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Factors Affecting Lactoferrin Concentration in Human Milk: How Much Do We Know?

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Abstract

Lactoferrin (LF) is a breast milk glycoprotein with antimicrobial and anti-inflammatory effects. Its beneficial properties in infants, especially in those born preterm, are currently being studied in clinical trials. However, the maternal and nursing infant factors that may affect the concentration of LF in breast milk are still not clear. We conducted a systematic review to investigate the factors that may affect LF concentration. We used a 2-step approach to identify the eligible studies according to inclusion/exclusion criteria and to determine which studies would be considered. We included 70 qualified articles from 29 countries with publication dates ranging from 1976 to 2015. We described the correlation between LF concentration in breast milk and lactation stage; 10 maternal factors, such as race, parity, among others; and 2 infant factors, infections and prematurity. Colostrum has the highest LF levels, but they decrease with days postpartum. No other factor has been consistently associated with LF concentration. A major limitation of the majority of the published studies is the small sample size and the different methods used to measure LF concentration. Therefore, there is a need for large, multicenter studies with standardized study design, sample collection, and LF measurement methods to identify clinically significant factors associated with LF expression in breast milk, which will help promote exclusive breastfeeding in preterm infants.

Keywords

Lactoferrin; breast milk; lactation; maternal factors; neonatal factors

Introduction

Breast milk is widely recommended as the exclusive source of infant nutrition during the first 6 months of life (Kramer and Kakuma 2012); it is also an important protective factor against infectious diseases and mortality in young children (Duijts et al. 2009; WHO 2000). Numerous bioactive proteins in human milk are responsible for this protection, including

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immunological factors such as IgA, live cells, cytokines, and active proteins and enzymes such as lysozyme and lactoferrin (LF) (Lönnerdal 2013). LF is a multifunctional protein that is involved in iron homeostasis and also has antimicrobial, immunomodulatory, and anti-inflammatory activity (Ward et al. 2005; Liu and Newburg 2013).

Several clinical studies have been conducted in children to demonstrate the effect of LF on different clinical outcomes. Protection against diarrhea and neonatal infections are the most likely relevant activities of LF in children (Ochoa et al. 2012). In light of published and ongoing clinical studies on LF supplementation for the prevention of neonatal infections (Turin et al. 2014) and the need to apply research into breast milk composition in infant formulas for children who cannot be breastfed (Lönnerdal 2013), it is important to determine the potential factors affecting LF levels. A recent systematic review showed longitudinal changes of LF concentration in breast milk from mothers around the world (Rai et al. 2014). However, there are no systematic reviews about other factors associated with LF concentration in breast milk. Therefore, we conducted a review of studies on maternal and infant factors that may influence the concentration of LF in human breast milk.

Methods

We conducted a systematic literature search from November 2014 to March 2016. Articles that fulfilled the following inclusion criteria were included: a) studies that quantified LF concentrations in human milk, b) well-defined populations of breastfeeding mothers around the world, and c) minimum sample size of 10 (we only considered those studies with less than 10 subjects that performed serial sampling). In order to be selected, the article had to provide the following information: geographical location, gestational age, stage of lactation, a thorough description of the studied factor, milk sampling methods, LF measurement method, and units used to express LF concentration. Articles that did not contain primary data (editorials, letters to the editor, narrative reviews), case reports or case series, or articles on methods for LF quantification were not considered for this review. Furthermore, we did not include articles that considered LF concentrations from donor human milk or from non-breastfeeding mothers; articles on the effect of storing, pasteurization or treatment of breast milk; articles on the activity or properties of LF; or papers that focused only on the relationship of LF levels in breast milk and in other fluids/tissues. Since breast milk protein content does not differ significantly throughout the day (Mitoulas et al. 2002), we did not limit the review based on the time of milk collection.

Prior to conducting this review, we performed a first search attempt. We noticed that while there are medical subject headings (MeSH) for lactoferrin and milk; and human terms (entry terms such as breast milk; breast; milk, breast; and human milk), there are also other articles that cannot be found using these MeSH terms. Therefore, in an attempt to include all available studies, we created a search strategy to capture articles on protein concentrations that included LF. We searched for the following MeSH and text words in Pubmed: breast milk; breastmilk; human milk; lactoferrin; levels; and concentration. All of the abstracts were screened to verify that they reported LF levels, and if this was not clear, the complete article was examined. Then, we repeated the electronic search in Google Scholar and

Embase. We limited the search to articles on humans, in English, and with full-text access. We used Zotero as a reference manager to exclude duplicates.

A two-step approach was used to identify all eligible studies. First, the titles and abstracts from search results were screened for initial eligibility by one reviewer (AV). Potentially eligible studies with full-text access were obtained, and articles whose eligibility was unclear based on title and abstract were assessed by TO, MR, CT, and AV. Second, the reviewers independently examined all the potentially eligible studies to determine whether they met the inclusion/exclusion criteria. We manually reviewed the list of articles cited in the selected papers, especially all papers cited in the systematic review by Rai et al. (2014). The decision to include each eligible study that passed the two-step screening described above was reached by the consensus of all of the investigators, with input from an experienced researcher (TO).

Results and discussion

We found a total of 97 articles that could be included in the review. However, 14 articles were excluded based on our inclusion/exclusion criteria, and 13 articles could not be considered for review because of lack of full-text access. Thus, we included 70 qualified articles from 29 countries with publication dates ranging from 1976 to 2015 (Table 1). All of the studies quantified LF in breast milk more than once. Five were randomized controlled clinical trials, and 67 (93%) were observational studies. Data were heterogeneous regarding the number of subjects included, the methods and timing for sample collections, and LF measurement. Variability was reported also within the same study.

Factors affecting LF concentration in breast milk

We describe studies on the correlation between LF concentration in breast milk and lactation stage, geographical distribution, and 12 main factors (10 related to the mother and 2 related to the nursing infant) (Table 2).

Lactation stage

Lactation stage is the most widely studied factor affecting LF concentration and study results are very consistent: LF concentration is highest in colostrum and decreases with days postpartum. The most important study to date is the systematic review elaborated by Rai et al. (2014), as it included a consolidation of data from 94 articles on LF concentration in term and preterm infants. After analyzing data from 2724 women, they found a higher LF concentration in early milk (<28 days after birth), mean ≈ 5 g/L, when compared to mature milk (≥28 days), mean ≈ 2 g/L. The highest LF concentration is in the first 5 days of life, mean ≈ 7 g/L, decreases to 50% by days 6–10, and plateaus out after the first month of life (Rai et al. 2014). This is consistent with the results from multiple studies (Hennart et al. 1991; Molinari et al. 2013; Reddy et al. 1977; Sanchez-Pozo et al. 1986; Shashiraj et al. 2006; Trend et al. 2015). Differing results were presented by Hsu et al. 2014; they found a stable LF concentration in the first month in the milk from mothers from Taiwan; however, they started collecting samples 5–7 days postpartum. Van der Strate et al. also found no significant difference during the course of lactation, although they highlight a 7-fold

decrease in LF concentration from colostrum compared to samples from 2 weeks after birth (van der Strate et al. 2001).

Figure 1 shows the decrease in LF concentration over time found in a prospective study done as part of an ongoing clinical trial in three main Neonatal Units in Peru conducted by our group (NEOLACTO, NCT01525316). This study included 346 mothers of neonates with a birth weight <2000g. Milk samples were collected in 4 stages: colostrum (0–7 days), transitional milk (8–14 days), mature milk at 1 month (23–37 days) and mature milk at 2 months (53–67 days). LF concentration was measured using a commercial ELISA kit. LF concentration significantly decreased over time (Ochoa et al. 2015).

Geographical distribution

The paper with the greatest number of subjects on this topic is also the systematic review by Rai et al. (2014), examining 228 mean values for human milk from all over the world. Most of the values came from Europe (77 values), followed by Asia (29 values), North America (26 values from the United States), South America (24 values), and Africa (16 values).

Regarding the term milk, they found the highest levels of LF in South America, attributable to elevated means from early stages of lactation. Between 2 weeks to beyond 12 months of lactation, levels were higher in Asia compared with the rest of the locations. The latter was also seen in preterm milk, owing to the greater number of studies in the first week of lactation.

Some studies have compared LF levels in breast milk from two different areas. Lönnerdal et al. found significantly higher levels in 50 Swedish mothers with a privileged socioeconomic status compared to 104 Ethiopian mothers from both a privileged and non-privileged socioeconomic status during the lactation period from 0.5 to 1.5 months (Lönnerdal et al. 1976a). Prentice et al. found higher concentrations of LF in breast milk in early stages of lactation from 152 mothers in Gambia compared to milk from 10 mothers in Cambridge (UK), but this was not tested for significance or adjusted to nutritional status (Prentice et al. 1983). In another study by the same authors, they did report LF levels significantly higher in mothers in Gambia compared to mothers in Cambridge at a specific season (Prentice et al. 1984a). Higher LF levels in breast milk have also been reported in Zairian mothers from urban areas compared to mothers from rural areas. This study also considered Belgian mothers and no difference was found between LF levels in milk from these mothers collected on lactation day 7 versus Zairian mothers with milk collected in the first 3 months of lactation (Hennart et al. 1991). Leelahakul et al. studied 14 Japanese and 15 Thai mothers and while they found slightly higher levels in the Japanese mothers, the differences were not significant (Leelahakul et al. 2009).

Evidence is not clear on whether geographical distribution is associated with LF concentration in breast milk. In order to assess this factor, a large number of subjects would be needed as well as all of the potential confounding factors such as race, socioeconomic status or even nutritional status would need to be addressed.

Maternal factors

Race/ethnicity—Breast milk composition may vary according to the mother' ethnicity. Houghton et al. (1985) evaluated the relationship between LF levels in breast milk and the maternal nutritional status in 45 Aboriginal and 34 non-Aboriginal Australian women, and they found no significant association with maternal race/ethnicity. Burch et al. (2013) have studied the association between the concentration of immune markers in breast milk and the maternal race in a cohort of 178 mothers. For analysis, the participants were classified as Caucasian (77), African American (20), and other (18); they found that African-American mothers had significantly increased levels of certain cytokines ($p<0.05$) (Burch et al. 2013). Ciardelli et al. (2007) have evaluated the production of cytokines and specific sIgA in breast milk of mothers of different ethnic groups (originally from Italy, Africa, Asia, and Eastern European Countries) who lived in Italy. They found a significant difference between the sIgA, IL-6, IL-8, and IL-10 concentration and maternal race. However, it remains unclear how strongly ethnicity affects the expression of immune factors in mothers' breast milk, such as LF and cytokines.

Age—In the study by Lewis-Jones et al. (1985a) of 47 healthy mothers from the UK, the highest mean LF concentration was found in mothers aged 17 to 21 years, but this was not significantly different than other age groups. The authors proposed that this could be explained by the presence of more physiologically active secretory cells within the mammary alveoli in younger mothers. Ella et al. (2009) also found higher LF levels in colostrum, transitional and mature milk of young mothers (<20 years old) of healthy infants. On the other hand, lower LF levels were reported in older mothers from Peru (Marquis et al. 2003); no significant differences in proteins concentrations, including LF, were found by Bachour et al. (2012) between mothers of different ages.

Parity—When investigating the association between parity and the concentration of LF in term breast milk, there are also mixed findings. Some studies found no association between these two variables (Montagne et al. 1999; Shashiraj et al. 2006). Montagne et al. (1999) reported that although LF levels did not seem to be affected by parity, they did find a bigger effect of prematurity on the levels of milk proteins during the first days of lactation in primiparous mothers compared to multiparous. On the other hand, three studies found higher LF levels with lower parity (Hennart et al. 1991; Lewis-Jones et al. 1985a; Prentice et al. 1983). Hennart et al. found higher levels in primipara than in multipara women (Hennart et al. 1991) and Lewis-Jones et al. found higher levels in mothers of parity 2 (Lewis-Jones et al. 1985a). However, only one of these studies found a statistically significant difference, reporting increased levels in mother of parity 1 and 2 vs. parity from 3 to 9 (Prentice et al. 1983). Two studies observed higher LF concentration in breastmilk of mothers with higher parity (Houghton et al. 1985; Marquis et al. 2003).

Socioeconomic status—There are contradictory data on the association between maternal socioeconomic status and LF levels in breast milk. Sanchez-Pozo and colleagues found an opposite association. They included 181 healthy Spanish mothers, classified into three socioeconomic status groups: upper, middle, and low. They found higher LF values in Spanish mothers of the low socio-economic group (Sanchez-Pozo et al. 1987). On the other

hand, Lonnerdal and colleagues analyzed LF levels in healthy Ethiopian mothers of two socioeconomic groups: non privileged (n=78) and privileged (n=26), and found no significant association (Lönnerdal et al. 1976a)

Nutritional status—It is of great interest to determine if maternal nutritional status is related to LF in breast milk. There are two studies that found no correlation with maternal nutritional status, based on different parameters such as weight/height index, hemoglobin, body mass index, arm circumference, serum albumin concentration (Hennart et al. 1991; Sanchez-Pozo et al. 1987). In Australia, a study did show a positive association between LF in breast milk and > 90% weight for height (Houghton et al. 1985), whereas in Japan, an association with lower fat intake was found (Leelahakul et al. 2009). Other studies have examined the effect of interventions on mothers´ nutritional status on breastfeeding. Herias et al. conducted a trial with undernourished mothers living in poor areas of Guatemala, providing high caloric cookies to one group and low caloric ones to another, and found no differences in the concentration of Lf in milk between the two groups (Herías et al. 1993, 1995) Likewise, Gambian mothers were given energy-rich dietary supplements, and LF levels did not differ from the non-supplemented mothers (Prentice et al. 1983, 1984b). When a high protein versus low protein diet was tried, no statistically significant differences in LF were found (Forsum and Lönnerdal 1980). Other kinds of supplementation to mothers such as pre-germinated brown rice diet (Sakamoto et al. 2007), a probiotic product (Mastromarino et al. 2015) and retinol supplement (Filteau et al. 1999) were evaluated and no association with LF levels in breast milk was found. Chao et al. did report a significant increase in LF levels with maternal supplementation with a protein-rich chicken extract (Chao et al. 2004). On the other hand, even with a marginal reduction of dietary protein intake in US mothers, no association was found (Motil et al. 1995).

Smoking—Smoking during breastfeeding has been related to a lower breast-milk volume, lower infant weight-gain (Vio et al. 1991) and exposure to detrimental substances through milk (Dahlström et al. 2004; García-Esquinas et al. 2011; Zanieri et al. 2007). There are only 2 studies that attempted to explain the effects of smoking in LF concentration in human milk. First, Milnerowicz et al. analyzed milk samples from 74 Polish women that were in the first 8 days postpartum; they found no association between LF levels and smoking (Milnerowicz and Chmarek 2005). On the same note, Bachour et. al (2012) analyzed milk samples from 66 Lebanese mothers and found no correlation, although their method for LF concentration measurement (SDS-PAGE) was not optimal.

Mastitis—We found 3 studies that showed a direct correlation between LF levels and maternal mastitis. Prentice et al. have conducted a study to assess the LF concentration in mothers with mastitis in Gambian villages. They reported 65 cases of mastitis from 40 women and collected samples at the time of presentation at the clinic (week 0), 1 week and 5 weeks later. At week 0 and week 5, there were no significant differences in LF concentration. However, at week 1, there was a significant higher LF concentration in mothers with mastitis (Prentice et al. 1985). Similarly, Semba et al. evaluated the LF concentration in 96 women with mastitis in Malawi and found higher LF levels in those women with infection (1,230 vs 565 mg/L, $p<0.0007$, among the women with and without

mastitis, respectively) (Semba et al. 1999). A third study conducted by Fetherston et al. has shown significant higher LF levels in breast milk of mothers with more extreme symptoms of mastitis in a cohort of 26 women followed during the first 3 months postpartum (Fetherston et al. 2006). This correlation is opposite to what Buescher and Hair found in a small study (Buescher and Hair 2001).

Chorioamnionitis, postpartum infections and other maternal infections—Most of the studies in the literature have focused on healthy women. In Peru, breast milk composition was compared between 34 mothers with acute postpartum infections and 23 healthy mothers, finding significantly lower LF levels in the former group. They included patients with urinary tract infections, chorioamnionitis, endometritis, skin infections, pneumonia and other respiratory tract infections, in the first 48 hours postpartum. The authors of this study suggested that infection might have reduced the levels by association of LF with inflammatory mediators such as macrophages within the mammary glands. Another possible explanation might be a “compensatory decrease”, because elevated levels of LF have been found in the amniotic fluid of mothers with chorioamnionitis (Heller et al. 1995; Otsuki et al. 1999; Pacora et al. 2000). Using the same previously mentioned criteria for acute infection, the breast milk of a similar population of mothers from Peru with breastfeeding infants aged 1 to 6 months, was studied. The LF concentration from this group was compared to that of healthy controls, and the two groups did not differ significantly (Zavaleta et al. 1995a). A more recent study in Australia had similar findings, they recruited 21 mothers-infant pairs and measured LF concentration in breast milk samples during and after any mother or child infection. They found no significant changes in LF in breast milk from different lactation stages when mothers presented infections including influenza-like illness, gastrointestinal infections, urinary tract infections, vaginal thrush, ear or eye infections (Hassiotou et al. 2013). Other infectious diseases that could potentially affect LF levels have also been examined. There was no association with leprosy (Duncan et al. 1983) or maternal HIV status (Shapiro et al. 2007), or with human CMV DNA in preterm breast milk (van der Strate et al. 2001).

Infant factors

Infection—There have been various studies focused on understanding the changes in LF in the face of infant infection. Prentice et al., in 1984, sampled 95 breastfeeding women from Gambia at three different times, each in a season with different environmental characteristics, food accessibility and infection risk. Their results showed a non-significant reduction in immunoproteins in the months when diarrhea prevalence was higher (Prentice et al. 1984a). Most recent studies have shown differing results regarding this subject. Hassiotou et al. in Australia found no correlation between infant infection and LF concentration but their results may be limited by the analysis of mother and infant infections as a combined entity, which may obscure the changes associated with child illness alone (Hassiotou et al. 2013). On the other hand, Breakey et al. studied 30 mother-infant pairs in Argentina and analyzed the association of LF amounts in breast milk and gastrointestinal and respiratory symptoms in the infants. They found that LF concentrations were higher when the infants had presented an infection in the past month or when they were about to become ill the following month. No changes in LF concentration were found at the time of

disease. This suggests a “predictive” nature of the immune components in breast milk, as well as a delayed onset of LF production (Breakey et al. 2015). Riskin et al. also found a positive correlation, although not significant, on LF levels in breast milk from a group of mothers from Israel with children than were hospitalized for fever or sepsis (Riskin et al. 2012). Finally, Ella et al. studied a sample of mothers from Nigeria and determined the LF concentration of colostrum, transitional and mature milk in mothers of infants with or without neonatal sepsis. They found lower LF concentration in the breast milk of mothers with sick children for all lactation stages, and they attribute the infant’s predisposition to infection to the lack of adequate LF intake from breast milk (Ella et al. 2009).

Prematurity—Many studies have been published to demonstrate a direct or indirect association between LF in breast milk and prematurity. Some researchers have found that LF concentration is higher in mothers of preterm infants than term infants (Dawarkadas et al. 1991; Goldman et al. 1982a; Mathur et al. 1990; Montagne et al. 1999). Goldman et al. conducted a study that included 13 mothers of term infants and 8 mothers of preterm infants, who donated milk samples at 2, 4, 6, 8, 10, and 12 weeks of lactation. LF concentration was greater in preterm milk than in term milk and the difference was particularly marked during the 10th and 12th week (Goldman et al. 1982a). Dawarkadas et al. also concluded that the mean LF levels were significantly higher in preterm (n=25) than in full term (n=10) colostrum (Dawarkadas et al. 1991). Similar results were reported by Mathur et al. (1990) in calostrum from 35 mothers. In addition, Montagne et al. (1999) found that primiparous mothers who delivered before 33 weeks of gestation had significantly higher LF concentrations (7.5 ± 4.8 g/l) than primiparous term mothers (5.1 ± 2.6 g/l). The fact that the preterm milk is of lower volume and higher immune composition would be beneficial for the immunologically immature infant. On the other hand, an Italian study conducted by Velona and colleagues have shown opposite results; they found higher LF levels in term milk compared to preterm milk (Velona et al. 1999). Similarly, Mehta & Petrova (2011) have found that very preterm delivery was associated with higher levels of sIgA, lysozyme and adiponectin in transitional milk, but lower LF and leptin ($p<0.05$). Moreover, some authors have also performed studies that did not find a significant difference between preterm and term milk (Britton 1986; Hsu et al. 2014; López et al. 1997; Molinari et al. 2013; Ronayne de Ferrer et al. 2000). Broadhurst et al. (2015) have published a recent study that included 30 mothers at their second and fifth week of lactation, organized in three groups according to gestational age (very premature, premature and full term), and found no significant differences. A limitation of the majority of studies is the small sample size and the different methods used to measure LF concentration. Therefore, larger and homogeneous studies should be to done to clarify this association, which will help promote exclusive breastfeeding in preterm infants.

Other factors

When multiple gestation was studied, LF levels in breast milk from one woman who delivered twins was significantly higher than levels from 4 other women during late pregnancy, but then similar values were reported after third day postpartum (Kulski and Hartmann 1981).

There is no study that focuses on the association between the type of delivery and LF levels in breast milk although elective cesarean section is becoming a growing trend among women around the world, even in the absence of a clear indication (Betran et al. 2016). Yet, Dizdar et al. studied the association of this factor and the amount of the main macronutrients in breast milk, they analyzed colostrum samples of 111 women that delivered vaginally and 93 by cesarean section. A statistically lower quantity of protein was found in the group that delivered by cesarean section, but they didn't determine the specific concentrations of the diverse group of proteins that can be found in human milk (Dizdar et al. 2014).

No association of LF levels in breast milk was found with exercise (Lovelady et al. 2003) or serum concentrations of prolactin (Hennart et al. 1991). Intrauterine arsenic exposure was related to lower levels of LF at 12 months of lactation (Raqib et al. 2009).

More recent studies have focused on maternal and infant health status. Gestational diabetes mellitus was studied, and while they did find higher N-glycosylation of LF in breast milk in mothers that had presented with diabetes, the concentrations in breast milk were not different (Smilowitz et al. 2013). Other recent studies have investigated allergic conditions. Hogendorf et al. found no association of LF concentration in breast milk and infant food allergy (Hogendorf et al. 2013) while Polonkai et al. found higher LF levels in allergic mothers and allergic infants (Polonkai et al. 2015). Higher concentrations were also seen in mothers of infants with atopy and eczema, and elevated levels were associated with fewer cases of upper respiratory infections in nursing infants up to 2 years old but more cases of lower respiratory infections throughout the first year of life. No association was reported with wheezing and asthma in infants (Zhang et al. 2014).

Methods for Analysis of Lactoferrin in breast milk

There are different methods for LF analysis in breast milk with no current internationally accepted consensus. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Sanchez-Pozo et al. 1986) and electrophoresis in agarose gel (Laurell 1966) are amongst the first methods described. Throughout the years and because of the potential clinical applications of LF, new approaches have been developed for its analysis in breast milk as well as in infant formula (Indyk and Filonzi 2005).

Immunological methods such as single radial immunodiffusion assay and enzyme-linked immunosorbent assay (ELISA) are now considered more suitable for LF measurement because they can discriminate the tertiary structures of proteins and thus differentiate intact from denatured LF, unlike the previously mentioned methods (Gokavi 2009). Further, ELISA can give quantitative measurements and is more sensitive than the immunodiffusion techniques (Bjoerck et al. 1993). Commercial kits for bovine and human LF ELISA are available although somewhat more expensive (Miller et al. 2013), making it the most used technique. In this review, the majority of studies (43%, 30/70) reported ELISA for LF analysis. However, there is no consensus on which approach should be used as a gold standard to measure LF concentration, and to validate new methods since they are actively evolving. For example, a recent study described an automated, sensitive biosensor-based

immunoassay for LF in bovine milk with surface plasmon resonance (SPR) optical detection with results comparable to those from ELISA (Indyk and Filonzi 2005).

Critical appraisal

Lactoferrin supplementation as a measure to prevent infection in premature neonates seems to be a promising strategy. Many ongoing clinical trials are evaluating its antimicrobial properties in more than 6000 infants (Turin et al. 2014). However, the factors that may affect the LF expression in breast milk are still not elucidated. To clarify this question, we performed an extensive review of literature. We found that the only factor that consistently affected the LF concentration was the number of days postpartum or lactation stage. No other factor has been firmly associated with LF levels. This is the first review that detailed the relationship between maternal and nursing infant factors and lactoferrin concentration in breast milk.

Limitations of our study included restricted access to some potentially eligible papers with no full-text access available or studies in languages other than English and Spanish. We were unable to perform a statistical analysis due to the heterogeneity of results in the studies included in this review.

We also found a number of limitations in the studies published to date. First, there is a considerable diversity in the methods used for milk sampling and for determining LF concentration. Moreover, the moment of sample collection varied significantly, ranging from a few days to years postpartum. Another important limitation was that, although there were some important factors that were analyzed by many authors, such as nutritional status and maternal and infant infections, the definition of the variables differed between studies, which further complicated an attempt to compare them. Furthermore, many studies included a small number of participants, although this could be overcome with more than one milk sample from each subject at different stages of lactation. Finally, none of the studies considered genetic factors that could affect the expression of LF such as single-nucleotide polymorphisms (SNPs) in the lactoferrin gene, which are associated with changes in milk composition in animals (Guo et al. 2010).

Conclusion

LF levels change with lactation stage, colostrum has the highest LF concentration, and it decreases significantly with days postpartum. This should be taken into consideration by physicians to encourage exclusive breastfeeding, especially in groups that are vulnerable to infection, like preterm infants. No other factor has been consistently associated with significant changes in breast milk LF concentration. Therefore, there is a need to conduct large, multicenter studies with standardized study design, sample collection, and LF measurement methods to investigate clinically significant factors associated with LF expression in breast milk. This will aid future LF clinical trials to exclude potential bias and to focus their efforts in children at risk for receiving lower amounts of LF and who may benefit from an adequate supplementation.

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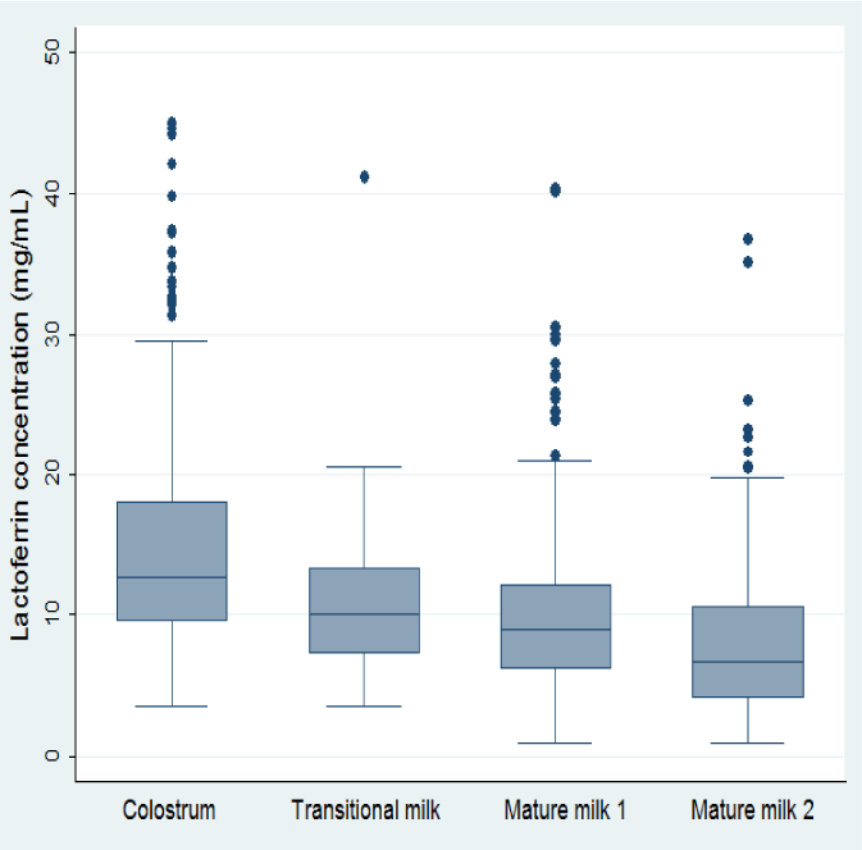

Figure 1.

Lactoferrin concentration according to stage of lactation for 695 milk samples: 277 colostrum, 55 transitional milk, 259 mature milk at 1 month, and 104 at 2 months. *, Significant difference in LF concentration between different stages of lactation ($p < 0.01$).

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Table 1

Included studies on factors associated to lactoferrin (LF) concentrations in human breast milk

Reference	Country	Study design	n	Analysis method	Factor(s) Studied
(Breakey et al. 2015)	Argentina	Case-control	30	ELISA	Infant Illness
(Mastromarino et al. 2015)	Italy	RCT*	67	ELISA	Probiotic administration
(Trend et al. 2015)	Australia	Mixed	96	ELISA	Late-onset neonatal sepsis, lactation stage
(Polonkai et al. 2015)	Hungary	Cohort	40	ELISA	Maternal and infant allergy
(Hsu et al. 2014)	Taiwan	Cohort	32	ELISA	Gestational age, lactation stage
(Zhang et al. 2014)	Australia	Cohort	142	ELISA	Infant atopy, eczema, wheezing and asthma
(Broadhurst et al. 2015)	New Zealand	Cohort	30	ELISA	Gestational age, lactation stage
(Mastromarino et al. 2014)	Italy	Cohort	48	ELISA	Gestational age, lactation stage
(Rai et al. 2014)	US	Systematic review	2724	Various	Lactation stage, geographical distribution, gestational age, methods for LF analysis
(Hassiotou et al. 2013)	Australia	Cohort	21	ELISA	Lactation stage, health of mother/child dyad, exclusive breastfeeding
(Smilowitz et al. 2013)	US	Case-control	24	Bradford assay	Gestational diabetes mellitus
(Hogendorf et al. 2013)	Poland	Cohort	84	ELISA	Infant food allergy
(Bachour et al. 2012)	Lebanon	Cross-sectional	66	Bradford assay	Smoking
(Molinari et al. 2013)	Australia	Cohort	25	SDS-PAGE	Gestational age, lactation stage
(Riskin et al. 2012)	Israel	Case-control	51	ELISA	Nursing infant infection
(Yuen et al. 2012)	China	Pilot study	25	ELISA	Lactation stage
(Mehta and Petrova 2011)	US	Cohort	20	EIA	Gestational age, lactation stage
(Ella et al. 2009)	Nigeria	Cohort	500	ELISA	Infant sepsis
(Leelahakul et al. 2009)	Japan, Thailand	Descriptive	29	SDS-PAGE	Geographical distribution, fat intake
(Raqib et al. 2009)	Bangladesh	Pilot study	140	ELISA	Intrauterine arsenic exposure
(Sakamoto et al. 2007)	Japan	RCT	41	ELISA	Pre-germinated brown rice diet
(Shapiro et al. 2007)	Botswana	Case-control	100	ELISA	Maternal HIV status
(Fetherston et al. 2006)	Australia	Cohort	26	ELISA	Mastitis
(Shashiraj et al. 2006)	India	Cohort	200	ELISA	Anemia, lactation stage, parity
(Milnerowicz and Chmarek 2005)	Poland	Case-control	74	ELISA	Mother smoking status
(Chao et al. 2004)	Taiwan	Case-control	30	ELISA	Supplementation with chicken extract
(Lovelady et al. 2003)	US	Mixed	53	ELISA	Exercise
(Marquis et al. 2003)	Peru	Case-control	727	ELISA	Breastfeeding/pregnancy overlap, age, parity

Reference	Country	Study design	n	Analysis method	Factor(s) Studied
(Montagne et al. 2001)	France	Cohort	64	MEN immunoassays	Lactation stage
(Buescher and Hair 2001)	US	Case-control	8	ELISA	Mastitis
(Strate et al. 2001)	Germany	Cohort	23	ELISA	Human CMV DNA in breast milk, lactation stage
(Ronayne de Ferrer et al. 2000)	Argentina	Cohort	46	SDS-PAGE	Gestational age, lactation stage
(Semba et al. 1999)	Malawi	Case-control	96	ELISA	Mastitis
(Montagne et al. 1999)	France	Cohort	74	MEN immunoassays	Gestational age, parity
(Filteau et al. 1999)	Bangladesh	RCT	212	ELISA	Maternal supplementation with retinol or b-carotene
(Velonà et al. 1999)	Italy	Case-control	16	SDS-PAGE	Gestational age, lactation stage
(López et al. 1997)	Argentina	Cohort	46	SDS-PAGE	Gestational age
(Lönnerdal et al. 1996)	Peru	Cross-sectional	57	ELISA	Maternal postpartum infections, chorioamnionitis
(Zavaleta et al. 1995b)	Peru	Case-control	29	Inmunoelectrophoresis	Iron status, iron supplementation
(Zavaleta et al. 1995a)	Peru	Case-control	74	ELISA	Acute maternal infection
(Motil et al. 1995)	US	Case-control	24	ELISA	Marginal reduction of dietary protein intake
(Herías et al. 1995)	Guatemala	RCT	70	SRID	Caloric supplementation
(Zapata et al. 1994)	Brazil	Case-control	28	ELISA	Iron supplementation
(Herías et al. 1993)	Guatemala	RCT	67	SRID	Caloric supplementation
(Dawarkadas et al. 1991)	India	Cohort	35	SRID	Gestational age
(Hennart et al. 1991)	Belgium	Cohort	127	Radioinmunoassay	Lactation stage, maternal nutritional status, maternal socioeconomic status, prolactin status, parity, place
(Mathur et al., 1990)	India	Case-control	35	SRID	Gestational age
(Sanchez-Pozo et al. 1987)	Spain	Cohort	181*	Electrophoresis	Maternal socioeconomic status, nutritional status
(Montgomery et al. 1987)	US	Cohort	6	SDS-PAGE	Lactation stage
(Britton 1986)	US	Cohort	108	Electrophoresis	Gestational age
(Pamblanco et al. 1986)	Spain	Cohort	53	SRID	Lactation stage
(Sanchez-Pozo et al. 1986)	Spain	Cohort	209	SDS-PAGE	Lactation stage
(Lewis-Jones et al. 1985a)	UK	Cohort	47	SRID	Parity, maternal age, gestational age
(Lewis-Jones et al. 1985b)	UK	Cohort	47	SRID	Lactation stage
(Houghton et al. 1985)	Australia	Cohort	79*	EIA	Nutritional status, parity, race, lactation stage
(Prentice et al. 1985)	Gambia	Cohort	40	SRID	Mastitis
(Prentice et al. 1984a)	Gambia, UK	Cohort	95	SRID	Seasonal variation, infant infection
(Prentice et al. 1984b)	Gambia	Cohort	162	SRID	Lactation stage, maternal nutritional status

Reference	Country	Study design	n	Analysis method	Factor(s) Studied
(Butte et al. 1984)	US	Cohort	10	EID	Lactation stage
(Duncan et al. 1983)	Ethiopia	Case-control	99	Radial inmunodifussion	Leprosy
(Prentice et al. 1983)	Gambia	Cohort	162	SRID	Lactation stage, seasonal variation, maternal nutritional status, place, parity
(Goldman et al. 1983a)	US	Cohort	5	EID	Lactation stage (throught the second year)
(Goldman et al. 1983b)	US	Cohort	7	EID	Lactation stage: weaning
(Goldman et al. 1982a)	US	Cohort	21	EID	Gestational age
(Goldman et al. 1982b)	US	Cohort	56	EID	Lactation stage (throught the first year)
(Kulski and Hartmann 1981)	Australia	Cohort	18†	SRID	Lactation stage in the peripartum period
(Forsum and Lönnerdal 1980)	Sweden	Case-control	3	Inmunoelectrophoresis	High versus low protein diet
(Reddy et al. 1977)	India	Case-control	250	Radial inmunodifussion	Iron supplementation, anemia, maternal nutritional status, lactation stage
(Lönnerdal et al. 1976a)	Ethiopia	Cohort	104	Inmunoelectrophoresis	Socioeconomic status
(Lönnerdal et al. 1976b)	Sweden	Cohort	50	Inmunoelectrophoresis	Lactation stage

Note: ELISA, enzyme-linked immunosorbent assay; SDS–PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; EIA, enzyme immunoassay; RCT, randomized controlled trial; SRID, Single radial immunodiffusion; EID, electroimmunodiffusion; MEN, microparticle-enhanced nephelometry immunoassays;

*n indicates number of samples collected;

†n indicates the number of breastfeeding mothers.

Table 2

Factors associated with lactoferrin (LF) concentration in breast milk

Variable	Findings	Reference
Lactation stage	Lower LF concentration with more days postpartum *	(Sanchez-Pozo et al. 1986) (Hennart et al. 1991) (Shashiraj et al. 2006) (Rai et al. 2014)
	Difference observed in full-term but not pre-term milk	(Mastromarino et al. 2014)
	No association	(Strate et al. 2001) (Hsu et al. 2014)
Geographical distribution	Unclear evidence	(Rai et al. 2014)
Maternal characteristics		
Age	Proteins in colostrum are higher in younger mothers	(Dizdar et al. 2014)
	LF lower in older mothers	(Marquis et al. 2003)
	No difference with age	(Lewis-Jones et al. 1985a) (Bachour et al. 2011)
Race/ethnia	No difference with race	(Houghton et al. 1985)
Nutritional status	LF higher with greater than 90% weight for height	(Houghton et al. 1985)
	LF higher with lower fat intake	(Leelahakul et al. 2009)
	No difference with variation in maternal nutritional status	(Hennart et al. 1991) (Sanchez-Pozo et al. 1987)
Socioeconomic status	LF higher in low socioeconomic status	(Sanchez-Pozo et al. 1987)
	No association with socioeconomic status	(Lönnerdal et al. 1976a)
Smoking status	No difference between smoker and non-smoker groups.	(Milnerowicz and Chmarek 2005) (Bachour et al. 2011)
Parity	LF higher with lower parity	(Prentice et al. 1983) (Lewis-Jones et al. 1985a) † (Hennart et al. 1991) †
	LF higher with higher parity	(Houghton et al. 1985) † (Marquis et al. 2003)
	No relationship with parity	(Montagne et al. 1999) (Shashiraj et al. 2006)
Chorioamnionitis and postpartum infections	LF lower in mothers with chorioamnionitis and postpartum infections	(Lönnerdal et al. 1996)
Mastitis	LF higher in infected mothers	(Prentice et al. 1985) (Semba et al. 1999) (Fetherston et al. 2006) †
	No association	(Buescher and Hair 2001)
Other maternal infections (diarrhea, pneumonia, UTI)	No relationship between LF and acute infections	(Zavaleta et al. 1995a) (Hassiotou et al. 2013)
Iron status	No relationship between LF and iron status/ anemia	(Zavaleta et al. 1995b) (Shashiraj et al. 2006)
Infant characteristics		
Prematurity	LF in preterm milk higher than term milk	(Goldman et al. 1982a) (Mathur et al. 1990) (Dawarkadas et al. 1991)

Variable	Findings	Reference
		(Montagne et al. 1999)
	LF in term milk higher than preterm milk	(Velonà et al. 1999) (Mehta and Petrova 2011)
	No difference between preterm and term milk	(Britton 1986) (López et al. 1997) (Ronayne de Ferrer et al. 2000) (Molinari et al. 2013) (Hsu et al. 2014) (Mastromarino et al. 2014) (Broadhurst et al. 2015)
Infant infection	No difference between mothers of ill versus healthy infants	(Hassiotou et al. 2013)
	LF higher in mothers of ill infants	(Riskin et al. 2012)[†] (Breakey et al. 2015)
	LF lower in mothers of ill infants	(Ella et al. 2009)

Note:

[*] Only the most important studies were included in the table. Other studies also showed a decrease in LF concentration with more days postpartum, including Lnnerdal et al. 1976b; Goldman et al. 1982b; Prentice et al. 1983; Butte et al. 1984; Houghton et al. 1985; Lewis-Jones et al. 1985b; Pamblanco et al. 1986; Sanchez-Pozo et al. 1986; Montgomery et al. 1987; Filteau et al. 1999; Velona et al. 1999; Ronayne de Ferrer et al. 2000; Montagne et al. 2001; Mehta and Petrova 2011; Yuen et al. 2012; Molinari et al. 2013; Broadhurst et al. 2015; Trend et al. 2015.

[†] was a tendency towards the findings, but the difference was not statistically significant.