Relationship of Subclinical Mastitis in Ghanaian Women and Breast Milk Intake by Infants

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Summary and Implications

Human subclinical mastitis (SCM) is inflammation of mammary tissue without any overt manifestations but is associated with lactation failure, sub-optimal infant growth during the early postpartum period, and increased risk of mother-to-child-transmission of HIV via breast milk. Subclinical mastitis (SCM) has been associated with infant growth faltering but the mechanism explaining this association remains unknown. We hypothesized that SCM is associated with reduced breast milk intake resulting in diminished growth.

Ghanaian mothers who were 3-6 months postpartum were screened for SCM using the California mastitis test (CMT). A CMT score of ≥ 1 was categorized as SCM positive (N=37); a CMT score < 1 was considered SCM negative (N=23). SCM diagnosis was confirmed by an elevated breast milk sodium-potassium ratio (Na/K > 1.0). We measured infants' 12-hour breast milk intake in both groups of mothers using the test weighing methodology.

Breast milk intake tended to be lower among infants whose mothers had elevated Na/K > 1.0 (-65.1 g; 95% CI: -141.3 g, 11.1 g). Infants whose mothers were positive for SCM with both CMT and Na/K criteria had significantly lower breast milk intake (-88.9 g; 95% CI: -171.1 g, -6.9 g) compared to those whose mothers tested either negative with both tests or positive on only one. However, in the multiple linear regression analysis, infant weight (p<0.01) and frequency of feeding (p<0.01) but not maternal SCM status were associated with breast milk intake (p = .12). When infant weight and feeding frequency were considered, the observed direct effect of SCM on infant breast milk intake was no longer significant. However, lower breast milk intake (p = .12 in MLRA) coupled with limited subjects and only 12 hr breast intake data warrant further investigation and concerns.

Introduction

There is compelling evidence that breastfeeding is associated with optimal infant growth and protection from diarrhea, respiratory infections, and other illnesses. However, some lactating women experience breastfeeding difficulties that may lead to early cessation of breastfeeding or introduction of complementary feeding before the recommended six months. A common problem that has been associated with lactation failure is subclinical mastitis (SCM). SCM is an inflammatory condition of the lactating breast that is thought to be caused by milk stasis or infections and has been associated with poor infant weight gain. SCM is commonly diagnosed as either elevated breast milk sodium or sodium potassium ratio (Na/K). Milk sodium is considered elevated when it is above 16 mmol/L while Na/K above 1.0 is considered elevated. Although SCM is most prevalent during early lactation, SCM rates of 12 % to 23% at 14 weeks postpartum have been reported. It is common knowledge in the dairy industry that cows diagnosed with SCM produce less milk. This evidence is further strengthened by the fact that calves fail to grow optimally when they are nursed by a cow with chronic mammary inflammation. Kitchen has reported a wide range of compositional and volumetric changes in dairy milk during SCM. In human lactation, however, little is known about the effects of compositional alterations in breast milk that are caused by SCM. The mechanism by which SCM affects weight gain among breastfeeding infants also remains unknown.

SCM commonly occurs in one breast. Milk output of the unaffected breast is capable of compensating for the affected breast without noticeable changes in common breastfeeding indicators. Conner has reported a single case in which an infant accepted milk from one breast with reluctance and nursed normally from the other. Milk from the rejected breast was reported to have a salty taste. Later analyses showed that the sodium content of the affected breast was 103 µmol/L compared to a typical breast milk sodium concentration of < 10 µmol/L in fully lactating women.

In this study we hypothesized that the mechanism linking infant growth faltering with SCM is the reduction of infant milk intake. The objective was to measure breast milk intake in infants of mothers with SCM and of mothers with normal breast health to determine whether reduced breast milk intake constitutes a plausible explanation linking infant growth faltering with maternal SCM.

Materials and Methods

Study area
Data for this study were collected in the Manya Krobo district of Ghana between July 2006 and February 2007. The Manya Krobo district is located in the Eastern region of Ghana and has a population of about 157,000. Sixty percent of households in the district live in rural communities. The major occupations of the district population include crop

farming, fishing, and trading. A relatively high HIV prevalence rate of about 5 % has been observed in Manya Krobo, double that of the national rate which is currently 2.7 %. Exclusive breastfeeding among three to six month old infants is common (83.1 %).

Participants

A total of 72 mother-infant pairs were recruited from seven child welfare clinics in the Manya Krobo district. Women included in the study satisfied the following criteria: at least 18 years old, singleton birth, less than 3 months postpartum, and intending to breastfeed beyond three months.

Data collection procedures

After obtaining informed consent from the mother, the field worker scheduled a home visit for a date when the infant would be between 3 and 6 months old.

Measurement of breast milk intake

Field workers spent 12 consecutive hours in the participant's home to measure infant breast milk intake using test weighing procedures. Test weighing involved the recording of the weight of the infant just before and then immediately after the infant received breast milk. Clothing or diapers worn by the infant were not changed between weighings. Test weighing measurements were recorded to the nearest 0.5 grams using the Sartorius EA15DCE-I digital scale (Sartorious Group, Goettingen, Germany). The scale was calibrated weekly using standard weight blocks.

Maternal and infant anthropometry

Anthropometric measurements that were recorded during the home visit included infant weight, length, head circumference, and mid-upper arm circumference as well as maternal height and weight. Infant weight was measured without any clothing. All weight measurements were recorded to the nearest 0.1 kilogram using the Tanita BWB800S digital floor scale (Tanita Corporation, Tokyo, Japan). Infant weights were measured by first having the mother stand on the scale without the infant, tarring the scale, and then recording the infant's weight while being held by the mother standing on the scale. Head circumference and mid-upper arm circumference were measured to the nearest 0.1 centimeter using a non-stretchable tape measure (Chasmors Ltd, London, United Kingdom). Both infant length and maternal height were measured to the nearest 0.1 centimeter using the Shorr infant/child/adult height/length measuring board (Shorr productions, Maryland, USA). Each anthropometric measurement was performed in duplicate by two field personnel who were trained and regularly standardized to perform maternal and infant anthropometric measurements using standard methods.

Morbidity and infant feeding

During the home visit, maternal and infant 7-day health histories were recorded. Mothers were asked to recall occurrence and frequency of any disease symptoms for both the mother (including fever, breast pain, engorgement, and sore nipple) and the infant (including diarrhea, cough, and fever) that were experienced over the last week. Any treatment for these symptoms and the source of treatment was also recorded.

A 7-day food frequency questionnaire was used to record all dietary intake of the infant including breast milk and other liquid and solid foods. Mothers recalled all foods or drinks taken by the infant and the frequency of intake.

Breast milk collection and handling

Breast milk samples were obtained one day prior to the scheduled 12-hour home visit. A sample of approximately 5 mL of breast milk expressed manually from each breast was obtained from each mother for analysis of sodium and potassium concentration. Before expressing breast milk, the women washed both hands thoroughly with disinfectant liquid hand soap and running water, rinsed with deionized water and dried their hands with clean paper towels. The first drops of expressed milk were discarded after which the nipple and surrounding areola were cleaned with cotton gauze soaked with 70 % ethyl alcohol. Milk expressed thereafter was collected in 60 mL plastic vials with snap-on caps. Upon completion of expression from one side, hand rinsing and breast surface cleaning was repeated as above before milk was expressed from the second breast.

About 2 mL of the milk sample were tested for SCM immediately after collection using the California mastitis test (CMT). The CMT is widely used by the dairy industry as an inexpensive and rapid 'cow side' screening test of SCM. We used the CMT to screen women for SCM during recruitment into the study. The SCM status was subsequently confirmed using sodium potassium ratio. The CMT involved mixing about 2 mL of milk with an equal amount of the CMT reagent in a test paddle and swirling the paddle in an anticlockwise fashion. Thickening or gelatinization of the mixture after about 10 seconds indicates SCM. Severity of SCM inflammation was categorized as the extent of gel formation which was recorded on a scale of 1 to 3, with 3 representing the thickest gel formation. No gel formation was scored as 0. The remaining milk sample was kept on ice in a sealed container and transported to temporary storage in the field laboratory at -18 ^{0}C. Subsequently, these samples were transported on ice to Accra for storage in a freezer at -32 ^{0}C until analyses for sodium and potassium content.

Sodium and potassium analyses were carried out using the Medica Easylyte ion-selective electrode analyzer (Medica Corporation, Bedford, USA). Samples were first thawed to room temperature (25 ^{0}C) and then homogenized before 0.1 mL of sample was aspirated into the Medica Easylyte ion-selective electrode analyzer for analyses. In

addition to internal saline standards used by the Medica Easylyte analyzer, analytical quality was monitored by simultaneous analyses of milk with a saline standard with known electrolyte concentration.

Statistical analyses

Descriptive statistics including arithmetic means and standard deviations were computed for continuous maternal and infant variables. Categorical data were summarized into frequencies. Sodium-potassium ratios were computed from sodium and potassium data and categorized into SCM as follows: Na/K ≤ 1.0 indicating no SCM and Na/K > 1.0 indicating SCM. A CMT score ≥ 1 was considered a positive diagnosis for SCM. Group differences for categorical variables were tested using Pearson's chi-square statistic. One-way Analyses of Variance was performed to test the difference in infant milk intake between SCM groups as well as the bivariate associations with other maternal and infant factors. Multiple linear regression was used to model the infant and maternal factors that predict 12-hour milk intakes.

Results

Sodium and potassium data and CMT scores were available for 67 and 65 mothers, respectively. Five mothers had sodium and potassium data but not SCM scores while 7 others had sodium and potassium data but not CMT scores. Only 60 out of the 72 mother-infant pairs had complete information and were included in this analysis. About 62 % of mothers tested positive for SCM in one or both breasts using CMT ≥ 1. However, using Na/K > 1.0 as the threshold, only 26.9 % of women tested positive for SCM in one or both breasts.

Table 1 compares the characteristics of participants based on their Na/K-defined SCM category. Women who tested positive for SCM tended to be younger (p=0.05) and were most likely to be primiparous (p=0.03). There were no other significant differences in maternal characteristics between the SCM groups. There were lower infant weight (p=0.05), length (p=0.04) and head circumference (p =0.04) among infants whose mothers tested positive for SCM.

Average breast milk intake for all infants during the 12-hour observation was 407.7 ± 127.2 g, with a range between 173.5 g to 683.5 g. There was no difference in breast milk intake between infants whose mothers were diagnosed with a positive CMT score and those whose mothers had a negative CMT score (403.9 ± 130.7 vs. 425.6 ± 121.8, respectively; p=0.52). Using Na/K for SCM diagnosis, however, there tended to be a lower milk intake (-65.1 g; 95 % CI: -141.3 g, 11.1 g) among infants whose mothers had Na/K > 1.0 compared to infants whose mothers had non-elevated milk Na/K. Figure 1 displays the distribution of milk intake for the SCM groups as diagnosed by either CMT or Na/K.

Eighteen percent (N=11) of mothers tested positive for SCM with both CMT and Na/K. Infants of these women

(Figure 2) consumed significantly less breast milk (-88.9 g; 95% CI: -171.1 g, -6.9 g) during the 12-hour observation period compared to those whose mothers were diagnosed either as negative on both SCM tests or positive for only one of the tests.

In the multiple linear regression analysis, factors that predicted infant milk intake included infant weight (p<0.01) and total number of breastfeeds (p<0.01) during the 12-hour observations. Other factors that were included in the model but which did not explain additional variance in milk intake included maternal SCM status, age, and body mass index as well as infant sex, length, head circumference, arm circumference and number of feeds .

Discussion and Conclusions

The objective of this study was to determine whether SCM status was associated with a decrease in infant milk intake and, therefore, elucidate the mechanistic pathway by which SCM contributes to infant growth faltering. Our results showed that infants whose mothers were diagnosed with SCM using two diagnostic criteria (CMT score > 1 and Na/K > 1.0) consumed significantly less milk (p=0.034). The observed association, however, disappeared when the model accounted for infant weight and breastfeeding frequency. (p =.12 given 2 other very high milk intake variants (wt and # feedings) and limited number of experimental units).

To our knowledge, this is the first study to investigate the relationship between SCM and infant intake of breast milk beyond early lactation. Manganaro and colleagues have recently reported an inverse relationship between breast milk sodium and infant milk intake during the first week postpartum. Their results were consistent with findings in dairy cattle in which SCM is known to reduce milk output and permanently impair lactational performance.

SCM typically occurs unilaterally and therefore it is possible for milk output from a healthy breast to compensate for the adverse effect of SCM on an affected breast. As reported by Connor, infants may be capable of differentiating between normal breast milk and that with elevated sodium and thus exhibit a preference for the latter. An ideal design for a study of the effect of SCM on lactational performance, therefore, would be to measure milk secretion from one breast independently of the other. Such a study should preferably impose minimal interference on the 'on-demand' feeding relationship between mother and infant. Daly et al. have demonstrated the capacity to measure milk output using a computerized breast measurement system that allows measurement of changes in breast volume changes. The technology and cost of such a study design is however not feasible in many laboratories and certainly not in the field setting that this study was performed.

Elevated Na/K was more prevalent among younger and primiparous mothers in this study. A study in Zambia has also reported that primiparous mothers had significantly

higher Na/K from the first through the 16th week postpartum. These findings demonstrated a need to focus interventions on supporting young and first time mothers to maintain optimal breast health during lactation.

In this study, we measured infant milk intake using test weighing as a proxy for milk output because it is a simple procedure and its effect on the maternal-infant feeding relationship is only minimal. Costs and logistics limited us to 12-hour test weighing sessions rather than a full 24-hour test weighing session. Matheny and Picciano have reported that a doubling of infant milk intakes observed during the day was not equivalent to intakes observed throughout 24 hour test weighing sessions. In this study, insensitive water loss was not estimated. Arthur et al. have demonstrated significant underestimation of infant test weighing without consideration for insensitive water loss. Although our comparisons across groups are not affected by not controlling for insensitive water loss, it is possible that there was underestimation of breast milk intake across all subjects in our study.

The CMT was used in this study as a screening test during recruitment because it is a simple and inexpensive diagnostic procedure that gives immediate results. We have observed in our studies in this community that the CMT overestimates the prevalence of SCM. Na/K which is commonly used to diagnose SCM was therefore used to confirm CMT scores. Either CMT or Na/K alone failed to demonstrate significant differences in milk intake between infants based on their mothers' SCM status. This may be explained by issues related to the thresholds for SCM diagnosis. Somatic cell count which forms the basis of the CMT is higher in cows than humans. The CMT's diagnostic thresholds were designed and optimized to detect elevated somatic cell counts in dairy milk and therefore may exhibit different sensitivity levels in human milk.

The Na/K diagnosis is typically made using a threshold of 1.0 which is considered to be equivalent to sodium concentration of 18 μmol/L. This level of milk sodium is observed in breast milk during mammary tight junction closure or weaning. However, it is not known whether the fluctuations in milk electrolytes observed during onset of lactation or weaning follows the same pattern as the acute changes occurring in the milk of non-weaning lactating women. A further limitation in the current study was our inability to recruit equal numbers of women with SCM and those without SCM.

Conclusion

In this setting, children of women having both elevated CMT and Na:K ratio >1 had significantly lower breast milk intake. We were unable to demonstrate a reduction in breast milk intake among infants whose mothers had SCM after infant weight and # of feedings were included in the linear regression model. However, milk intake trended lower (p =0.12) and this combined with limited subjects and only 12 hr data warrant concern and further investigation of the relationship of SCM on breast milk intake and child growth and development.

Table 1. **Characteristics of Ghanaian lactating women and their infants by SCM status[1]**.

| Characteristics	SCM status[1]		p-value[3]
	Negative (N=46)	Positive (N=14)	
Maternal			
Age, y	26.8 (6.0)[2]	23.7 (4.7)	0.02
Education, y	7.6 (3.8)	7.5 (2.8)	0.23
Body mass index, kg/m^2	24.0 (3.1)	25.6 (5.0)	0.14
Primipara, %	32.6	64.3	0.02
Ill in last 7 days, %	23.9	7.1	0.08
Fever in last 7 days, %	4.3	7.1	0.28
Infant			
Age, mo	3.2 (0.1)	3.2 (0.1)	0.46
Weight, kg	6.2 (0.9)	5.8 (0.6)	0.05
Length, cm	60.5 (2.1)	59.5 (1.8)	0.04
Head circumference, cm	40.1 (1.2)	39.6 (0.9)	0.05
Mid-upper-arm circumference, cm	13.6 (1.2)	13.3 (0.6)	0.19
Exclusively breastfed, %	87.0	78.6	0.18
Male infants, %	43.5	50.0	0.34

[1] SCM positive defined as Na/K > 1.0; SCM negative defined as Na/K≤

[2] Mean (SD) or %

[3] Group differences in continuous and categorical characteristics were tested with Student's t-test and Pearson's χ^2 respectively; Fisher's exact test used for three variables: Ill in last 7 days, Fever in last and Exclusively breastfed
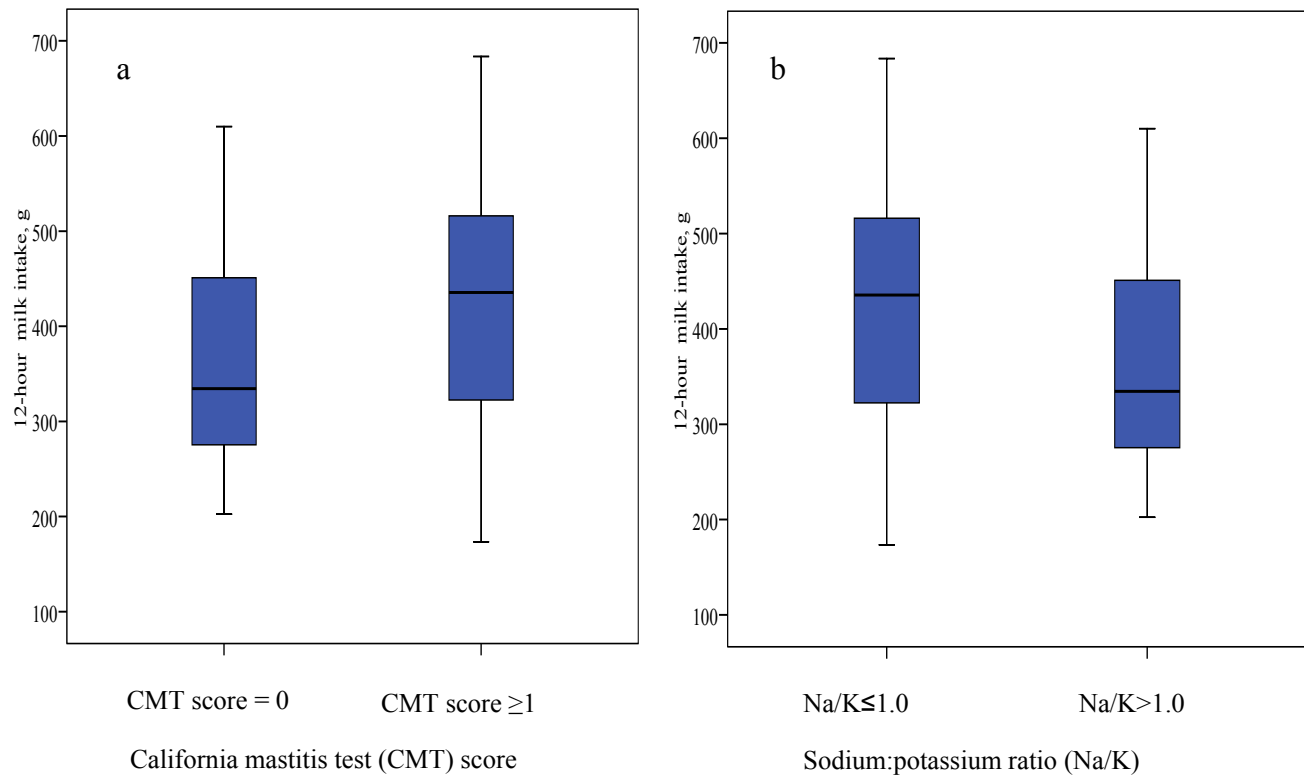
Figure 1. 12-hour breast milk intake of Ghanaian infants by test of subclinical mastitis:
(a) intake compared among California mastitis test (CMT) score groups; CMT=0 (n=37), CMT≥1 (n=23); (b) intake compared among sodium:potassium ratio (Na/K) groups; Na/K≤1.0 (n=46), Na/K>1 (n=14). The 25th and 75th percentiles are demarcated by the box; the median is represented by the dark horizontal line; the whiskers represent 1.5 multiplied by the interquartile. There were no significant differences between groups.
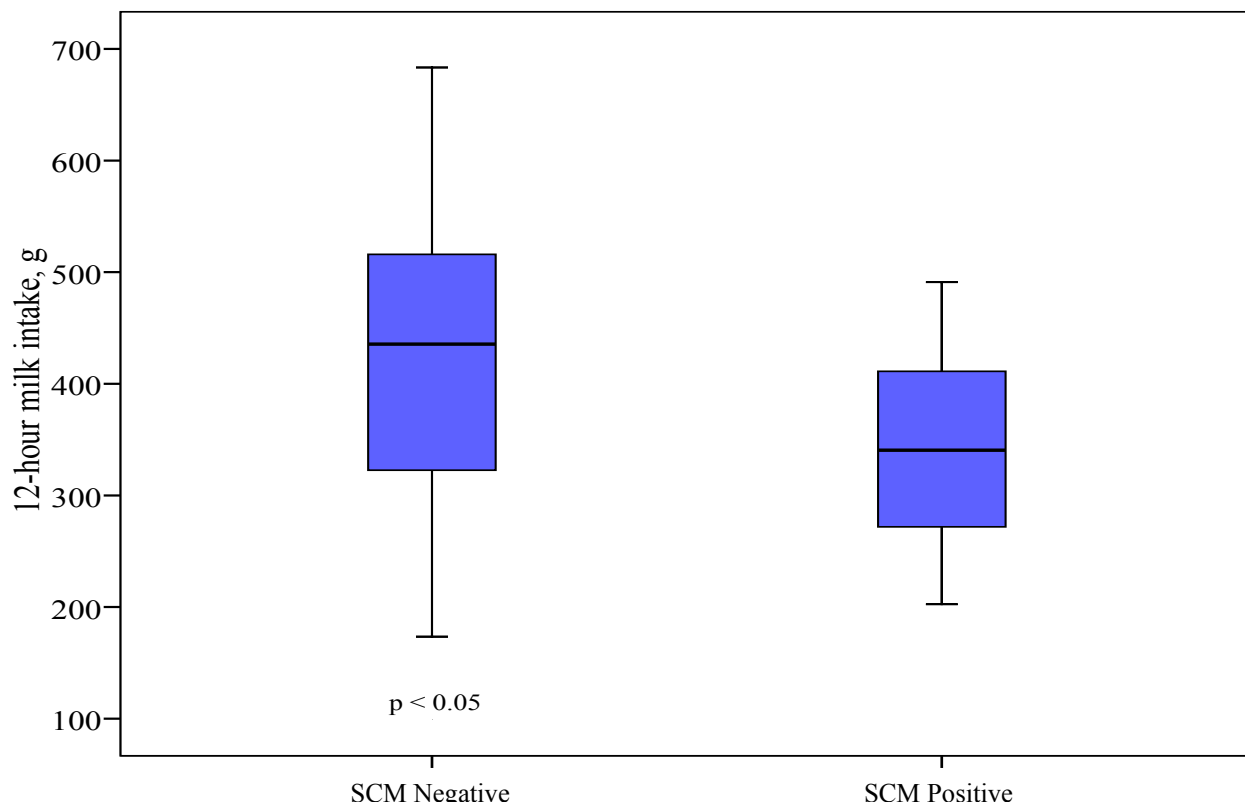
Figure 2. 12-hour breast milk intake of Ghanaian infants by subclinical mastitis diagnosis
SCM negative (n=49), SCM positive (n=11). SCM positive indicates mothers who had CMT score>1 and Na/K>1.0 in at least one breast; SCM negative indicates all other mothers excluded from the SCM positive group. The 25th and 75th percentiles are demarcated by the box; the median is represented by the dark horizontal line; the whiskers represent 1.5 multiplied by the interquartile. Milk intake from women with SCM was significantly lower (p < .05).