

Lysozyme, lactoferrin, and secretory immunoglobulin A content in breast milk: influence of duration of lactation, nutrition status, prolactin status, and parity of mother¹⁻³

Philippe F Hennart, Daniel J Brasseur, Josiane B Delogne-Desnoeck, Michèle M Dramaix, and Claude E Robyn

ABSTRACT Milk lactoferrin (LF), lysozyme (LZ), and secretory IgA (sIgA) were measured cross-sectionally in 127 Zairean mothers, lactating ≥ 18 mo. The 54 urban mothers were of marginal nutrition status [body mass index (BMI) 22.6 ± 2.6 kg/m² and albumin 33.1 ± 4.5 g/L]. The neighboring rural mothers were of significantly ($P < 0.001$) poorer nutrition status (BMI 20.5 ± 2.2 kg/m² and albumin 27.7 ± 5.4 g/L). In both urban and rural mothers, as lactation progressed LF decreased by 33% and 55% whereas sIgA remained unchanged and LZ steadily increased. There was more LZ and sIgA in rural milk, contrasting with the poorer maternal nutrition. As calculated from individual milk yields, the urban infants were fed daily with twice as much LF and sIgA but with similar amounts of LZ as were the rural infants. In the early stage of lactation, the milk of both groups of Zairean mothers contains more sIgA than that of a group of west European (Belgian) mothers ($n = 20$), but the LF and LZ contents were rather similar. *Am J Clin Nutr* 1991;53:32-9.

KEY WORDS Human milk, lysozyme, lactoferrin, secretory IgA, nutrition, prolactin, parity

Introduction

Various epidemiological studies have established that human milk is beneficial for infants. Breast-fed infants experience fewer infectious diseases of both the digestive and the respiratory tracts. This increased resistance is due to a passive protection conferred by antimicrobial substances present in human milk. Among these, lactoferrin (LF), lysozyme (LZ), and secretory immunoglobulins A (sIgA) play a key role (1).

LF acts as an iron-chelating protein with potent antibacterial activity against a wide range of microorganisms requiring iron for growth (2). LZ is an enzyme that cleaves to the cell wall and the outer membrane of a variety of microorganisms, causing lysis (3). The antibacterial activities of these two nonantibody proteins are considered to be the most important factors of the nonspecific immunity.

Milk sIgA appear to provide passive immunoprotection against antigens of microorganisms present in the gastrointestinal tract of the mother (4). sIgA are secreted in milk by plasma cells after attachment of dimeric IgA to a secretory component, making

the protein more resistant to proteolytic enzyme breakdown. When in close contact with the intestinal epithelium, the sIgA prevent antigen binding and uptake (4, 5).

Maternal diet and nutrition status may influence the quantity of milk and to some extent its quality. Malnourished mothers usually produce less milk that is of marginal nutrition value (low in protein, calcium, fat, and water-soluble vitamins) (6).

Few studies have concentrated on the protective factors present in human milk and more precisely on the influence of the mother's nutrition status on the production of protective factors in milk. Most were short-term studies performed on a limited number of mothers suffering from marginal energy deprivation rather than from protein depletion.

The populations settled in the highlands of the Kivu province in Zaire live in a poor rural environment characterized by subsistence economy, rapid demographic expansion, and rudimentary sanitation (7). Malnutrition prevails among infants, including breast-fed infants, and among pregnant and lactating mothers (8). This deficient nutrition status is particularly dramatic for women because they are in charge of heavy physical work in the fields. They suffer throughout life from an endless alternation of childbearing and breast-feeding, two highly energy-consuming processes (9). In this context, the infants are breast-fed for long periods: 100% and 80% are breast-fed at 9 and 18 mo, respectively (10). In this population the antibacterial factors of human milk are of particular interest (11).

The present study was designed to investigate 1) the influence of deprived maternal nutrition on the antibacterial factors of human milk at different stages of long-term lactation (> 18 mo) in a region where protein-energy malnutrition is endemic (12;

¹ From the School for Public Health; the Brussels Children's Hospital; and the Human Reproduction Unit, Hôpital Saint-Pierre, Université Libre de Bruxelles (ULB), Brussels; and the Centre Scientifique et Médical de l'ULB pour ses Activités de Coopération (CEMUBAC), Centre de Recherches en Sciences Naturelles (CRSN), Lwiro, Zaire.

² Supported by research grant 3.4501.81 from the Fonds National Belge de la Recherche Scientifique Médicale.

³ Address reprint requests to PF Hennart, ESP, Campus Erasme, Route de Lennik 808, B-1070 Brussels, Belgium.

Received June 23, 1989.

Accepted for publication January 31, 1990.

P Hennart, unpublished observations, 1983) and 2) the impact of prolactin status and of parity on milk LF, LZ, and sIgA contents in the same population of mothers.

Subjects and methods

Mothers

The study was performed at Kabare Hospital in a mother and child care unit: 54 mothers living in the city of Bukavu (Zaire) and 73 mothers living in the neighboring rural area were observed for 24 h. The number and duration of sucklings were recorded and the total amount of milk fed to the infant during this time was estimated with the test-weighing technique (6; P Hennart, unpublished observations, 1983). Other foods given to the infants were not recorded.

When the urban and the rural areas are compared, the following differences are observed: the duration of breast-feeding is longer, the daily number of suckling times is higher, and supplements are introduced at a lower age (6; P Hennart, unpublished observations, 1983). The primary food supplement is banana; boiled sorghum is added sometimes, and cassava is added rarely.

Descriptive data of the populations under investigation are given in Table 1. Note that primipara in this rural milieu have a higher mean age than do their urban counterparts.

In the rural area the classic adult diet comprises red beans, sweet potatoes, cassava, and banana (fruit or beer). In the city of Bukavu, palm oil, meat, and fish are usually added to this diet.

The experimental protocol was approved by the Centre de Recherches en Sciences Naturelles du Zaire (CRSN); it was a part of the World Health Organization (WHO) collaborative study on breast-feeding (6).

Milk and blood samples

Milk samples (5 mL) and blood samples (5 mL) were collected from each of the 127 Zairean mothers. Blood samples were collected between 1000 and 1600 without standardization of the time in relation to the last meal or to the last suckling. The mean time interval between blood sampling and the initiation of the preceding suckling was 87 min. Milk samples were collected daily during the first postpartum week from 20 Belgian mothers at the Maternity of Saint Pierre Hospital, Brussels. Merthiolate (0.1 mL/L) was added to all serum and milk samples, which were stored at -25°C and eventually transferred to Brussels under dry ice. Serum albumin concentrations were determined by using a paper-electrophoresis technique (13).

LF, LZ, and sIgA were measured by a radioimmunoassay (RIA) by using the second-antibody technique (14). The three proteins were purified to homogeneity as previously described (15, 16) and iodinated with ^{125}I (Amersham Int, Lpc, Amersham, UK) by the chloramine T method (17). The labeled proteins were separated from free iodide by column chromatography with Sephadex G 75 (Pharmacia, Uppsala, Sweden; LF and LZ) or with G 50 column (sIgA). Rabbits were immunized by intramuscular injection with complete Freund adjuvant (Difco Lab, Detroit, MI). The National Research Council's guide for the care and use of laboratory animals was followed.

Standard solutions (200 μL) or milk samples at the adequate dilution (200 μL) were incubated for 24 h at 4°C in disposable

TABLE 1

Descriptive data of the Zairean mothers from the rural and the urban areas of the Kivu province*

	Rural area (n = 73)	Urban area (n = 54)
Age (y)	28.1 \pm 6.7	22.3 \pm 5.5†
Parity	5.2 \pm 3.1	3.1 \pm 2.6
Number of sucklings/24 h	6.8 \pm 1.6	10.1 \pm 4.8†
BMI (kg/m ²)	20.5 \pm 2.2 (15.0–26.5)	22.6 \pm 2.6† (18.0–34.7)
Arm circumference (cm)	24.9 \pm 2.1 (20.0–31.0)	25.8 \pm 2.2‡ (22.6–34.0)
Serum albumin (g/L)	27.7 \pm 5.4 (1.18–3.81)	33.1 \pm 4.5† (2.15–4.65)

* $\bar{x} \pm \text{SD}$; range given in parentheses.

†‡ Significantly different from rural area: † $P < 0.001$, ‡ $P < 0.05$.

glass tubes (12 mm \times 75 mm) with 500 μL of diluted anti-LZ (1/15 000, vol:vol), anti-LF (1/120 000, vol:vol), or anti-sIgA (1/40 000, vol:vol) antiserum. These dilutions were prepared in nonimmunized rabbit serum at 1/1500 (vol:vol) in phosphate-buffered saline (PBS, 0.01 mol/L and pH 7.0). Thereafter, 100 μL of the corresponding iodinated milk protein diluted at 500 cpm/ μL were added to all tubes for a second incubation of 24 h at 4°C . Finally, 200 μL of a sheep antirabbit IgG serum diluted in PBS at 1/75 (vol:vol) was added to all tubes except to those containing the total count of tracer alone (14). After a third incubation of 48 h at 4°C , 4 mL PBS (at 4°C) was added and the tubes were centrifuged at $3330 \times g$ for 60 min. After the supernatant was discarded, ^{125}I in the precipitate was counted (Ultrogamma 1280, LKB, Produktor Ab, Bromma, Sweden). In all three RIAs, the nonspecific binding was $< 5\%$.

The fractions of tracer bound to the polyclonal antibody ranged from 30% to 45%. The sensitivity was 0.125 ng in the RIAs of LF and LZ and 1.0 ng in the RIA of sIgA. In the three RIAs the dilution curves obtained with milk samples were parallel to the standard curves obtained with the highly purified preparation of the corresponding milk protein. In the RIAs of LF and LZ, the dilution curves obtained with a variety of biological fluids (bile, seminal fluid, synovial fluid, serum, cerebrospinal fluid, and urine) were also very similar to those obtained with the corresponding standards. Mean serum LF ($n = 10$) was $253 \pm 39 \mu\text{g/L}$ ($\bar{x} \pm \text{SEM}$). Native LF and iron-saturated LF reacted similarly in the RIA of this milk protein. Some 25% cross reaction was found with bovine LF in the RIA of human LF: the slope of the standard curve obtained with bovine LF being much flatter than that obtained with human LF. No cross reactions were found among LF, LZ, and sIgA. The intraassay coefficients of variation under routine conditions were 7% for LZ, 8% for LF, and 9% for sIgA. The interassay coefficients of variation under the same conditions were 11% for LZ, 13% for LF, and 15% for sIgA.

Serum prolactin was measured by a double-antibody RIA with a polyclonal rabbit antiprolactin serum as previously described (18).

All milk samples from the same Belgian mothers were tested in the same assay. All milk samples from the Zairean mothers



were included in one assay for each of the three antimicrobial proteins.

Statistical evaluation of the data was performed after logarithmic transformation of the individual values by using regression and variance analyses and conducted with the *SPSS 9PO* programs (19) and the usual method of one-way and two-way analyses of variance (20).

Results

Antimicrobial proteins in the milk of Zairean mothers during long-term lactation

Milk LF concentration decreased during the first year of lactation by 33% in the urban mothers and by 55% in the rural mothers (Fig 1). The milk from the urban mothers contained on average more LF (0.70 g/L, SEM 0.67–0.76) than did the milk from the rural mothers (0.60 g/L, SEM 0.57–0.64). The difference is very small but was present at all stages of lactation except one (Fig 1). By use of two-way variance analysis, both the decrease in milk LF concentration with time and the difference between urban and rural mothers are statistically significant ($P = 0.013$ and $P = 0.040$, respectively).

On average the urban mothers had much higher milk yields (612 ± 27 mL/d, $\bar{x} \pm$ SEM) than did the rural mothers (307 ± 16 mL/d). The difference is significant at all stages of lactation (Fig 2). For both rural and urban mothers, the daily milk outputs did not decline with time during 18 mo of lactation (Fig 2).

Parallel to milk concentration, the total amount of LF fed daily to the infant also progressively declined (Fig 1). However, this change is not statistically significant. The urban mothers fed their infants with about twice as much LF (0.45 g/d, SEM 0.40–0.47) as did the rural mothers (0.19 g/d, SEM 0.17–0.20). This difference is highly significant ($P = 0.001$) and was demonstrated at all stages of lactation (Fig 1).

The milk LZ concentration increased progressively and significantly with time ($P = 0.001$), starting at 130 mg/L (SEM 106–163) during the first trimester to reach on average 350 mg/L (SEM 289–426) between the 15th and 18th postpartum month (Fig 3). The milk of the rural mothers contained some 1.7 times ($P = 0.001$) more LZ (250 mg/L, SEM 224–280) than did the milk of the urban mothers (150 mg/L, SEM 139–164). This difference varied according to period of lactation. During the first trimester there was less LZ in the milk of the rural mothers. Thereafter, the trend was inverted (Fig 3).

The amount of LZ fed daily to the infant also increased significantly with time ($P = 0.001$). For LZ there were no systematic differences between the urban and the rural mothers (Fig 3). However, at 0–3 mo and at 15–18 mo more LZ was fed to the infants by the urban mothers (111 and 169 mg/d, respectively) than by the rural mothers (33 and 109 mg/d, respectively): these differences are statistically significant ($P < 0.001$).

The concentration of sIgA was stable throughout the 18 mo of lactation and fluctuated around 1.8 g/L (Fig 4). The milk concentration of sIgA was slightly (25%) but significantly higher ($P = 0.021$) in the rural mothers (2.0 g/L, SEM 1.9–2.1) than in the urban mothers (1.6 g/L, SEM 1.5–1.7).

The amount of sIgA fed daily to the infant was not influenced by the stage of lactation (Fig 4). However, at each time considered, urban mothers transferred more sIgA (1.0 g/d, SEM 0.9–

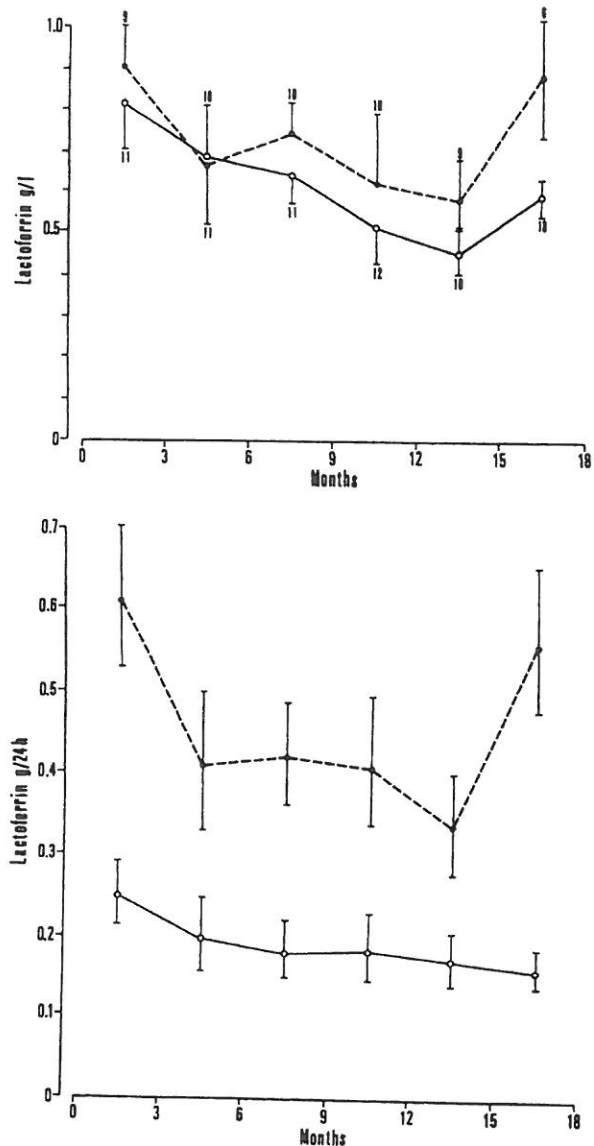


FIG 1. Mean milk lactoferrin concentrations and total amounts fed to the infant per 24 h by lactating mothers from Kivu (Zaire) during 18 postpartum months. The mothers were from a rural (●) or an urban (○) area. Means are calculated by 3-mo intervals. Number of mothers is indicated on top of the vertical bars representing SEMs.

1.1) than did rural mothers (0.6 g/d, SEM 0.5–0.7): this difference is highly significant ($P = 0.001$).

Antimicrobial proteins during the first postpartum week in the milk of Belgian mothers

The milk concentrations of sIgA, LF, and LZ were highest in the colostrum collected on the first day postpartum (Fig 5). When the concentrations of the three milk proteins were compared, the highest was that of sIgA (130 g/L, SEM 103–174). The concentrations of LF and LZ were ~30 and 600 times lower, respectively. During the first 3–4 d of lactation, the milk concen-



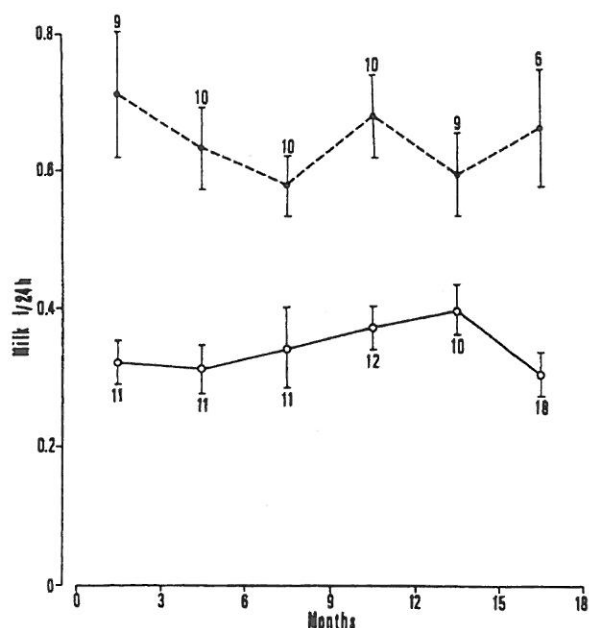


FIG 2. Milk yields (L/24 h) of rural (●) and urban (○) mothers from Kivu (Zaire) at various stages of lactation. Number of mothers is indicated on top of the vertical bars representing SEMs.

trations of all three antimicrobial proteins rapidly decreased. The fall was much more dramatic for sIgA (100-fold) than for LF and LZ (threefold). At the end of the first postpartum week, the milk concentrations of the three proteins had already reached a constant concentration: 76 mg/L for LZ and 1.6 g/L for sIgA and LF (Fig 5). These concentrations of LF and LZ in Belgian mothers are similar to those observed during the first trimester in the milk of the Zairean mothers (Table 2). However, the sIgA concentration was almost two times higher in the milk of the Zairean mothers.

Influence of the parity, the endocrine status, and the nutrition status of the mother

The mean LF concentrations and the daily amounts of LF fed to infants tended to be higher in primipara (0.72 g/L, SEM 0.67–0.78, and 0.31 g/d, SEM 0.27–0.35) than in multipara (0.63 g/L, SEM 0.60–0.66, and 0.26 g/d, SEM 0.24–0.28), but these differences are not statistically significant. sIgA concentration was significantly ($P < 0.05$) higher in multipara (1.90 g/L, SEM 1.79–2.01) than in primipara (1.52 g/L, SEM 1.39–1.66). For the total amount of sIgA fed daily to infants, the difference was only significant in the group of the rural mothers: 0.68 g/d (SEM 0.63–0.74) for the multipara and 0.36 g/d (SEM 0.31–0.42) for the primipara ($P = 0.004$).

By use of calculation of partial correlation coefficients adjusted for the duration of lactation, no significant relationship could be found between the serum concentrations of prolactin (Table 3) and the milk content of LZ, LF, or sIgA in either of the groups of Zairean mothers.

There were also no significant relationships between the milk concentration of LZ, LF, or sIgA and nutrition status as expressed

by body mass index, arm circumference, or serum albumin concentration.

Discussion

The milk concentrations of LZ and sIgA reported in this study are similar to those found by others using immunodiffusion techniques (21–25), radioimmunoassay (26), or enzyme immunoassay (27). We report concentrations of LF, in the milk of both Belgian and Zairean mothers that are lower than those published by Reddy et al (22) and by Prentice et al (28). However, Goldman et al (24), Houghton et al (29), and Lewis-Jones et al (25) found milk LF concentrations very similar to ours. Such

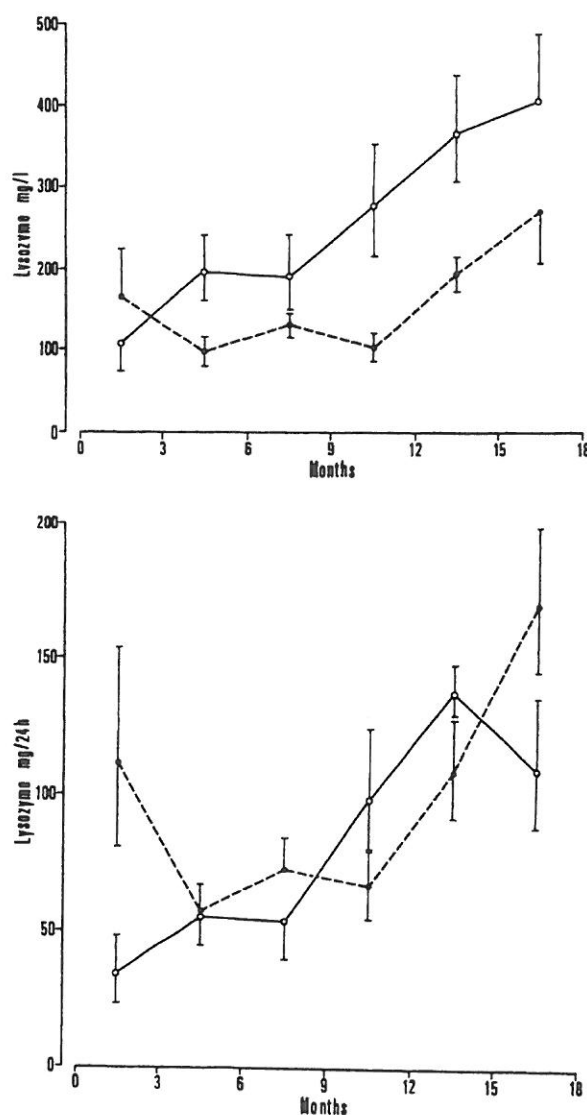


FIG 3. Mean milk lysozyme concentrations and total amounts fed to the infant per 24 h by lactating mothers from Kivu (Zaire) during 18 postpartum months. The mothers were from a rural (●) or an urban (○) area. Vertical bars represent SEMs.



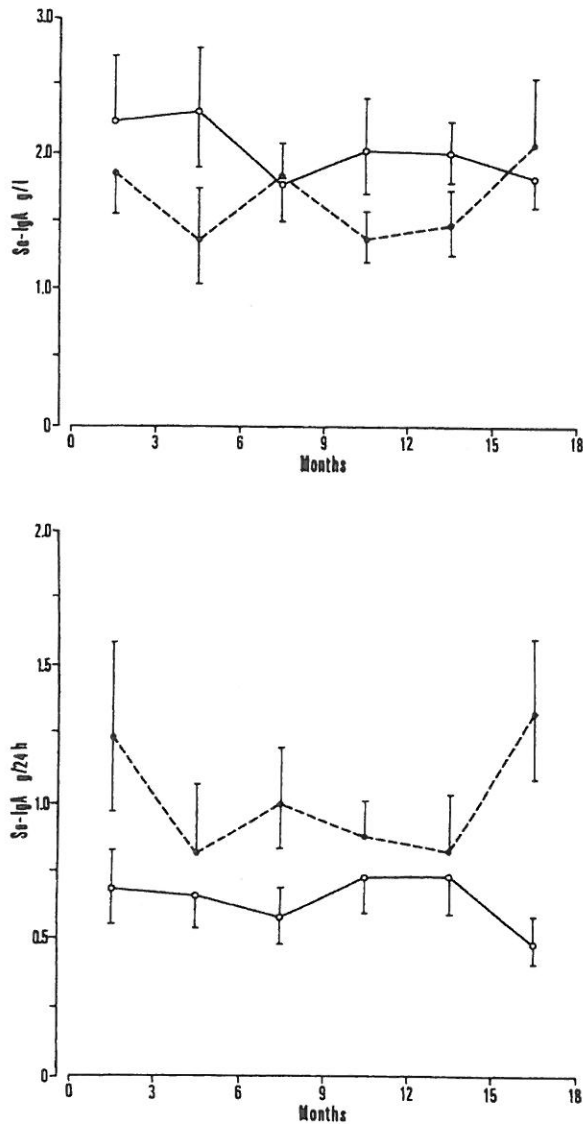


FIG 4. Mean milk secretory IgA concentrations and total amounts fed per 24 h to the infants by lactating mothers from Kivu (Zaire) during 18 postpartum months. The mothers were from a rural (O) or an urban (●) area. Vertical bars represent SEMs.

discrepancies are likely due to differences in the specificity of the radioimmunoassays or in the standard preparations used. Most authors did not mention the LF concentrations found in biological fluids other than milk. The RIA for LF described here has been applied to the measurement of this protein in serum: the mean value found in normal adults (250 $\mu\text{g/L}$) is similar to that reported when systems specifically designed for serum determinations are used (30–35). In addition, the dose-response curves obtained with LF present in various biological fluids were parallel to those obtained with highly purified LF preparations used both as standard and for labeling.

The sIgA concentrations in human milk reported here in terms of a pure sIgA standard preparation obtained from milk cannot

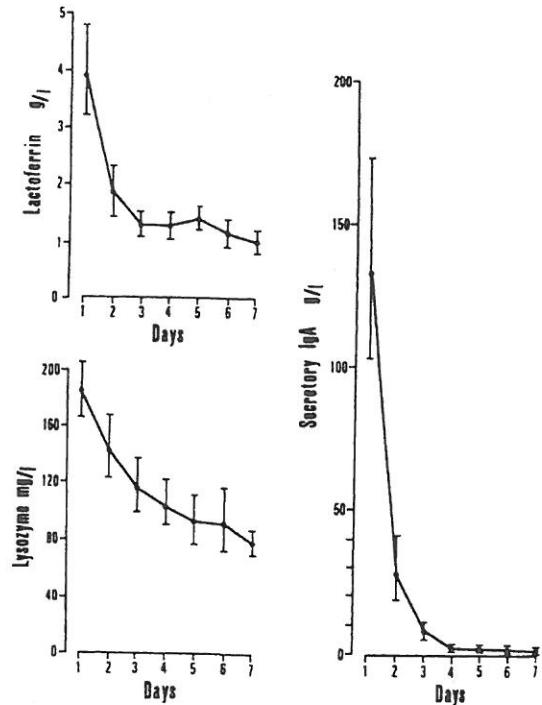


FIG 5. Mean milk lactoferrin, lysozyme, and sIgA concentrations measured daily from 20 Belgian mothers studied during the first postpartum week.

be compared with those reported by Prentice et al (28) for Gambian mothers using serum IgA as reference preparation. Prentice et al (28) reported that in Gambian mothers the concentrations of the antimicrobial factors present in milk were not influenced by the nutrition status. However, the highest concentrations of LF in the milk collected from aboriginal mothers within 15 d after delivery were associated with a weight-for-height index above the 90th percentile (29).

The milk collected during the first 3 postpartum months from the Zairean mothers contains about the same amounts of LF and LZ but about twice as much sIgA as the milk collected on day 7 postpartum from the Belgian mothers. This observation is of even greater interest because final milk (that from Zairean

TABLE 2

Lactoferrin, lysozyme, and secretory IgA (sIgA) concentrations in milk from Belgian and Zairean lactating mothers*

	Lactoferrin	Lysozyme	sIgA
	g/L	mg/L	g/L
Belgian mothers (postpartum day 7)	1.0 (0.8–1.2)	76 (69–85)	1.6 (0.8–2.5)
Zairean mothers (first postpartum trimester)	0.90 (0.8–1.0)	131 (106–163)	2.0 (1.8–2.3)

* Geometric \bar{x} (– and + SEM).

TABLE 3
Prolactin status of the Zaïrean mothers from the rural and the urban areas of the Kivu province at different postpartum stages

	Rural area		Urban area	
	<i>n</i>	Prolactin <i>mU/L</i>	<i>n</i>	Prolactin <i>mU/L</i>
0-5 mo	22	1095 (612-1961)*	18	1276 (1021-1596)
6-11 mo	19	1129 (677-1883)	19	1377 (1017-1866)
≥12 mo	24	726 (332-1590)	15	842 (511-1388)

* Geometric \bar{x} (- and + SD).

mothers) is expected to contain somewhat less antimicrobial protein than that of transitional milk (that from Belgian mothers) (23). Thus, the tremendous socioeconomic and nutrition differences existing between these two populations of mothers have no impact on the milk concentrations in antimicrobial proteins.

In Zaïrean mothers we were unable to demonstrate any correlation between maternal nutrition status and milk concentration of any of the three antibacterial proteins investigated. In our opinion, poor maternal nutrition does not seem to interfere with the production and/or the transfer of these factors into milk. Our findings are in agreement with the data published by Miranda et al (33). These authors observed no differences in milk LZ or IgA contents between well- and malnourished mothers. There only was more IgA in the colostrum of well-nourished mothers. Reddy et al (22), addressing the same issue in Indian women, did not report differences between well- and malnourished mothers for LF, LZ, or IgA even in colostrum. Because no milk yields were reported in these studies, interpretation of the data remains speculative. The critical endpoint is the amount of antimicrobial proteins fed daily to infants. As reported here the main factor influencing the amount of antimicrobial proteins effectively fed to infants is the milk yield.

The milk yield is influenced by the maternal nutrition status, which is better in industrialized countries than in developing countries (6). In developing countries, nutrition conditions are better in urban than in rural areas (6). In Kivu the urban mothers produced daily about twice as much milk as did the rural mothers (27). The nutrition status of the rural mothers, when evaluated by body mass index, arm and thigh circumferences, and serum albumin, was dramatically deficient when compared with that of the urban mothers (Table 1). Protein deficiency plays a major role in the type of malnutrition prevalent in this part of the world (36). The studies performed in similar populations also demonstrate a strong association between maternal serum albumin concentrations and milk production (P Hennart, unpublished observation, 1983). The present cross-sectional study involved groups of mothers with significant differences in their nutrition status, at various stages of their lactation (up to 18 mo). In this way we could demonstrate that the nutrition status, so different between urban and rural mothers, exerts no direct influence on the milk content of antibacterial proteins but has an indirect effect on the daily intake of these proteins. The av-

erage milk LF and sIgA concentrations of the urban mothers were close to those of the rural mothers. Because of a much higher milk yield, the urban mothers breast-feed their infants with about twice as much LF and sIgA per day as do the rural mothers. The situation is different for LZ. The rural mothers, with the poorer nutrition status, have about twice as much LZ in their milk as do the urban mothers. As a consequence of the difference in milk yields, the amount of LZ fed daily to infants is about the same for both groups of mothers.

Differences other than nutrition status exist between urban and rural mothers and could be responsible for the observed milk-yield differences. In rural mothers suckling is less frequent but is of somewhat longer duration than in urban mothers (P Hennart, unpublished observation, 1983). Rural infants are frequently left alone at home while the mothers are working hard in the fields. This partly explains why the suckling frequency (Table 1) and thus also the milk output is lower in these rural mothers. However, the difference in suckling frequency does not account for the whole difference in milk yield. The infants born in the rural area have birth weights lower than those of infants born in the city. Thus, ability to suckle and growth requirements may differ. Rural infants suffer more from early protein-energy malnutrition and their albumin concentrations are lower (P Hennart, unpublished observations, 1983); they are also supplemented earlier in life, and the beikost they receive is highly contaminated with pathogenic microorganisms.

The antibacterial properties of breast milk are very stable during long-term lactation. Only the LF content declines and only declines very slightly with time, actually much more slowly (10-fold) than reported for a group of English lactating mothers (25). The sIgA concentration remains unchanged and that of LZ increases approximately twofold during long-term lactation. The increase in LZ is greater (fourfold) in rural mothers with the worst nutrition status. Similar profiles of milk LZ and sIgA also were reported by Reddy et al (22), Goldman et al (24), Prentice et al (28), and Lewis-Jones et al (25). Our results also agree with previous observations reported by Lepage et al (37) that claimed that even after 1 y lactation maternal milk still protects the infants against gastrointestinal infections.

The independent developments of LZ and LF contents in breast milk during long-term lactation suggest that the production and/or the transfer of these two proteins are controlled by different mechanisms. Both proteins are closely associated in the cytoplasm of polymorphonuclear neutrophils: LF is found in secondary granules whereas LZ is found both in primary and secondary granules. The plasma concentration of LZ is more closely related to the granulocyte turnover rate, whereas that of LF is related more to the total number of neutrophils in blood (38). Thus, some differences likely exist in the release mechanisms of these intragranular proteins from neutrophils. LF and LZ are also found in many glandular cells and in their external secretions: saliva, tears, bile, pancreatic juice, and seminal and synovial fluids (39-42). The mechanisms of transfer and/or local production of LF and LZ in epithelia have not yet been elucidated. Green and Pastewka (43) showed that prolactin stimulates the synthesis of LF from mouse mammary gland explants cultured in a synthetic medium containing insulin and hydrocortisone. However, we could not find any significant correlation in vivo between maternal serum prolactin concentration and milk LF concentration.



We report here a higher sIgA concentration in milk from multipara than from primipara mothers. In aboriginal mothers but not in the Zairean mothers, milk LF concentrations were correlated with parity (29). Both observations are at variance with those reported by Prentice et al (44), who suggest that the ability to secrete protective factors in milk decreases with increasing parity. Thus, previous pregnancies and breast-feeding experiences may differently influence the antimicrobial content of maternal milk according to local and/or environmental conditions.

In conclusion, the milk content in antimicrobial proteins remains fairly constant (LF and sIgA) or even significantly increases (LZ) throughout long-term lactation. The nutrition status of the mother has no direct influence on milk content of LF, LZ, or sIgA. However, a deficient nutrition status together with other factors seems to exert a negative indirect influence on the amount of antimicrobial proteins fed daily to infants by reducing milk yield. Serum prolactin concentrations appear to have no influence on the milk content of antimicrobial proteins, not even of lactoferrin. In the present study milk sIgA content was higher in multiparous than in primiparous mothers. ■

We thank the National Institute of Diabetes, Digestive and Kidney disease; the Center for Population Research of the National Institute of Child Health; and the Agricultural Research Service of the US Department of Agriculture for the reagents used in the radioimmunoassay of human prolactin. We are grateful to HL Vis for continuous encouragement and financial support. We thank C Nandance for excellent technical assistance.

References

1. Welsh JK, May JT. Anti-infective properties of breast milk. *J Pediatr* 1979;94:1-9.
2. Reiter B. The biological significance of lactoferrin. *Int J Tissue React* 1983;5:87-96.
3. Jolles P, Jolles J. What's new in lysozyme research? *Mol Cell Biochem* 1984;63:165-89.
4. Kleinman PE, Walker WA. The enteromammary immune system: an important new concept in breast milk host defense. *Dig Dis Sci* 1979;24:876-82.
5. Walker WA, Isselbacher KJ. Intestinal antibodies. *N Engl J Med* 1977;297:767-73.
6. World Health Organization. Collaborative study on breast-feeding: the quantity and quality of breast milk. Geneva: WHO, 1985.
7. Vis HL, Pourbaix P, Thilly C, Van der Borgh H. Analysis of the nutritional status of the traditional societies in the region of lake Kivu: the Shi and the Havu. *Ann Soc Belg Med Trop* 1969;49:353-419 (in French).
8. Vis HL, Bossuyt M, Hennart P, Carael M. The health of mother and child in rural Central Africa. *Stud Fam Plann* 1975;6:437-41.
9. Vis HL, Hennart P, Ruchababisha M. Some issues in breast-feeding in deprived rural areas. *Assignment Children (UNICEF)* 1981;55/56:183-200.
10. World Health Organization. Contemporary patterns of breast-feeding. Report of the WHO collaborative study on breast-feeding. Geneva: WHO, 1981.
11. Briscoe J. The quantitative effect of infection on the use of food by young children in poor countries. *Am J Clin Nutr* 1979;32:648-76.
12. Hennart P, Ruchababisha M, Uwayitu N, Christophe C, Robyn C, Vis HL. Influence of pregnancy, breast-feeding and mother/child interrelationship on the nutritional status of the child in Central Africa. *Med Entwicklungsländern* 1982;11:165-82.
13. Sonnet J, Rodhain J. Study of serum proteins by paper electrophoresis: I. Technique and new results. *Rev Belge Pathol Med Exp* 1952;22:226-40 (in French).
14. Robyn C, L'Hermite M, Petrusz P, Diczfalusy E. Potency estimates of human gonadotrophins by a bioassay and three immunoassay methods. *Acta Endocrinol (Copenh)* 1971;67:417-33.
15. Barel AD, Prieels JP, Maes E, Looze Y, Leonis J. Comparative physiological studies of human α -lactalbumin and human lysozyme. *Biochim Biophys Acta* 1972;257:288-96.
16. Prieels JP, Pizzo SV, Glasgow LR, Paulson JC, Hill RL. Hepatic receptor that specifically binds oligosaccharides containing fucosyl α 1-3 N-acetylglucosamine linkages. *Proc Natl Acad Sci (USA)* 1978;75:2215-9.
17. Hunter WM, Greenwood FC. Preparation of iodine¹³¹ labelled growth hormone of high specific activity. *Nature* 1962;194:495-6.
18. Badawi M, Bila S, L'Hermite M, Perez-Lopez FR, Robyn C. Comparative evaluation of radioimmunoassay methods for human prolactin using anti-ovine and anti-human prolactin sera. In: International Atomic Energy Agency. *Radioimmunoassay and related procedures in medicine*. Vol 1. Vienna: International Atomic Energy Agency, 1974:411-22.
19. Hull CM, Nie NH. *Statistical package for the social sciences*. Update 7-9. New York: McGraw-Hill, 1981.
20. Snedecor GM. *Statistical methods applied to experiments in agriculture and biology*. 6th ed. Ames, IA: Iowa State University Press, 1957.
21. Peitersen B, Bohn L, Andersen H. Quantitative determination of immunoglobulins, lysozyme and certain electrolytes in breast milk during the entire period of lactation, during a 24-hour period, and in milk from the individual mammary gland. *Acta Paediatr Scand* 1975;64:709-17.
22. Reddy V, Bhaskaram C, Raghuramulu N, Jagadeesan V. Anti-microbial factors in human milk. *Acta Paediatr Scand* 1977;66:229-32.
23. McClelland DBL, McGrath J, Samson RR. Antimicrobial factors in human milk. Studies of concentration and transfer to the infant during the early stages of lactation. *Acta Paediatr Scand [Suppl]* 1978;271:1-20.
24. 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.
25. 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.
26. Gross SJ, Buckley RH, Wakil SS, McAllister DC, David RJ, Faix RG. Elevated IgA concentration in milk produced by mothers delivered of preterm infants. *J Pediatr* 1981;99:389-93.
27. Cruz JR, Carlsson B, Garcia B, et al. Studies on human milk. III. Secretory IgA quantity and antibody levels against *Escherichia coli* in colostrum and milk from underprivileged and privileged mothers. *Pediatr Res* 1982;16:272-6.
28. Prentice A, Prentice AM, Cole TJ, Paul AA, Whitehead RG. Breast-milk antimicrobial factors of rural Gambian mothers. I. Influence of stage of lactation and maternal plane of nutrition. *Acta Paediatr Scand* 1984;73:796-802.
29. Houghton MR, Gracey M, Burke V, Bottrell C, Spargo RM. Breast milk lactoferrin levels in relation to maternal nutritional status. *J Pediatr Gastroenterol Nutr* 1985;4:230-3.
30. Bennet RM, Chitra M. A solid-phase radioimmunoassay for the measurement of lactoferrin in human plasma: variations with age, sex and disease. *J Lab Clin Med* 1976;88:156-66.
31. Hanse NE, Malmquist J, Thorell J. Plasma myeloperoxidase and lactoferrin measured by radioimmunoassay: relations to neutrophil kinetics. *Acta Med Scand* 1975;198:437-43.



32. Malmquist J. Serum lactoferrin in leukemia and polycythaemia vera. *Scand J Haematol* 1972;9:305-10.
33. Miranda R, Saravia NG, Ackerman R, Murphy N, Berman S, McMurray DN. Effects of maternal nutritional status on immunological substances in human colostrum and milk. *Am J Clin Nutr* 1983;37:632-40.
34. Olsson I, Olofsson T, Ohlsson K, Gustavsson A. Serum and plasma myeloperoxidase, elastase and lactoferrin content in acute myeloid leukemia. *Scand J Haematol* 1979;22:397-406.
35. Sykes JAC, Thomas MJ, Goldie DJ, Turner GM. Plasma lactoferrin levels in pregnancy and cystic fibrosis. *Clin Chim Acta* 1982;122:385-93.
36. Hennart P, Hofvander Y, Vis H, Robyn C. Comparative study of nursing mothers in Africa (Zaire) and in Europe (Sweden): breast-feeding behaviour, nutritional status, lactational hyperprolactinaemia and status of the menstrual cycle. *Clin Endocrinol (Oxf)* 1985;22:179-87.
37. Lepage P, Munyakazie C, Hennart P. Breast-feeding and hospital mortality in children in Rwanda. *Lancet* 1981;2:409-11.
38. Hansen NE, Malmquist J, Thorell J. Plasma myeloperoxidase and lactoferrin measured by radioimmunoassay: relations to neutrophil kinetics. *Acta Med Scand* 1975;198:437-43.
39. Masson PL, Heremans JF, Dive CH. An iron-binding protein common to many external secretions. *Clin Chim Acta* 1966;14:735-9.
40. Mason DY, Taylor CR. Distribution of transferrin, ferritin and lactoferrin in human tissues. *J Clin Pathol* 1978;31:316-27.
41. Bennet RM, Quartey EAC, Holt PJJ. Lactoferrin—an iron binding protein—in synovial fluid. *Arthritis Rheum* 1973;16:186-90.
42. Gilette TE, Allansmith MR. Lactoferrin in human ocular tissue. *Am J Ophthalmol* 1980;90:30-7.
43. Green MR, Pastewka JV. Lactoferrin is a marker for prolactin response in mouse mammary explants. *Endocrinology* 1978;103:1510-2.
44. Prentice A, Prentice AM, Cole TJ, Whitehead RG. Determinants of variations in breast milk protective factor concentrations of rural Gambian mothers. *Arch Dis Child* 1983;58:518-22.

