IgA levels in early colostrum may be as high as 2000 mg/100 ml but that the average concentration in mature milk ranges from 25 to 50 mg/100 ml.

No significant changes in the IgG content were seen during the three stages tested. There are reports of IgG content decreasing (*22,23*) but these authors compared colostrum to samples collected 1 to 3 wk postpartum. It appears that the decrease in IgG concentration occurs within 1 d postpartum which is before our initial sampling time.

The IgM content (Table 2) decreased from 7.6 mg/100 ml at the early transitional stage to 5.8 and 3.8 mg/100 ml at the transitional and mature stages, respectively. These means are in agreement with other values in the literature. Yadav et al. (*27*) reported 7.0 mg/100 ml in day 7 milk, whereas McClelland et al. (*19*) reported 10 to 20 mg/100 ml in milk collected 4 to 7 d postpartum and found no detectable levels in late samples. It should be noted that Ogra and Ogra (*21*) studied IgA, IgG and IgM contents of human milk from 1 to 180 d postpartum. Their results for 30-d milk, given in mg/g of protein, can be converted using the Handbook No. 8 (*24*) value of 1.03 g protein/100 g giving means of 26, 2.2 and 4.2 mg/100 g for IgA, IgG and IgM, respectively. These concentrations are in close agreement with our results for mature human milk.

Lactoferrin concentrations of 551, 457 and 311 mg/100 ml for early transitional, transitional and mature milk, respectively, (Table 2) are in the same range as previously reported values. Nagasawa et al. (*20*) found the lactoferrin concentration to decrease from 490 mg/100 ml in colostrum (2 to 5 d postpartum) to 450 mg/100 ml in transitional milk (6 to 10 d postpartum) and 210 mg/100 ml in mature milk (11 to 60 d postpartum). Their study (*20*) showed a mean value of 160 mg/100 ml in milk collected 61 to 210 d postpartum, indicating that the lactoferrin content continues to decrease even after the mature lactational stage is reached. Goldman et al. (*10*) reported a similar trend, although somewhat lower results, with the concentration decreasing from 195 mg/100 ml 2 wk postpartum to 85 mg/100 ml 12 wk postpartum.

The results of the processing and storage study are given in Table 3. The total IgA, IgM and lactoferrin levels were significantly decreased by all heat treatments. Heating at 62.5°C for 30 min did not affect the IgG content; however, the other heat treatments significantly reduced IgG concentration. All heat treatments reduced IgM content to a level that was undetectable. For total IgA and IgG, no significant difference was found between the 62.5°C for 30 min treatment and the 72°C for 15 s treatment. There was also no difference between the 88°C for 5 s and the 100°C for

5 min treatments for these milk components. The two highest temperatures of treatment reduced the total IgA concentration to a level that was undetectable. The lactoferrin concentration decreased with increased temperature up to 100°C where none was detected after 5 min of heating. These results show the same trend that other authors have reported. Evans et al. (*6*) and Eyres et al. (*7*) both indicated that, while holding the time of heating constant at 30 min, increasing the temperature resulted in decreased concentrations of IgA and lactoferrin. Evans et al. (*6*) also reported a decrease in IgG concentration with increased temperature of heating. Ford et al. (*9*) heated human milk at 56 and 62.5°C for 30 min and at 65, 70, 75 and 80°C for 15 min. IgA content decreased with increased temperature. Lactoferrin content also decreased with increased temperature of treatment; however, at temperatures of 70°C and above, virtually all of the lactoferrin was destroyed. Results reported here for IgM levels agree with those of Ford et al. (*9*) who showed that IgM was not detectable after the milk was heated at 62.5°C or above.

While there is still considerable debate over whether or not human milk donated to milk banks should be heat processed before being fed to the neonate, the results of this study indicate that both time and temperature of heating must be considered. At the times and temperatures selected for this study, the LTLT and lower of the two HTST treatments were less detrimental to the protective factors than the higher temperature HTST and sterilization treatments. The two higher temperature treatments resulted in essentially complete inactivation of all four protective factors. In summary, it appears that either a 62.5°C for 30 min or 72°C for 15 s treatment would be better than the higher temperature treatments for processing human milk from the standpoint of retaining protective factors in the milk. It is possible that between the LTLT and the 72°C for 15 s processes there is an optimum time-temperature relationship. Further experimentation is necessary to determine the adequacy of the heat treatments from a microbiological standpoint and to optimize the processing of human milk.

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