Effects of maternal subclinical mammary inflammation on infant growth

by

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A dissertation submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Nutritional Sciences

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2007

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Acknowledgements

I will forever remain in the debt of many wonderful people both at Iowa State University and in Ghana whose assistance and encouragement made the completion of this dissertation possible. Firstly, my warmest gratitude goes to my major advisor, Dr. Grace S. Marquis whose relentless encouragement and guidance spurred me on throughout my doctoral program. I especially commend my program of study committee members: Drs. D. Lee Alekel, Manju B. Reddy, Mack Shelley II, and Marcus Kehrli for their support and positive criticism offered right from the beginning and throughout the course of my studies and research. Dr Leo Timms was instrumental in the training for laboratory analysis that I performed on breast milk samples and has become a good and supportive friend. I owe him much for completing my studies successfully. I am also grateful to all faculty, staff, and fellow graduate students in the Department of Food Science and Human Nutrition (FSHN), especially those with whom I shared office space with and who helped me in diverse ways.

To all the wonderful mothers and infants who were included in the studies reported in this dissertation, I extend immense appreciation to you for your being available and patient with me and the field staff during the data collection activities. Many thanks to Professor Anna Lartey and Dr Lucy Brakohiapa who were my supervisors in the field during data collection. I had the opportunity to work with a wonderful team in the field: Kwame Opoku, Abigail Nartey, Victoria Chantey, and Beatrice. I am also grateful to the RIING project and its entire staff for supporting my study in Ghana.

Last, but not the least, I am grateful for the love, encouragement, and understanding of my family for allowing me to be away from home during the last four years to pursue this study. I am really grateful, really!

Abstract

Breastfeeding is unquestionably the best nourishment for infants. Current epidemiological as well as experimental evidence has repeatedly demonstrated the nutritional, immunological, and psychosocial benefits attributable to breastfeeding. However, suboptimal breast health may compromise maternal ability to breastfeed and the well-being of breastfeeding infants. Subclinical mastitis (SCM) is an asymptomatic inflammatory condition of the lactating breast that is associated with a number of adverse outcomes including lactation failure, infant growth faltering during the early postpartum period, and increased risk of mother-to-child transmission of the human immunodeficiency virus (HIV).

Three studies were carried out in Ghana with the overall aim of describing the mechanistic pathway(s) linking SCM and infant growth faltering. The specific objectives of the studies were to determine: 1) the prevalence of SCM among lactating women in Ghana, 2) whether SCM is associated with reduced breast milk intake by the infant, and 3) whether SCM occurring beyond the third month postpartum had an adverse effect on infant growth between the third and sixth month postpartum. Two cross-sectional studies were designed with respect to the first two objectives. Data were collected in a longitudinal study to examine the third objective.

All data from the three studies were collected from infant-mother pairs residing in the Eastern region of Ghana. Maternal data included demographic, health, and anthropometric data as well as breast milk samples that were analyzed to determine maternal SCM status. Maternal SCM was primarily diagnosed as elevated breast milk sodium:potassium ratio (Na/K) above 1.0. California mastitis test (CMT) was also used in the cross-sectional studies to diagnose SCM. Infant breast milk intake was estimated using the test weighing procedure. Infant data included feeding, health, and growth measured as weight, length, head circumference, and mid-upper arm circumference.

In the first cross-sectional study, SCM (Na/K>1.0) was observed among 45.3% of women at three or four months postpartum. About 30% of the observed SCM occurred in only one breast. In the second cross-sectional study, infants whose mothers had Na/K > 1.0 as well as CMT score ≥ 1 had significantly lower breast milk intake (-88.9 g; 95% CI: -171.1 g, -6.9 g). However, the observed milk intake difference across SCM groups disappeared when infant weight and feeding frequency were controlled in multivariate analyses. The longitudinal study did not find an association between maternal episodes of SCM (Na/K) occurring between the 3rd and 6th month postpartum and infant growth occurring during the same period.

In conclusion, our results indicate that SCM is a common condition among women in the Eastern region of Ghana. However, we did not find a reduction in breast milk intake of infants whose mothers had SCM. There was, also, no association between maternal SCM occurring between the 3rd and 6th postpartum and infant growth during the same period.

Chapter 1. General Introduction

The dissertation is organized into seven chapters as follows: general introduction, a review of the relevant literature, general methods, and a chapter each for manuscripts of three reports prepared in a format suitable for submission to peer-reviewed journals. The final chapter is general conclusions. An appendices section detailing the data collection tools and informed consent documents is included at the end. All references cited in the dissertation, excluding those in the three journal manuscripts, are listed in Arabic numeric order in a references section to be found after the general conclusions. References cited in the journal manuscripts are included at the end of each respective chapter.

Background

Breastfeeding is unquestionably the best nourishment for infants. Current epidemiological as well as experimental evidence has repeatedly demonstrated the nutritional, immunological, and psychosocial benefits attributable to breastfeeding.[1-3] These benefits are derived from the unique formulation of anti-microbial, immunoactive, and growth factors as well as the highly bioavailable macro- and micronutrients in human milk.[4-7] It is no surprise, therefore, that study after study in many different settings continue to report, unambiguously, the strong relationship between breastfeeding and reduced risk of infant malnutrition, morbidity, and mortality.

In recognition of the numerous benefits attributed to breastfeeding, global efforts have been instituted to promote and sustain breastfeeding practice. Beginning from the early 1980's, the international code on the marketing of breast milk substitutes was adopted by the 34th session of the World Health Assembly in 1981.[8] The code was designed as a set of regulations to preserve and promote appropriate breastfeeding practice and also to stem the tide of rapid breastfeeding decline that was being fueled by unethical marketing of commercial breast milk substitutes. The Innocenti declaration in 1990 and subsequently the

Baby Friendly Hospital Initiative (BFHI) instituted by the United Nations Children's Fund (UNICEF) in 1991 are two other notable efforts to promote breastfeeding practice.[9]

The World Health Organization currently recommends that all infants, with the exception of those born to HIV-infected mothers, be breastfed exclusively during the first six months of life with subsequent introduction of complementary feeding starting around the sixth month.[10] Current estimates indicate that breastfeeding is initiated by more than 90% of women globally.[11] However, only 39% of infants less than six months are exclusively breastfed.[12]

The primary reason given by most women for suspending breastfeeding is maternal concern about inadequate milk supply.[13,14] Maternal factors such as late onset of lactation, breast/nipple soreness and infant factors including gender, latch problems, and poor weight gain have also been cited as reasons for breastfeeding cessation or early introduction of complementary feeds.[15-17] Where available, lactation support to women during pregnancy or after delivery has been effective at reducing breastfeeding problems and subsequently improving breastfeeding practice.[18-21]

Successful breastfeeding may also be affected by maternal conditions that are not obvious to the mother. Subclinical mastitis (SCM) falls into this category. SCM is an inflammation of the lactating breast and has only recently been clearly characterized in the human lactation literature.[22] In dairy cattle, however, SCM has been described and extensively studied because of its adverse effects on milk production.[23] Interest in maternal SCM has grown because of the reported association of SCM with elevated risk of HIV transmission.[24,25]

SCM may occur for several reasons including localized inflammation of mammary tissue resulting from milk stasis, localized breast infection, physical breast tissue trauma,[26] mammary gland involution due to reduced breast milk production,[27] micronutrient deficiencies,[28,29] and systemic infection.[30] Poor lactation practice and weak suckling by the

infant have been associated with SCM.[27,31] The mammary tissue inflammation occurring during SCM leads to involuntary opening of the tight junctions cementing alveolar cells. Consequently, there is a net leakage of inflammatory cells and other plasma components including sodium and chloride ions into breast milk and a simultaneous efflux of potassium ions out from milk into plasma to maintain ionic balance.[32-34] An elevation in breast milk sodium potassium ratio (Na/K) has been found to correlate significantly with the inflammatory cytokine, interleukin 8, and therefore Na/K is considered a marker of mammary inflammation.[28]

In a study of lactating women in the United States, Morton[35] found an association between elevated breast milk sodium content and lactation failure. Children of the affected women gained less weight at one month postpartum than those whose mothers did not have elevated sodium. Similar infant growth faltering associated with elevated breast milk Na/K has subsequently been reported among infants in Bangladesh and Zimbabwe at 3 months and 4.5 months, respectively.[36,37]

The epidemiology of SCM is not as well characterized as clinical mastitis which is well described in the human lactation literature. Bilaterally elevated breast milk sodium is typical of early lactation and reduces by the 14th day postpartum.[5,34,37] This reduction is attributed to the closure of paracellular pathways in the mammary epithelium just before onset of lactation.[34] However, the unilateral elevation in breast milk sodium, typical of SCM, is persistent and occurs in response to mammary inflammation.[38] Moderately elevated Na/K between 0.6 and 1.0 has been reported in 16% of Bangladeshi women at 4 weeks postpartum.[21] In Zimbabwe, a moderate increase in Na/K was reported among 10% to 15% of HIV uninfected women and 12% to 21% of women infected with HIV during the first 4 months postpartum.[37] The rate of severely elevated Na/K (> 1.0) has a wide range: 9% at 1 month[36] and 22.4% at 6 weeks in South Africa.[24]

Typically, the rate of elevated Na/K declines over time. At 3 months postpartum, reported rates of elevated Na/K include 11% in Tanzania[28] and 12% in Bangladesh.[21] Willumsen et al.[24] have reported unusually high rates of severe unilaterally elevated Na/K of 22.9% among lactating HIV-infected women at 14 weeks postpartum. In this group of women, prevalence of severely elevated Na/K in one or both breasts was 25.3%, excluding those with moderate Na/K who usually constitute a larger proportion. Another study assessing SCM as elevated breast milk sodium (> 12 mmol/L) found about 16% of lactating women in Malawi had SCM at six weeks postpartum.[4] Another study has suggested that markers of SCM may be observed even at 12 months postpartum.[39] Studies by Dewey et al.[31] have reported that the weaning period is associated with elevated milk sodium levels, indicative of SCM. These studies suggest that SCM is a common occurrence among lactating women. There is a need, therefore, for further studies to fully understand factors that result in SCM as well as the pathways that lead to the stated infant and maternal outcomes of mammary inflammation.

Currently, the mechanism relating SCM with infant growth faltering remains undefined. In lactating cattle, SCM is associated with decreased milk production. It has also been reported that calves nursed by beef cows with SCM experience significant decrease in weight gain.[40] Kirkbride[41] had earlier reported that the weight that calves fail to gain due to mammary infection can be recovered with mastitis treatment. These studies suggest that SCM may be associated with a decline in the ability of mammary epithelium to produce enough milk to meet the needs of the growing calf. A similar mechanism may be occurring in human infants although there is no evidence from human studies. Prentice et al.[26] have reported that clinical mastitis may be associated with destruction of secretory mammary tissue with consequences for subsequent lactational performance.

Another mechanism that may explain infant growth faltering as a result of maternal SCM is decreased milk acceptability by the infant that translates into decreased milk intake.

Conner[42] has reported the case of an infant who preferred sweet milk produced from a normal breast as opposed to 'salty' milk produced by the mother's other breast that had elevated sodium. In such a situation, infant milk intake may be affected directly or indirectly through reduced emptying of the affected breast, which is known to affect milk output.[43] In such a situation, the bulk of the infant's intake will then be coming from the unaffected breast. Whether the unaffected breast alone is capable of producing enough milk to meet the nursing demands of the infant depends on the ability of the mother to practice breastfeeding behaviors that increase her milk supply. However, studies have shown that when mothers have concerns about low milk supply, they are likely to introduce other foods or wean their children.[16,17] We hypothesize therefore that SCM affects infant growth by reducing infant milk intake through two possible pathways: 1) reduced acceptability of milk and 2) reduced milk output resulting from either involution of mammary tissue, inadequate breast emptying or both.

To understand the relationship between SCM and infant growth faltering, we carried out three studies on infant growth and subclinical mastitis in Ghana between 2005 and 2006. The first study was a rapid survey to determine the burden of SCM among Ghanaian lactating women. Subsequently, we tested our hypothesis that SCM will reduce infant milk intake by recruiting a different sample of lactating women and their infants to observe infant milk intake. Our third study examined the association between SCM and infant growth faltering among breastfeeding infants beyond early lactation in a cohort of infant-mother pairs.

Previous studies investigating the association between SCM and infant growth have focused on the first three months postpartum period. Our current studies investigated the effects of SCM beyond the early lactation period. This period was also of interest because it coincided with the introduction of other foods (i.e., mixed feeding) to the child. Willumsen et al. (2000) have shown that mixed feeding is associated with elevated risk of SCM.[44]

Specific aims and hypotheses

The specific aims and hypotheses of the studies included in this dissertation are:

1. To determine whether SCM is prevalent among lactating Ghanaian women.

Hypothesis: Prevalence of SCM beyond 3 months postpartum is in excess of 20% among lactating women in Ghana.

2. To determine the relationship between maternal SCM and infant breast milk intake.

Hypothesis: SCM is associated with a decrease in infant breast milk intake.

3. To determine the relationship between maternal SCM and infant growth beyond the third month postpartum.

Hypothesis: SCM is associated with a decrease in weight and length gain in breastfeeding infants.

Chapter 2. Literature Review

Breastfeeding is recommended as the ideal way of feeding infants.[10] Breast milk provides essential nutrients, immunoactive proteins, antimicrobial factors, and other bioactive constituents that have been associated with reduced risk of morbidity and mortality among breastfed infants.[45] Many studies have shown that infants benefit from breastfeeding in a dose-dependent manner;[46] exclusive breastfeeding during early lactation as well as longer breastfeeding duration are both associated with less risk of infant morbidity and mortality. These findings form the basis for the recommendation of breastfeeding as a critical public health intervention to reduce infant morbidity and mortality, especially in low income settings where the risk of infections is often high.[1]

Breastfeeding and young child survival

Many studies have shown that breastfeeding is associated with increased infant survival, especially for infants below 6 months.[45,47] Children who are breastfed derive nutritional, psychosocial, as well as immunological benefits from breast milk. Observational studies in low income settings have demonstrated that breastfeeding, either exclusively or partially, is associated with decreased risk of many childhood diseases among both full term and preterm infants.[48,49] Protective effects of breastfeeding have been observed specifically for diarrhea,[50] respiratory infections,[51] and necrotizing enterocolitis.[52]

These benefits have been demonstrated in experimental studies as well. In a quasi-experiment in which Iranian women were given breastfeeding education, Froozani et al.[53] reported a lower mean number of days of diarrhea ($p < 0.004$) among infants in the experimental group who had a higher exclusive breastfeeding (EBF) rate (54%) than those in the control group in which EBF was low (6.5%). In the same study, children in the experimental group also had mean weights and lengths that were significantly higher ($p < 0.05$) than those in the control group. A recent breastfeeding trial in Belarus assigned mothers with intention to breastfeed to either a control group or an enhanced baby friendly hospital

initiative intervention.[54] After 12 months of follow-up, the study demonstrated a 40% reduction in risk of gastrointestinal infection among infants in the intervention group who had increased rates of EBF at both 3 and 6 months postpartum.

Breastfeeding is also known to reduce the mortality risk due to diarrhea and respiratory diseases by almost four-fold.[2] Some studies indicate that the risk of all-cause mortality is greatest in the first few months if a child is not breastfed.[46,55,56] Because the protection offered by breastfeeding increases with the dose of breastfeeding, the greatest mortality risk occurs among non-breastfed[46,57] and bottle-fed infants.[58] Non-breastfed children below 5 months have a 5-fold higher risk of dying compared to breastfed children of similar age.[46]

In a study of Latin America and Caribbean infants, EBF during the first 3 months and partial breastfeeding during the remainder of the first year of life prevented 55% of infant deaths related to diarrhea and acute respiratory disease and 66% of deaths among infants below 3 months due to both diarrhea and respiratory infections.[59] In the same group of children, about 14% of all-cause infant mortality was prevented by EBF. A meta-analysis of prospective and case-control studies of breastfeeding demonstrated that breastfeeding decreases the risk of mortality due to diarrhea (odds ratio 6.1; 95% CI, 4.1-9.0) and acute respiratory infections (2.4; 95% CI, 1.6-3.5) of children in the first 6 months of life.[60]

<u>Benefits of breastfeeding beyond infancy</u>

The benefits of breastfeeding persist beyond infancy. In a meta-analysis of 20 studies, Anderson et al.[61] reported that children below 16 years old, who had been breastfed as infants, demonstrated superior motor and cognitive abilities compared to formula-fed, even after adjusting for child age and maternal socio-demographic factors. Among this age group, the evidence supported a dose-dependent effect of breastfeeding on cognitive function. In a prospective cohort of Australian children, the risk of asthma at 6 years of age was less if the duration of exclusive breastfeeding was at least 4 months.[62]

Several studies have also demonstrated an association between having been breastfed as an infant and a reduced risk of developing various chronic disorders including premenopausal breast cancer,[63,64] leukemia and lymphoma,[65] and other cancers.[66] Beneficial breastfeeding outcomes have also been reported for the risk of cardiovascular diseases,[67,68] diabetes mellitus,[69] and obesity in adulthood.[70]

<u>Effects of breastfeeding on maternal health</u>

Among women, breastfeeding is associated with maintenance of a healthy body weight postpartum and a reduced risk of obesity.[71] Kramer et al.[72] have reported significant postpartum reductions in hip circumference among women breastfeeding either exclusively or partially, although the mechanisms involved have not been established. Brewer et al.[73] have reported significant reductions in postpartum weight of lactating women between the third and sixth month only among EBF women compared to formula-feeding or mix-feeding. A recent study by Kac et al.[74] concluded that prolonged breastfeeding contributes to a decrease in postpartum weight retention among Brazilian women. This is in agreement with an earlier study among Australian women in which excess postpartum weight gain was associated with early cessation of breastfeeding.[75] Among another cohort of Brazilian women who were observed over a period of five years postpartum, both body mass index and percentage fat mass, determined by bio-impedance, were lowest for women who breastfed for 6-11 months and highest for those who breastfed for less than one month.[76]

However, a prospective study of lactating mothers failed to establish a consistent relationship between lactation and weight loss between 6 weeks and 12 months postpartum.[77] Parker and Abrams[78] have suggested that postpartum weight retention may be racially determined. Their study found that black mothers were twice as likely to retain at least 20 pounds more weight postpartum than white mothers. However, since breastfeeding duration was not controlled in this study, the results may only be reflective of low breastfeeding practice among black women.[79] Differences in cultural perceptions of body composition and

weight loss my also explain the differences across races. However, the effects of cultural differences on postpartum weight loss have not been reported.

On-demand breastfeeding is associated with reduced incidence of ovulation[80] and thus reduced fertility. The biological basis underlying this phenomenon is explained by the disruption of the normal pattern of gonadotropin releasing hormone secretion during breastfeeding, which results in reduced luteinizing hormone secretion from the pituitary gland.[81] Ultimately, a failure of follicle development is induced. This physiological process forms the basis of the birth control technique known as the lactational amenorrhea method (LAM). LAM has been demonstrated in a trial in Honduras where women who breastfed exclusively for four to six months had longer amenorrhea than those who introduced solid foods at 4 months.[71]

There is also evidence suggesting that breastfeeding is linked with reduced risk of some cancers that affect women. Many studies have demonstrated a protective effect of breastfeeding for ovarian cancer;[82-84] often the risk reduction due to breastfeeding was associated with duration of breastfeeding. Evidence for the effect of breastfeeding on breast cancer is however less consistent. In recent studies, breastfeeding either had no effect,[85] or only had a small protective effect when parity, age at first breastfeeding, and age at menopause were controlled.[86,87] A recent review of the available evidence concluded that women who have a history of breastfeeding had reduced risk of both ovarian and breast cancer.[88]

Breastfeeding is also associated with greater vascular stress response, lower perceived stress levels, and fewer depressive symptoms among lactating women.[89] In a study on maternal stress and mood, investigators observed that breastfeeding mothers reported lower perceived stress than bottle-feeding mothers.[90] The same study also reported that breastfeeding was associated with a decrease in 'negative mood' while bottle-feeding was associated with a decrease in 'positive mood'.

In 2001, Nduati and colleagues[91] reported the disturbing outcome of a randomized trial on HIV progression among Kenyan women. They found a 3-fold elevation in mortality among women in the breastfeeding arm of the study compared to women feeding their infants with formula. The findings reported by Nduati et al.[91] have been criticized for two reasons: 1) women who were practicing mixed-feeding were included in the breastfeeding group, and 2) almost 50% of women in the breastfeeding arm of the trial had low CD4$^+$ count at baseline. The later critique is strengthened by findings of a study by Otieno et al.[92] which concluded that although breastfeeding was associated with a significant CD4$^+$ decline (p=0.01) up to 24 months postpartum, mortality was not significantly different between breastfeeding and formula-feeding women. Analyses of data collected among HIV-infected South African women did not find an elevated maternal mortality risk due to breastfeeding.[93] Also, a meta-analysis of data from a number of randomized trials also failed to find excess mortality due to breastfeeding compared to not breastfeeding among HIV$^+$ lactating women.[94]

Characteristics and composition of human breast milk

Human breast milk is a non-uniform fluid with a composition that varies with stage of lactation, time of day, stage of feeding at which it is sampled, maternal nutrition, and individual differences.[95] The first flow of milk after birth is known as colostrum. The onset of colostrum secretion is variable but occurs mostly between 38 to 98 hours after birth. Colostrum is a thicker fluid compared to mature milk with a creamy or yellowish appearance.[95] It is exceptionally rich in lipid soluble vitamins, antioxidants, and immunoglobulins. However it has less lipid content compared to mature milk. The concentration of sodium, chloride, and magnesium in colostrum is higher than mature milk[27] whereas the levels of lactose and potassium are lower.

Colostrum is important for the establishment of bifidus flora including *Lactobacillus bifidus*. The immunological benefits derived from colostrum are especially crucial for

protecting the infant from infections during the immediate postpartum period when the immune system is not fully developed. Colostrum gradually turns into transitional milk towards the end of the first week postpartum.[95] Transitional breast milk has a lower concentration of total proteins and immunoglobulins and higher lactose and lipid content than colostrum, resulting in an increase in the total energy provided by the milk. By the end of the second week postpartum, transitional milk has almost been replaced by mature milk.

About 90% of mature breast milk is in the aqueous phase.[96] Mature milk is packaged as emulsified globules coated with a membrane, proteins in colloidal dispersion as micelles, and most minerals and all the lactose in true solution.[97] Human milk contains all the major nutrients, including lipids, proteins, carbohydrates, vitamins, and minerals. The presence of immune factors, enzymes, hormones, growth factors, and other bioactive factors makes breast milk superior to breast milk substitutes.

The lipid content of mature human breast milk is about 4% and is considered the most variable constituent of breast milk.[98] Most of the lipids in breast milk (98%) are present as triacylglycerol.[96] Other major lipid components in breast milk include cholesterol, fatty acids, phospholipids, and sterols. Total lipid content of human milk may be influenced by factors including stage of lactation, diet, health status, parity, and gestational age.[99] Masters et al.[100] have shown that supplementation with conjugated linoleic acid decreases breast milk total lipid content. Breast milk lipids are important as energy stores and as sources of essential fatty acids and cholesterol for the infant, and important for promoting intestinal absorption of fat-soluble vitamins. The long chain polyunsaturated fatty acids, docosahexaenoic acid, and arachidonic acid in human milk have been found to be especially vital in normal neurological development,[101] development of vision, as well as synthesis of eicosanoids.[102]

Milk protein content varies among mammals. In humans, proteins constitute less than 1% of mature milk content.[95] The highest concentration of milk proteins is found in colostrum.[103] Major proteins in milk include β-casein, albumin, α–lactalbumin,

β–lactoglobulin, and immunoglobulins.[95] Unlike α–casein in cow's milk, β–casein in human milk is easily digested. Some milk proteins may not provide nutritional benefit, but play critical roles as immune factors. Several immunoprotective proteins occur in human milk including lactoferrin, lysozyme, secretory immunoglobulin A, epidermal growth factor, and interleukin 8.[104] Based on their function, these proteins have been classified as either direct-acting antibacterial factors, anti-inflammatory factors, or immunomodulating bioactive compounds.[104] Lactoferrin is a glycoprotein with both bacteriostatic and bactericidal properties and is found in high concentrations in human milk.[105] Lysozyme is capable of breaking up bacterial cell walls.[106] Interleukin-8 is one of two chemokines found in relatively high concentrations in human milk. Interleukin-8 is secreted in response to inflammatory activity and plays a role in recruitment and activation of lymphocytes and neutrophils.[107] Interleukin-1 has also been measured in high quantities. During inflammatory conditions such as mastitis, high levels of interleukin-8 are secreted into milk.[28]

Lactose is the principal carbohydrate present in human milk[95] and supplies almost half of the energy (40%) available from breast milk. Lactose exerts 60 to 70% of the total osmotic pressure of milk and its secretion is accompanied by secretion of large amounts of water, making it a key determinant of milk volume.[27,108] The quantity of water secreted along with lactose is enough to meet the needs of breastfeeding infants without intake of water from other sources.[109] This finding justifies the recommendation of EBF without giving water during the first 6 months postpartum.[110] Another osmotic benefit of milk lactose is that because it is a disaccharide, its digestion and absorption is less likely to cause postprandial osmotic stress. In addition to lactose, other simple carbohydrates present in milk include glucose and galactose, both of which are present in relatively small quantities. Breast milk contains an array of moderate-chain-length carbohydrates (oligosaccharides and gluco-conjugates), as well as conjugated carbohydrates including glucoproteins, glycolipids, and nucleotide sugars. Some of these carbohydrates protect the gut environment by promoting the

growth of lactobacilli, limiting colonization by bacterial pathogens, and inhibiting bacterial toxins.[111]

The major minerals found in human milk include calcium, magnesium, sodium, potassium, phosphorus and chloride.[95] Other essential minerals such as iron and zinc are found in lesser quantities and their concentration in milk tends to decrease during the first four months after birth.[5,27] Although the concentrations of iron and zinc in breast milk are relatively low, the percentage absorbed is higher among exclusively breastfed infants,[112] compared to those whose receive other foods, because of the high bioavailability of these minerals in human milk. Current evidence suggests that the robust homeostatic physiology of the lactating woman ensures that the mineral content of her milk is not affected when she is deficient in minerals.[113] However, maternal iodine and selenium deficiency depletes milk of these minerals and maternal dietary intake has been shown to improve the content of these minerals in breast milk.[114,115]

Adequacy of vitamin supply in breast milk is vitamin specific; generally, breast milk vitamin content is sufficient during early lactation.[116] With the exception of low vitamin K in breast milk, other fat soluble vitamins are supplied adequately by breast milk during the first few months postpartum. Breast milk content of most water soluble vitamins are significantly affected by maternal status with the exception of folate.[113] Vitamin B_6 content in milk as well as vitamin A has been found to respond to changes in maternal status.

Enzymes, hormones, and other non-nutritive factors that are especially important in neonatal digestion and growth have been assayed in human milk.[117,118] These non-nutritive components of breast milk play critical roles in modulation of inflammatory processes, enhancement of neural transmission, and nutrient synthesis, assembly, and utilization.

Potentially harmful substances have also been identified in breast milk including medication taken by the mother, chemical and radioactive exposures like caffeine, nicotine, aflatoxins, and viruses like HIV. Several studies have reported the presence of unusually

large amounts of environmental pollutants in breast milk, especially, polychlorinated organic compounds.[119]

Definition of breastfeeding

Until 1990, breastfeeding practice was not clearly defined. As a result, there was much variation in the indicators used for describing breastfeeding behavior, thus making comparisons among studies difficult.[120] In 1990, Labbok and Krasovec[121] published a framework in which breastfeeding behavior was defined to include three broad categories, namely: full, partial, or token. Full breastfeeding was described either as 'exclusive' or 'almost exclusive'. EBF described the practice of feeding infants with breast milk only; no other food, drink, vitamin drops, medicines, or water was provided.[10] When vitamins, minerals, water, juice, or other ritualistic feeds are given infrequently, in addition to breast milk, the practice was described as 'almost exclusive'.[122] The term 'partial' breastfeeding was used to describe the practice of giving other liquid or solid foods to the breastfeeding infant. Partial breastfeeding was further categorized as high, medium, or low depending upon the proportion of breast milk that constitutes the total nutrient intake of the child. When breastfeeding occurred irregularly and for short durations, and essentially to pacify, console, or comfort a child, it was described as 'token breastfeeding'. The WHO later published a similar breastfeeding definition framework in which the term 'complementary feeding' was used to describe any breastfeeding practice that involves giving the infant other liquid or solid foods.[122]

Global efforts to promote appropriate infant feeding

Until the past decade, wide disparities existed in breastfeeding practices between regions, countries, and even communities within countries. The WHO and United Nations Children's Fund (UNICEF) have championed global efforts to improve infant feeding behaviors, especially among developing countries. Some recommendations that were instituted to promote optimal infant feeding included the International Code on Marketing of

Breast Milk Substitutes, the Innocenti Declaration on the Protection, Promotion and Support of Breastfeeding,[9] and the Baby Friendly Hospital Initiative.[8]

In 1981, the WHO and UNICEF in consultation with governments, professional organizations, non-governmental organizations, and the infant feeding industry prepared the International Code on marketing of Breast Milk Substitutes that was passed as World Health Assembly resolution 34.22.[9] The goal of resolution 34.22 was to ensure appropriate marketing of breast milk substitutes, feeding bottles, and teats. The code was necessary to address the threat posed by marketing of breast milk substitutes to breastfeeding practice. The code prohibited promotion of infant formula through hospitals, free distribution of formula samples to mothers, and the use of marketing strategies that idealize bottle feeding. Most countries have endorsed and have either passed resolution 34.22 into law or are in various stages of passing it into law. However, a few countries, including the United States of America, Iceland, Chad, and Somalia have taken no action on the resolution.[123] The International Baby Food Action Network has been monitoring violations of the resolution, some of which have been described as substantial violations by major baby food and infant feeding bottles and teats manufacturers.[124]

In 1990, participants at the WHO/UNICEF policymakers' meeting on breastfeeding issued a statement in support of breastfeeding. This statement which became known as the Innocenti Declaration recognized that breast milk provides adequate nourishment for infants and contributes to their healthy growth and development.[9] It also noted that the benefits of breastfeeding increased with exclusive breastfeeding during the first four to six months of life and, thereafter, breastfeeding with addition of complementary foods. Additionally, the declaration urged national health authorities to reinforce breastfeeding culture, protect the erosion of breastfeeding culture, and to integrate breastfeeding into overall health and development policies.

The BFHI was launched in 1991 by UNICEF, with the support of other United Nations agencies as a strategy to promote breastfeeding in health institutions. The goal of the BFHI was to create an environment that supports breastfeeding in hospitals and free standing maternity wards.[8] Health facilities designated 'Baby Friendly' must prohibit free or low cost distribution of breast milk substitutes and must pass an external assessment for the implementation of 10 specific breastfeeding promotion steps. Some of the requirements of the 10 steps include putting infants to the breast within one hour of delivery, giving newborn infants no food or drink other than breast milk (with exceptions when medically indicated), practicing rooming-in (that is, allow mothers and infants to remain together 24 hours a day), and encouraging breastfeeding on demand.

The current WHO Global Strategy for infant and young child feeding recommends exclusive breastfeeding for the first six months, breastfeeding with complementary feeding starting at six months of age, and continued breastfeeding into the second year of life and beyond.[10] These recommendations were built on the international infant feeding ideals described above and were designed to serve as basic guidelines for more comprehensive, country-specific approaches to improve infant and young child feeding. The strategy also recognizes the important influence of maternal health and nutrition on young child growth and urges efforts to address maternal under-nutrition. Specific reference is given to the impact of HIV on infant feeding and proposes guidelines to address children in this unique situation.

Current breastfeeding behavior

Current evidence shows that there has been a positive global response to efforts to protect and promote breastfeeding practice. Currently, in many countries where data exist, more than 90% of infants are introduced to breast milk.[11,125] The greatest improvements in breastfeeding initiation have been reported in Europe and North America where breastfeeding initiation rates were previously very low.

The push for EBF of infants during the first few months postpartum has also yielded positive results. Between 1990 and 2000, EBF increased marginally in all regions of the world.[126] East Asia and Pacific regions recorded the highest rates of EBF. However, the greatest improvement in EBF was observed in the Sub-Saharan Africa region, which together with the North Africa and Middle East regions, currently, have the lowest regional EBF rates (34%). Within countries, there were greater improvements in EBF among urban rather than rural dwellers. Despite these improvements, EBF rates still remain low globally; only 39% of infants less than six months are EBF.[12]

Challenges to breastfeeding

Although the ability to breastfeed successfully comes naturally to many women, especially in cultures where breastfeeding the newborn is the norm, there are also many women who need support in order to breastfeed successfully. Young and first time mothers, especially, need expert support to overcome problems that affect their ability to breastfeed successfully. The source of breastfeeding problems may be attributed to cultural and socioeconomic factors such as maternal education, where the baby was delivered, availability of alternative infant feeds, lack of social support, misperceptions about breastfeeding from spouse, family, or friends, as well as the prevailing infant feeding practices in the community that may discourage breastfeeding practice.[127,128] Breastfeeding problems that derive from biological factors may include breast pain, sore nipples, breast swelling, and congenital defects that limit the ability of the infant to suckle effectively. Psychological barriers to breastfeeding include peer conformity pressure and the belief of 'inability to produce enough milk'.[13,14]

Subclinical mastitis

SCM is a common asymptomatic inflammatory condition of the lactating breast and has only recently been clearly characterized in the human lactation literature.[22] Interest in SCM has grown because of its association with elevated risk of HIV transmission from

mother to child through breast milk.[24,25] SCM may occur for several reasons including localized inflammation of mammary tissue resulting from milk stasis,[129] localized breast infection, physical breast tissue trauma,[26,27] mammary gland involution due to reduced breast milk production,[27] micronutrient deficiency,[28,29] and systemic infection.[30] Poor lactation practices and weak suckling by the infant have also been associated with SCM.[27,31] The mammary tissue inflammation occurring during SCM leads to involuntary opening of the tight junctions cementing alveolar cells. The result is a net leakage of inflammatory cells and other plasma components, including sodium and chloride ions, into breast milk.[32,33,34] Simultaneously, there is efflux of potassium ions from milk spaces into interstitial spaces to maintain ionic balance.

SCM may progress to clinical mastitis that is diagnosed with symptoms of fever, breast pain, reddening of affected breast area, and engorgement of the breast.[95] Clinical mastitis, unlike SCM, has been studied more extensively and has been associated with factors that affect ineffective emptying of the breast, including persistent breastfeeding from a preferred side of the breast, weaning, wearing tightly fitting brazziere/clothing,[130,131] use of pacifiers,[132] and a prone sleeping position.[133] The role of poor hygiene and infection in the causation of clinical mastitis has also been reported. [134]

Subclinical mastitis epidemiology

Bilaterally elevated breast milk sodium is typical of early lactation and usually becomes reduced by the 14th day postpartum.[34] The subsequent unilateral elevation in breast milk sodium, however, is persistent and occurs in response to mammary inflammation. High prevalence ranging from 12% to 25% during the first 14 weeks postpartum has been reported in different settings.[24,37,44] For example, a study in Bangladeshi women has shown that 25% and 12% of healthy breastfeeding women had elevated sodium:potassium ratios at 2 weeks and 3 months postpartum, respectively.[36]

Another study that diagnosed SCM using elevated breast milk sodium reported a prevalence of 16% among HIV-infected women in Malawi at six weeks postpartum; the rate reduced to 3% by the 6th month postpartum.[25] Some studies have reported that markers of SCM may be observed even at 12 months postpartum.[39] Studies by Dewey et al.[31] have also reported that the weaning period was associated with elevated breast milk sodium content.

Assessing subclinical mastitis

In human studies, SCM has commonly been diagnosed as elevated breast milk sodium,[25] sodium-potassium ratio (Na/K),[24,37,135] somatic cell count,[136] or Interleukin-8.[28] Inflammation of mammary tissue triggers a series of physiological changes that eventually results in the elevation of breast milk sodium ion and chloride ion concentrations as well as a decline in potassium ion concentration.[32,33] These changes form the basis for the use of milk sodium, Na/K or electrical conductivity as markers of SCM. Na/K, rather than sodium concentration alone is commonly used for SCM diagnosis because: 1) it is assumed that the ratio of sodium to potassium will control for assay variations that may occur due to unequal distribution of the ions in the aqueous and lipid phases of milk and 2) the ratio accounts for the normal and almost parallel decline in both sodium and potassium ions during early lactation.[5]

Normal human milk sodium and potassium concentration are tightly regulated and range between 5 to 6 mmol/L and 13 to 14 mmol/L respectively.[5,34,137] Adcock et al.[138] have noted, however, that there is a gradual decline in breast milk electrolyte concentration over time postpartum. The highest sodium concentration has been observed in colostrum. The reported extremes for sodium in human milk are a low of 2 mmol/L and a high of 94.6 mmol/L.[139,140]

Part of the variation in the reported sodium or potassium concentration is due to the different assay methods used including flame photometry, atomic absorption/emission spectroscopy,[24,28,37] and ion-selective electrode methods.[4,34] The procedures used in flame

photometry and atomic emission spectroscopy are well established and are considered standard methods.[141] However, ion-selective electrode methods allow for rapid and inexpensive analysis of large number of samples.

In the study reported by Semba[4] in Malawi, SCM was diagnosed when sodium concentration was above 12 mmol/L. About 16% of the 96 lactating women in the study were diagnosed with SCM at six weeks postpartum. In an earlier study, Morton[35] had screened women with lactation problems using sodium concentration of 16 mmol/L as the diagnostic threshold. When Na/K is used for SCM diagnoses, ratios below 0.6 are considered normal. Na/K between 0.6 and 1.0 are described as moderately elevated while Na/K > 1.0 is considered severely elevated.[24,37,44] These cutoffs were selected based partly on the distribution of Na/K and also on published levels of sodium and potassium in the available literature. Willumsen et al.[44] have argued that Na/K > 1 is equivalent to 18 mmol/L of sodium which is indicative of mastitis after the first few days postpartum and in the absence of weaning.

In dairy cows, the primary cause of mastitis is bacterial infection, with S*taphylococcus aureus* identified as the most common pathogen.[142] Bacterial infection leads to inflammation in the mammary tissue similar to mastitis in human mammary inflammation. Bacterial cultivation is therefore considered the 'gold standard' for diagnosing mammary infection.[143] However, other methods have been developed for diagnosing mammary infection including electrical conductivity,[144] somatic cell count,[145,146] and the California mastitis test (CMT).[147]

<u>Subclinical mastitis, infant feeding, and growth</u>

A few studies highlight the association between infant feeding practice and SCM. Aperia et al.[137] have reported elevated breast milk sodium in clinical situations where ineffective breast emptying occurred on or before the first three days postpartum. They noted that a high sodium content in breast milk was related to potential lactation failure that was

reversible with effective breast emptying using breast pumps. Willumsen et al.[44] have also reported that SCM was more prevalent among mothers who practiced mixed-feeding compared to EBF, especially during the first few days postpartum.

Morton[35] has reported that infants in the United States whose mothers had elevated breast milk sodium concentration (> 16 mmol/L) gained less weight within the 3rd and 8th days postpartum. This finding has been replicated among mother-infant pairs recruited into a vitamin A trial in Bangladesh.[36] In this trial, low weight gain was significantly associated with elevated breast milk IL-8 content. IL-8 is a marker of SCM and has been found to be significantly correlated with Na/K. Currently, the mechanism relating SCM with infant growth faltering remains undefined.

 In the dairy cows, SCM is associated with decreased milk production.[148] In beef cattle, it has been reported that calves nursed by cows with SCM experience a significant decrease in weight gain.[40] Lents et al.[149] have reported that the effect of SCM on calf weight was limited to early lactation, but disappeared by weaning age. Kirkbride[41] had reported that growth faltering in calves could be reversed with mastitis treatment. However, the study by Lents et al.[149] failed to observe this outcome. A recent study by Dahl et al.[150] reported that among 3 week post-parturition dairy cattle, frequent milking led to increased milk volume and decreased milk cell count. These studies suggest a number of outcomes due mammary inflammation: 1) SCM may be associated with a decline in the ability of the mammary epithelium to produce enough milk and 2) increased milk output with frequent milking may lead to better flushing of pathogens out of the gland. Poor growth outcomes in the beef calves may be due to avoidance of nursing by the lactating cow due to pain or by the calf due to changes in milk flavor or composition. Frequent milking can increase milk output as well as relieve mammary inflammation and hence increase the supply of milk to the calf. A similar mechanism may be occurring in human lactation although no evidence from human studies currently exists. Prentice et al.[26] have reported that clinical mastitis in the human breast may

be associated with destruction of secretory mammary tissue with adverse consequences for subsequent lactational performance.

Another mechanism that may explain infant growth faltering as a result of maternal SCM is decreased milk acceptability by the infant that translates into decreased milk intake (Figure 2.1). Conner[42] has reported the case of an infant who preferred the sweet tasting milk produced by the mother's normal breast compared to 'salty' milk produced from the other breast that had elevated sodium. In such a situation, reduced emptying of the affected breast, from which the infant feeds less frequently, will lead to reduced milk output.[150] The bulk of the infant's breast milk intake come from the unaffected breast. Whether the unaffected breast alone is capable of producing enough milk to meet the nursing demands of the infant depends upon the ability of the mother to practice breastfeeding behaviors that increase her milk supply. However, studies have shown that when mothers have concerns about low milk supply, they are likely to introduce other foods, which will further decrease milk intake, or wean their children.[16,17]

<u>Control of subclinical mastitis</u>

Animal studies have demonstrated a protective role of micronutrients including selenium and vitamin E against SCM.[29,151] Similarly, studies in rural Tanzanian women have demonstrated a reduction in SCM in response to vitamin E-rich sunflower oil but not to vitamin A-rich palm oil supplementatin.[28] Another trial in Zimbabwe showed a non-significant decrease in the prevalence of subclinical mastitis among HIV-positive women less than 4.5 months postpartum who were receiving multivitamin supplementation according to the U.S. dietary recommended intakes (DRI).[37]

A possible mechanism for the protective role of micronutrients is to decrease the inflammatory cytokines as well as the tissue damage resulting from free radicals that may be produced during inflammation.[44,152] In a recent micronutrient trial, pregnant women in Nepal were assigned to receive either routine iron and folate supplements or multi-micronutrient

tablets containing 15 micronutrients.[153] This study did not find any effect of multiple micronutrient supplementation on the contents of Na/K in breast milk collected at one month postpartum.

Flores and Filteau[21] have reported a non-randomized trial among Bangladeshi women in which the intervention group participated in a single lactation counseling session with a trained counselor. Counseling sessions were carried out any time around delivery (antenatal, on day of delivery or postnatally) and covered topics including the importance of giving colostrum, good positioning and attachment, importance of breastfeeding soon after birth, feeding on demand, and the need for breastfeeding exclusively for 4-6 months postpartum. At four weeks postpartum, women who had received lactation counseling had significantly lower geometric mean Na/K (14%) than those in the control group who did not have any counseling. Since this study was published, there have been no reports confirming the outcome of this study in the same or other populations. The efficacy of this intervention also needs to be tested in a randomized experiment to establish its application in the control of SCM.

A recent study in Malawi[136] assessed the effect of antibiotics on reducing breast milk HIV viral load by reducing SCM. Seventy-five HIV-1 infected women who were diagnosed with subclinical mastitis (leukocyte count $> 10^6$ cells/ml) at two weeks postpartum were treated with oral amoxicillin/clavulanic acid. The women were followed up at scheduled clinic visits during which milk samples were collected to assess SCM status. After one week post treatment, leukocyte count had declined significantly ($p < 0.001$) by more than 58%. However, there was a non-significant increase in breast milk sodium at the same time. Further leukocyte decline was subsequently observed between the 4th and 12th week post treatment ($p < 0.001$), and at this time, the leukocyte decline was accompanied by a significant decline in sodium concentration ($p < 0.05$). The outcome of this study was in

agreement with the use of antibiotics in the reduction of mammary inflammation in clinical mastitis.[154]

Milk biosynthesis, secretion, and regulation

The functional milk secreting unit of the mammary gland is the alveolus. Milk components are synthesized in the mammary gland by a single layer of epithelial cells lining the alveoli.[155] The content of the alveoli are drained by a network of ducts that export milk from the site of production to the nipple through which milk exits the breasts. The alveoli are surrounded by myoepithelial cells that provide the contractile force needed to expel milk from the alveolar lumen. A rich supply of capillary tissues and fat cells that surround the alveoli together provide the substrate needed for milk synthesis.

Substrates required for milk synthesis originate from circulating blood and may reach milk ducts by two main routes: 1) a trans-cellular pathway which allows substrates to be transported across both the basolateral and apical membranes of the alveolar cell into milk ducts and 2) a para-cellular pathway that allows substrates to 'leak' through extra-cellular spaces. Alveolar epithelial cells, however, are connected to each other by apical junctional complexes (adherens) and tight-junctional elements that inhibit exchange of substances between vascular and milk compartments via para-cellular pathways.[156] The para-cellular pathway thus becomes functional only under certain physiological conditions that will be discussed subsequently.

Milk secretion into alveolar ducts involves five distinct pathways based on the chemical structure of the substrate. Endogenously generated milk proteins are primarily secreted by the exocytotic pathway.[155] Other milk components including water, lactose, oligosaccharides, phosphate, calcium, and citrate are secreted by this pathway. Similar to other exocytotic secretion mechanisms, these milk components are packaged into membrane-coated secretory vesicles. The vesicles move to and become attached to the apical membrane of the alveolar cell where its contents are subsequently released into the milk duct. A trans-

cellular pathway has also been suggested for the secretion of some proteins including immunoglobulin A into milk.[157] This pathway is accomplished by endocytosis and exocytosis at the basolateral and apical membranes, respectively. Trans-cellular secretion of prolactin, transferrin,[158] and low-density lipoprotein into milk have been reported.[159]

Using substrate from fat cells and circulating plasma lipids, milk lipids are synthesized in alveolar cells, which have a well developed lipid synthesis capacity. Milk lipids are secreted as protein coated lipid droplets. These droplets subsequently coalesce with the alveolar apical membrane and are discharged into the milk duct as milk fat globules in a unique budding process described by Mather and Keenan.[160]

Solute-specific membrane secretion pathways exist for ions, glucose, and amino acid transport and involve specific transporters located at both the basolateral and apical membranes of the alveolar cell.[155] While transporters for sodium, potassium, and glucose have been identified on both the alveolar cell's basolateral and apical membranes[161,162], only basolaterally located channels have been described for calcium and amino acids.[163] At present, it is not entirely clear how calcium and amino acids are secreted into milk. This pathway has also been associated with secretion of certain drugs into milk.[164]

The paracellular pathway is associated with bi-directional transfer of both low and high density milk components into and out of milk spaces. This pathway is typically closed during established lactation among humans and other mammals by tight junctional complexes.[155] The tight junctions may become leaky during pregnancy and in lactation as a result of mammary inflammation. Solutes such sodium, chloride, and to a limited extent magnesium and potassium are secreted at a higher concentration towards the end of pregnancy and during the first few days postpartum, prior to the closing of the tight junctions.[27] A similar process occurs during the weaning period that triggers mammary inflammation, resulting in increased milk concentration of sodium and protein.

<u>Regulation of milk secretion</u>

Milk secretion is a highly regulated physiological function. Hormonal regulation of mammary tissue is initiated during puberty.[165] During early pregnancy, estrogen, progesterone, prolactin [166,167] and also growth hormone[168] regulate mammary tissue preparation for secretory activity by influencing ductal proliferation and alveolar tissue re-organization. The onset of milk secretion at birth is however regulated by a decrease in progesterone[169] in the presence of appropriate quantities of prolactin and cortisol[170] just around parturition. The decrease in progesterone serves as a trigger for the closure of the tight epithelial junctional complexes that limit para-cellular transport pathways.[170] While there is incomplete understanding of the hormonal control of all individual milk solutes, prolactin has been linked with survival of alveolar cells, maintenance of epithelial junctions, synthesis of milk proteins and lactose as well as the transport of several milk components including iodide,[171] some amino acids[172,173] and glucose.[161]

The individual milk components are assembled in the alveolar ducts to form milk during the onset of lactation. Milk ejection from the breast is triggered by suckling stimulus of the infant on the areola, which in turn stimulates oxytocin release. Circulating oxytocin causes the myoepithelial tissue surrounding the alveolar cells[174,175] to contract for milk ejection to occur. Hence, termination of the suckling stimulus can lead to milk stasis and eventually mammary tissue involution.

Once lactation has been established, local factors become involved in regulating the rate and amount of milk secreted.[176] Infant demand has been shown to be an important regulator of milk secretion.[177] The mechanism for control of milk secretion by infant appetite is explained by the strong association between the rate of milk secretion and the extent of breast emptying, breast storage capacity, and variations in the short-term rates of milk synthesis.[43,178] As a consequence, introduction of complementary food leads to a reduction in the amount of milk secreted.

Studies by Daly et al.[43] indicate that the capacity of the mammary gland to produce an adequate amount of milk for the infant is independent of either breast size or total milk storage capacity. Hence, women with small or large breast sizes are capable of breastfeeding their children successfully. It has long been known that children of small-breasted women were able to meet their daily milk requirements by increasing their milk intake frequency.[179]

It has also been suggested that an autocrine mechanism may be responsible for the short-term regulation of milk secretion. Studies in goats have suggested that feedback inhibitor of lactation (FIL) may be responsible for the local control of milk secretion.[180-182] FIL is a small whey peptide, produced by the mammary epithelium and has been shown to be sensitive to changes in the volume of the mammary gland without effecting significant changes in the composition of the milk secreted.[182] FIL content increases and acts in a negative feedback mechanism to reduce milk synthesis when the mammary tissue is filled with milk. On the contrary, when mammary tissue is emptied, FIL concentration is reduced to increase milk synthesis.

Typically, the 24-hour milk output increases from onset of lactation until it reaches a fairly constant volume of about 800 ml among exclusively breastfeeding women, at six months postpartum.[183] Variations in milk output across women may be attributed to both behavioral and biological influences. The most common complaint of women who have problems with breastfeeding is inadequate milk output.[13,14] Several other factors have been associated with lactation failure. In a study among low income Mexican women, lack of confidence in breastfeeding, delayed onset of milk production, poor maternal education, multiparity, sore nipples, early introduction of formula to the previous child, and the mother having been breastfed as a child were factors that were significantly associated with perceived insufficient milk.[17] Menella and Beauchamp[184,185] have reported a 20% decrease in infant breast milk consumption within the 3-4 hours following alcohol consumption. Menella[186] has also observed a decrease in milk intake after alcohol consumption without

alteration of milk quality. Moderate to heavy smoking is also associated with reduced milk secretion.[187]

Assessment of breast milk synthesis and infant intake

Test weighing is traditionally used to assess adequacy of infant milk intake.[188] Test weighing involves determination of the difference in infant weight before and after breastfeeding as an indication of infant milk intake. The accuracy of this method assumes that the losses in weight due to crying, insensible water loss through sweat, and spillage of milk during feeding are either minimized or considered in the analyses.[178] Typically, test weighing is carried out over a period of 24 hours to account for the variation in both milk intake within a day and the between-feed duration.

The advantage of test weighing to the assessor is that it involves a fairly simple procedure for measuring the weight of the infant just before and after breastfeeding. The use of test weighing is however associated with inherent difficulties. Test weighing is considered to be incapable of distinguishing between the ability of the infant to consume milk and the mother's ability to synthesize the same.[188] Savenije and Brand[189] have argued that although test weighing is an accurate procedure, its precision is low and even more so in situations where breastfeeding frequency is high and milk intake per feed is low. Test weighing is therefore not recommended for assessment of breast milk intake in clinical situations.

Furthermore, test weighing imposes an intrusion on the breastfeeding demand-and-response cycle occurring between the infant and mother. To reduce these intrusions, some studies have estimated breast milk intake using abbreviated test weighing methods that use 12-hour rather than 24-hour observations.[190,191] While Brown et al.[192] have reported that breast milk intake between 6 am and 6 pm among Bangladeshi infants accounted for 46–58% of the 24-hour total intake, Matheny and Picciano[190] found that more than half of the daily intake was consumed within the same period among American infants.

An indirect test weighing procedure that involves weighing both the mother and infant before sleep and in the morning, to avoid the inconvenience of night time measurements, also has proved inadequate.[190] Breast milk intake has also been assessed using stable isotope (^2H_2O, $^2H_2{}^{18}O$) dilution methods in either the mother or infant. While dilution methods allow unobtrusive measurement of milk intake, it is unsuitable for short-term assessment of milk intake.[193] Other less obtrusive methods that have been used for milk intake assessment are the swallow counter which is useful for assessing the pattern of short-term milk transfer[194] and the flow meter that consists of a miniature ultrasound flow transducer fitted into a nipple shield to measure the rate of milk transfer.[195] The flow meter, however, is considered to be less accurate in measuring milk intake compared to test weighing.[196]

To assess milk synthesis, researchers in Australia have developed methods that involve the measurement of breast volume using topographic technologies. These methods measure the increase in breast volume from the end of one breastfeed to the beginning of the next. A measure of the mother's short-term rate of milk synthesis is then estimated as the ratio of change in breast volume to the duration between breastfeeds. Arthur et al.[197] have described a method for measuring short-term milk synthesis known as Moire topography. The process involves illumination of the breast by passing a light source through a special grid that creates a 'Moire' effect. Photographs of the images created on the breast are captured to create a three-dimensional topograph of the breast that is then used to estimate breast volume. The precision of this procedure depends on the ability to re-position the breast between measurements. The performance of the method also falters with larger breast sizes. The data processing for this method was rather slow, making it impractical for larger sample sizes.

Subsequently, the computerized breast measuring system (CBM) was developed and successfully used to assess the rate of short-term milk synthesis.[43] The CBM uses a

computerized system to analyze video images of the breast upon which structured light patterns have been projected to create counters. The CMB overcomes the limitations of the Moire topography method by including features that minimize breast re-positioning deviations. The data created can also be analyzed more rapidly. Despite the potential utility of the CBM, it is currently an expensive technology that is not yet available to many research laboratories.

Infant growth and childhood malnutrition

Nutritional status of children is commonly assessed using weight and length measurements.[198,199] In emergency situations where rapid assessments are needed, mid-upper arm circumference (MUAC) has been found useful.[200] Traditionally, indices computed from anthropometric measurements have also been used to report populations at risk for malnutrition.[201] Three anthropometric indices that are commonly used to assess infant and child growth status are length-for-age, weight-for-age, and weight-for-length.[202] To determine the nutritional status of children, these indices are compared with a reference population. The use of a reference population allows comparison of child growth in different age and gender groups.

Until recently, the National Center for Health Statistics (NCHS)/WHO reference population was used in most countries.[201] This reference was generated using data collected from North American Caucasian children who were largely bottle-fed rather than breastfed and were measured at 3 month intervals.[203] The characteristics of the reference population were considered inappropriate as a reference for the optimal growth of children elsewhere, whose mothers are encouraged to practice EBF during the first six postpartum.[204]

In 2006, the WHO published new child growth standards.[205] Data for the new growth standards were obtained from six multi-center surveys covering a wide diversity of ethnicity and socio-demographic backgrounds. Unlike the growth references that were in use previously, the new WHO standards adopt a prescriptive approach that defines how children

'should' grow rather than 'how they grow' within the influences of their environment.[206] As expected, important differences have been observed when the growth patterns of the same children were compared using either the WHO growth standards, the NCHS/WHO references, or the CDC references.[205,207] Some of the differences were limited to specific growth indicators. Differences were also attributed to the overall nutritional status of the population being studied as well as the specific age group that was studied. For instance, the prevalence of low weight-for-age was higher when estimated using the WHO standards compared to either the NCHS, or the CDC references.[205,207]

Comparison to a reference population is made using either the z-score, percent of the median, or percentiles of the growth indices.[208] The z-score is considered a better indicator of physiological changes underlying growth than percentage of median or percentiles. The WHO recommends the use of the z-score by its member countries for the assessment of growth deficits.[209] The z-score is computed as the ratio of the deviation from the median of a reference population of the same age and sex, to the standard deviation of the reference population. A z-score less than -2.0 standard deviations is conventionally used as the threshold below which children are considered to have growth deficit. This cutoff is useful for identify the children most at risk of adverse child outcomes. It is important to note that the cut-off for z-scores is arbitrary and is should be determined based on the purpose for which it is to be used. For example, in emergency situations where there are limited resources, a more stringent cut-off such as z-score of 3.0 may be used to identify infants most at risk of mortality.

A low length-for-age z-score, described as stunting, is indicative of long-term under-nutrition and reflects retardation in skeletal growth.[201] Weight loss or a failure to increase body mass due to recent under-nutrition and/or disease is indicated by a low weight-for-length z-score (i.e. wasting). Weight-for-age (underweight) is commonly used as a screening tool in emergency situations because it does not involve the measurement of height or length.

However, interpretation of weight-for-age is less useful compared to length-for-age and weight-for-length because a low weight-for-age may result from either acute or chronic malnutrition or both. Compared to length-for-age, weight-for-age and weight-for-length have been shown to underestimate long-term malnutrition.[209]

In the year 2000, the WHO global database on child growth and malnutrition reported stunting in about 30% of all children less than five years of age in developing countries.[210] Stunting was most common in East Africa (44.4%) and South-Central Asia (39.7%). With the exception of Sub-Saharan Africa, there was a consistent downward trend for stunting in all developing countries between 1990 and 2000. Latin America and the Caribbean region had the lowest level of stunting (13.7%). In the same analyses, comparatively less underweight as compared to stunting was found in all regions of the world. About 22% of children below five years were found to be underweight globally. In developing countries, however, underweight was reported among 25% of children. About 74% of all underweight children, a total of 101 million, were residing in Asia. Of this number, about 72% were in South-Central Asia. The prevalence of wasting in developing countries was 9%. The highest prevalence of wasting was observed in West Africa (15.6%) and South-Central Asia (15.4%).[202] These measures of growth serve as useful indicators of child well-being and development.[211]

Outcomes of poor infant growth

There is strong epidemiologic evidence supporting the relationships between poor growth and a number of human development outcomes including delayed mental development,[212] poor school performance and reduced intellectual achievement,[213] functional impairment in adult life[211], and reduced work capacity.[214]

Prevalence of wasting above 5% in a population is often associated with substantial increases in mortality.[211] Analyses of community-based longitudinal studies have demonstrated that about 46 and 80% of death among children result from mild-to-moderate

malnutrition.[215] The commonest causes of preventable deaths in children below 5 years include pneumonia (19%), diarrhea (18%), measles (4%), malaria (8%) and neonatal conditions (37%) such as preterm birth, infections and birth asphyxia.[216] Yet, about 61% of diarrhea, 57% of malaria and 53% of pneumonia is attributed to being underweight.

The UNICEF framework on malnutrition identifies behavioral and environmental factors that increase the risk of infant morbidity and mortality[217] including inadequate access to food, poor child feeding practices, access to health services, availability of potable water and other utility services that are associated with disease prevention. The effect of these factors are often made worse by household and socio-economic factors that include poor maternal education status, low household incomes, large household sizes, poor child care practices, and harmful cultural practices.[218]

Determinants of poor infant growth

Infectious diseases and poor nutrition constitute the primary determinants of growth faltering among children in developing countries.[217] The World Bank in 1993 attributed between 20 and 25% of the global burden of disease in children to undernutrition. Other researchers have made projections that suggest that malnutrition may even account for as much as 50% of the global burden of disease.[219] The interactions between inadequate dietary intake and infectious diseases create a vicious cycle that ultimately overcome the child's immune system.[202] A poor micronutrient status has been shown to correlate with poor growth in children. For instance, zinc deficient children have a 13 to 21% elevated risk of dying from diarrhea, pneumonia, and malaria.[220] Perhaps the greatest impact of malnutrition on infant survival is the increased risk of case fatality.

The direct determinants of poor nutrition among children under five years have been identified as low prevalence of exclusive breastfeeding, delayed initiation of breastfeeding, introduction of complementary feeding before six months postpartum, delayed introduction of complementary feeding beyond six months postpartum, feeding foods with low energy

and nutrient density.[10,221,222] Several studies have demonstrated clearly that timely initiation and practice of exclusive breastfeeding during the first few months postpartum are associated with optimal growth outcomes in children.[223] Attachment to the breast as early as possible after birth as was well as timely introduction of complementary feeding just after the child becomes six months old are considered appropriate infant feeding behavior. Long-term intake of inadequate diets is associated with several outcomes of poor health and growth faltering in children, by compromising immune response and consequently increasing the risk and severity of infections.[224]

Deficiency of most nutrients have an effect on lowering immunity, giving way to increased incidence, severity, and duration of diseases. For example, vitamin A deficiency affects epithelial membranes and potentiates diarrhea and respiratory tract infections.[225] Although the results of high dose vitamin A supplementation trials have not yielded conclusive outcomes, it appears that the incidence of both diarrhea and lower-respiratory-tract infections may be lowered by vitamin A supplementation.[226,227] Other studies have shown that children with protein-energy malnutrition have increased episodes and duration of diarrhea.[228] Most diseases also reduce appetite and food intake, causing further weakening of the immune system and perpetuation of the vicious cycle.

Complementary feeding

Breast milk alone becomes inadequate to meet the nutritional needs of growing infants and it is recommended that complementary feeding start by the sixth month after birth.[10] Typically, non-human milk, semi-solid or solid foods are provided as sources of nutrients to complement nutrients that are obtained from breast milk.[229] Decades of studies in poor income settings show that the period of complementary feeding, especially the period between six and 12 months after birth, is associated with significant infant growth faltering and susceptibility to gastrointestinal and respiratory infections.[230]

It is thought that the adverse growth outcomes observed during the period of complementary feeding can be attributed to a number of related factors including caregivers' ignorance of appropriate complementary foods, how much food should be given to the infant, and the frequency of feeding.[202] The nutritional quality of the food is a key component of current definition of optimal complementary feeding. Analysis of complementary foods in a number of developing countries indicates a low level of lipids and micronutrients, especially zinc, iron and vitamin B_6.[229] The methods employed in food preparation and the way in which it is presented to the child also constitutes an important risk factor for inadequate complementary feeding. Additionally, feeding frequency, scheduling of feeding, as well as the environment in which the feeding process takes place, constitute other factors that are used to judge the adequacy of the complementary feeding process.

Dietary characteristics including energy density, sweetness, and viscosity have been found to affect the amount of complementary food intake among young children.[231] Because children tend to consume more food if it tastes sweet and are presented in a less viscous matrix, there have been efforts to study the effects of promoting these characteristics in complementary foods. Bennett et al.[232] have demonstrated that the use of the enzyme, amylase, to promote liquefaction of high density and high viscosity porridge resulted in increased daily energy intake among Peruvian children.

Intake of complementary foods is also influenced by the child's appetite. Childhood morbidities like diarrhea and malaria can lead to a loss of appetite and hence a reduced intake of complementary foods. It is estimated that diarrhea may reduce the intake of complementary foods by up to 30%.[233] It has also been reported that zinc deficiency is associated with impaired taste and smell sensations and may depress appetite in children.[234]

Physiological characteristics of the child during this early stage of life may also limit optimal intake of complementary foods. For instance, the small gastric capacity of infants

limits the total amount of food that can be consumed at each feeding event. Thus infants may require frequent feeding to meet their nutrient needs.

Caregiver interaction, encouragement, and responsiveness during infant feeding in a non-distracting environment are associated with increased total intake of complementary foods. Studies among Bangladeshi children found that responsive feeding behaviors may include active physical help and verbalization during feeding. Role-playing and persistence were associated with higher rates of food acceptance.[235] On the other hand, lack of responsive feeding behaviors led to more frequent rejection of food by the infant. The realization of improved infant well-being through improvements in infant feeding behaviors depends on the ability of caregivers to adopt these positive infant feeding behaviors.

HIV/AIDS and infant feeding

In addition to the commonly known infectious diseases that threaten infant and young child well-being, HIV and Acquired Immune Deficiency Syndrome (AIDS) constitute a new challenge to global efforts to improve maternal and child nutrition and health. HIV/AIDS was first observed in 1981[236] and has since become the most challenging public health problem in modern history. At the end of 2006, an estimated 39.5 million people were living with HIV/AIDS globally of which 2.3 million are children less than 15 years of age.[237]

Most HIV infection occurs in low income countries. Sub-Saharan Africa is home to 24.7 million people infected with HIV, representing more than 60% of all infections.[237] In 2006 alone, an additional 4.3 million people were diagnosed with HIV of which 530,000 were children below 15 years. Currently, almost 22% of all HIV infected persons live in Asia. HIV/AIDS is the leading cause of mortality worldwide for adults aged 15-59 years. AIDS has so far resulted in the death of about 26 million people; 2.9 million of AIDS mortality occurred in 2006 alone.[237] One out of every eight deaths due to HIV/AIDS involved a child less than 15 years old in 2006.

Poverty serves both as a determinant and an outcome of HIV. Among low income populations, high risk behavior is frequently practiced, in some cases dictated by food and livelihood insecurity. These high risk behaviors coupled with a compromised ability to negotiate safe sex lead to increased risk of infection.[238] Vulnerable groups, like women and sexually active adolescents have higher risk of infection. Elevated rates of infections are also found in high risk groups including commercial sex workers, men who have sex with other men, and intravenous drug users.

HIV and challenges to infant feeding

The major mode of HIV transmission for both men and women continues to be unprotected heterosexual relations.[237] Transmission may also occur through blood transfusions, intravenous drug usage, use of unsterilized invasive clinical equipment, and breastfeeding. The presence of HIV in breast milk was first reported by Thiry et al.[239] Subsequent studies confirmed this finding and also demonstrated clear evidence of HIV transmission via breast milk.[240] Other studies later found elevated risk of transmission among breastfeeding compared to formula feeding infants.[241] Mother-to-child transmission (MTCT) of HIV can occur via three pathways: placental transfer during pregnancy, mucosal transfer during delivery, and post-natally through breastfeeding.

Maternal factors that promote MTCT of HIV include elevated maternal plasma viral load that is a characteristic of primary infection, low $CD4^+$ ($< 400/mm^3$), failure to receive anti-retroviral therapy during pregnancy, maternal nipple lesions, and mastitis.[242] Among infants, oral thrush, prolonged breastfeeding, and inflammation of the gastrointestinal tract possibly resulting from mixed feeding may increase the risk of HIV transmission.[243] An estimated one-third worldwide [244] and one-half in Africa[241,243] of all MTCT of HIV and are due to breastfeeding.

Determination of the route of HIV transmission in infants is complicated by difficulties in determining the timing of infection. Early antibody tests developed for HIV

testing in infants were incapable of differentiating between infant and maternal antibodies during the first 12 months postpartum. Even with the use of polymerase chain reaction (PCR) techniques, HIV tests can be reasonably determined only after one month of age.[245] It has also been reported that the risk of transmission via breastfeeding is twice as high for women infected before or during pregnancy.[246] This is because initial infection is associated with high viral load.

Several studies have reported that risk of HIV transmission through breastfeeding may continue as long as the child is breastfed.[94,247] The use of advanced analytic techniques in a number of studies among larger populations has indicated an elevated risk of postnatal transmission through prolonged breastfeeding.[243] Mathematical modeling based on these studies thus propose a shorter duration of breastfeeding (three to nine months) as a way of reducing transmission via breastfeeding.[248] Considering the important role played by breastfeeding in the early postpartum period, implementing shorter breastfeeding policy and early complementary feeding may, however, increase the risk of morbidity and mortality.[60,249]

Several studies in developing countries have found that mothers in Sub-Saharan Africa and other poor settings who cannot afford commercial infant foods face the challenge of balancing risks. On one hand, there is the risk of infecting their infants through breastfeeding. On the other hand, there is the risk of infant morbidity and mortality as well as HIV/AIDS-related stigmatization that is associated with not breastfeeding.[250] In some communities, the decision to not breastfeed raises suspicions about HIV infection and the mother may suffer social, emotional, and physical consequences of stigmatization.

Most HIV-infected mothers often breastfeed or plan to breastfeed their infants regardless of a positive HIV status.[251-253] The choice of infant feeding option by HIV-infected African mothers is arrived at with considerations of several factors including financial constraints, fear of losing confidentiality of HIV status, lack of access to breast milk

substitutes, and limitations associated with preparing commercially prepared infant food.[253] The WHO has recently reviewed the recommendation on infant feeding following a technical consensus meeting. The available evidence led to the following recommendations for infant feeding among women who are HIV positive: "Exclusive breastfeeding is recommended for HIV-infected women for the first six months of life unless replacement feeding is acceptable, feasible, affordable, sustainable, and safe for them and their infants before that time".[254] The current recommendation differs little from earlier ones because it still places responsibility of infant feeding choice on the mother/caregiver with support from existing health services. The availability and effectiveness of supportive infant feeding counseling is vital to the application of this recommendation in low income settings. Achieving this requires more trained counselors and communication of new evidence in the prevention of transmission of HIV. However the final decision resides with the mother. The counselor is expected to provide the mother with current information about the risks associated with the feeding method of her choice, as well as provide support for whatever choice the mother makes.

Mechanisms of MTCT via Breastfeeding

The mechanisms that account for HIV access into the blood stream are dependent on breaches in both infant and maternal immune defenses. Infant oral thrush, a condition of candidal infection, has been associated with elevated postnatal transmission.[242] Infant oral thrush typically affects the epithelium of the mouth and allows HIV in breast milk to enter the infant's circulation. There is also evidence that compromised epithelial integrity of infant and fetal gastrointestinal mucosal lining is associated with MTCT. For example, a study by Cocchi and Cocchi[255] has shown that the conified layer of the infant skin is very thin compared to that of the adult. Hence, the infant gut becomes easily vulnerable to increased permeability leading to elevated risk of MTCT.[256] Dreyfuss and Fawzi[257] have demonstrated that vitamin A and zinc deficiencies are associated with epithelial impairment in the gastrointestinal tract. Thus, deficiency of these nutrients can elevate risk of viral transfer.

Maternal factors that may increase the infant's vulnerability to HIV transmission include nipple diseases like eczema, sore nipples, cracked nipples,[258] and subclinical mastitis.[44,259] Willumsen et al.[24] have reported the existence of higher HIV RNA viral load in the breast milk of women with elevated Na/K at one and 14 weeks postpartum. A recent study in Zambia has confirmed the relationship between elevated Na/K and high breast milk viral load.[135] Studies among HIV-1 infected women have shown that a 10-fold elevation in breast milk viral load was associated with a two-fold increase in HIV-1 transmission risk.[260]

In an attempt to describe the mechanism for MTCT of HIV, Rousseau et al.[261] determined the concentration of HIV-1 infected breast milk cells per milliliter of milk and found this concentration to be higher in colostrum versus mature milk. The authors concluded that infected breast milk cells played a greater role in HIV-1 transmission than cell-free virus. The essence of this finding is that among HIV infected women, when SCM persists, leakage of plasma constituents including infected somatic cells into milk may explain the higher risk of transmission to the infant through breastfeeding.

Anti-retroviral treatment in HIV

Short course anti-retroviral drugs have proved most effective at reducing the whole spectrum of MTCT of HIV.[250] ARV therapy reduces transmission of HIV-1 by decreasing its levels in both maternal blood and maternal mucosal secretions, resulting in reduced infant exposure to the virus.[260] The goal of ARV therapy is to reduce viral load as quickly as possible in order to: 1) prevent mutations which may lead to resistance to drug therapy, 2) reduce viral transport to other areas of the body where current medications are ineffective, and 3) allow for immune system recovery and thus slow the progression to AIDS and death.

The greatest benefit of anti-retroviral therapy in preventing HIV transmission was observed in studies involving non-breastfeeding women.[262] Studies among breastfeeding African mothers have also demonstrated reduced transmission using different regimens. The DITRAME trials in La Cote d'Ivoire and Burkina Faso found a 9.54% greater reduction in

HIV transmission at six months postpartum among women receiving zidovudine during pregnancy, labor, and at one week postpartum compared to women receiving placebo.[263] The difference in transmission was reduced to 8.5% at 15 months postpartum. Another anti-retroviral trial in Uganda involving 626 pregnant women randomly assigned one group to receive Nevirapine at onset of labor with the infant also receiving Nevirapine within 72 hours of delivery.[264] The other group of women received Zidovudine at onset of labor and every three hours until delivery and the infants received oral Zidovudine twice per day for all of the first week postpartum. Almost all the infants (99%) initiated breastfeeding. Between 14 and 16 weeks of age, 13.5% of the Nevirapine group was HIV-positive compared to 22.1% in the Zidovudine group. At 18 months, 15.7% and 25.8% from the Nevirapine and Zidovudine respectively were infected. Although anti-retroviral therapy has proven effective at reducing transmission of HIV,[265] it is still an expensive intervention for economically impoverished sub-Saharan African countries.[266]

Study area, Ghana

Ghana is a West African country located between latitude 4.0^0 N and 11.0^0 N and longitude 3.1^0 W and 1.1^0 E in the West African sub-region. Ghana shares international borders with La Cote D' Ivoire to the west, Togo to the East, Burkina Faso to the north and has the Gulf of Guinea as its southern border. The total land area of 238,539 square kilometers supports a population of 21.4 million people that is growing at a rate of 3%.[267] Total fertility rate is 4.5 children per woman and life expectancy is estimated at 56 years with infant mortality of 52.22 deaths/1,000 live births. Malaria accounts for about 22% of all deaths in children less than 5 years and 44% of all out-patient clinic attendances. Agriculture is the major economic activity of Ghanaians. An estimated 32% of the population lives below the poverty line.

Breastfeeding practice in Ghana

The 2003 demographic and health survey (DHS) provides the most current national data on infant feeding in Ghana.[267] Breastfeeding has always been a common infant feeding practice among Ghanaian women. Although 97% of Ghanaian women initiate breastfeeding sometime after birth, more than half of these women put their children to the breast well after one hour of delivery, contrary to optimal breastfeeding recommendations. For about one quarter of women who initiate breastfeeding, the process is initiated after the first 24 hours after delivery.

In the last five years, the DHS shows that the proportion of infants who were introduced to the breast within one hour after delivery has increased from 54 to 75%.[267] During the period before the first breastfeed, 19% of infants are given pre-lacteal feeds prior to the initiation of breastfeeding. Children who were delivered in a health facility compared to those delivered at home were more likely to be breastfed immediately after birth, and less likely to be given pre-lacteal feeds.

The Ghana Health Service (GHS) recommends that all children, unless medically contraindicated, be exclusively breastfed during the first six months. However, only 53.4% of Ghanaian infant below six months are exclusively breastfed. Nevertheless, this rate represents an improvement in EBF recorded in earlier surveys for the same age group (31.1% in 1998). Commonly, the exclusive breastfeeding rate starts declining between the second and third month postpartum in Ghana. The median duration of any breastfeeding in Ghana is 23 months. The proportion of children who have been weaned from the breast increases rapidly after the first year of life. While less than 5% of infants are not breastfeeding at the end of the first year, more than 70% of children are no longer breastfeeding by the end of the second year. Some children may breastfeed for as long as the 35th month. Children living in rural communities are breastfed longer than their counterparts in urban communities. Breastfeeding duration is higher among low income and less educated women.

About 63% of Ghanaian infants between the ages of six and nine months are fed solid foods (Figure 2.2) in addition to breast milk as recommended by the WHO.[267] At this stage, however, 5% of infants are still fully breastfed. Infant feeding problems identified include mother's complaints of inadequate breast milk production, earlier-than-recommended introduction of complementary feeding, use of feeding bottle, and delayed initiation of breastfeeding.

Complementary feeding in Ghana

The GHS encourages initiation of complementary feeding at six months postpartum. Data from the 2003 Demographic and Health Survey indicate that very few Ghanaian breastfeeding infants (less than 10% at six months) receive infant formula. Forty-seven percent of breastfeeding infants have been introduced to solid or semi-solid food by the time they reach five months old. A majority (53%) of these infants consume foods made from grains. Koko, a porridge made from fermented corn or millet, is the most common food given to complement breast milk and is given about once a day through the first year and twice or more beyond the first year of life. A blend of corn, millet, soybeans, and groundnuts is recommended as a nutrient-rich substitute for koko preparation rather than the traditional corn meal. However, the consumption of this blend was not described in this survey.

In addition to its six months EBF policy, the GHS promotes the addition of safe and affordable foods to complement the nourishment provided by breast milk beyond the 6th month. This recommendation is encouraged until the child is two years old. Thereafter, the infant may be weaned completely from breast milk. These recommendations are first presented to most Ghanaian mothers when they start attending child welfare clinics (CWC) at the sixth week postpartum. For a few mothers who have access to BFHI institutions, there may be an opportunity to learn these recommendations earlier than their first CWC visit. The BFHI was first initiated in Ghanaian health facilities in 1993. Most major hospitals and clinics have been trained and certified as baby friendly institutions. However, a majority of

women lack both physical and economic access to these hospitals. Ghana has in place legislation under the Food and Drugs Law to control the marketing of breast milk substitutes by baby food manufacturers in support of the global code on marketing of breast milk substitutes.

<u>Trends of HIV infection in Ghana</u>

HIV infection was first reported in Ghana in 1986 and the infection rate has since been increasing steadily.[268] HIV-1 is the predominant infecting agent (94.8% of cases) among Ghanaians. Currently, 2.3% of adult Ghanaians are infected with HIV, representing a modest decrease from 3.1% at the end of 2004.[269] There is, however, regional variation in prevalence that ranges from 1.2% in the northern regions to 4.7% in the Eastern region. Unusually high rates (19%) have been noted among prison inmates[270] and prison officers (8.5%). Current estimates of 350,000 infected include 180,000 adult women (15-49 yr) and 24,000 children less than 15 years of age. While most HIV infection occurs among females, male infection has been rising steadily. At the end of 2005, about 14,449 AIDS cases were recorded in Ghana, contributing to a cumulative 104,995 AIDS cases since the epidemic was detected. The main driving force of the infection in Ghana is unprotected heterosexual intercourse.

<u>HIV control strategies in Ghana</u>

Prevention is the core strategy of HIV control in Ghana. The Ministry of Health through the national AIDS control program, and with support from non-governmental agencies, is promoting a three stage control strategy involving abstinence from casual sex, faithfulness to sexual partners, and the use of condoms (ABC program). Starting in 2001, the ministry of health provided facilities at major public hospitals that provide services for prevention of MTCT. The number of such facilities continues to expand as the facilities and infrastructure become acceptable for use in programs to prevent MTCT throughout the country.

Pregnant mothers attending antenatal clinic at institutions that provide MTCT prevention services are offered voluntary counseling and testing for HIV. In a limited number of institutions where treatment is available, anti-retroviral therapy is provided to pregnant women who test positive for HIV. Beyond 28 weeks of pregnancy, HIV-positive women with $CD4^+ > 350$ cells/m^3 receive a double treatment of Zidovudine through labor till the seventh day postpartum. Pregnant women with $CD4^+ < 350$ cells/m^3 receive a triple treatment regimen. Additionally, both the mother and infant are given a single dose of Nevirapine prophylaxis intrapartum. The infant is subsequently given Zidovudine during the first seven days postpartum (personal communication, Dr Brakohiapa, L).

In addition to ART, policies have been put in place to help expectant mothers make informed choice about infant feeding that will protect the infant from possible infection through breastfeeding. There are, however, problems with operationalization of these policies since the cost of infant formula is beyond the purchasing power of most mothers. Also, the fear of stigma attached to not breastfeeding and related suspicion of HIV infection makes it difficult for mothers to exercise their choice of infant feeding, if it is an alternative to breastfeeding. HIV-infected mothers receive counseling on risks and benefits of breastfeeding and on other infant feeding options. The mother is then expected to make a decision on a feeding option that is feasible, safe, and sustainable. The mother then receives support to practice her chosen feeding option.

Manya Krobo

The studies reported in this dissertation were conducted in both rural and peri-urban communities in the Manya Krobo district of Ghana. The Manya Krobo district is one of 17 administrative districts in the Eastern region of Ghana (Figure 2.3). Manya Krobo district has a population of 165,409 living mostly in rural communities. Most communities in the district have access to pipe-borne water obtained from community stand pipes. A majority of residents in the district are involved in farming, fishing, trading and artisanal pottery. Most

households in larger towns have access to pipe borne water. Otherwise, water is obtained from hand dug wells where available. Breastfeeding is the norm for most infants from birth and continues beyond twelve months. However, introduction of liquids and semisolids starts early. Breastfeeding is commonly complemented with koko, a fermented maize porridge by three months postpartum.[267]

The district is served by three hospitals and a number of health centers. Health services include prenatal counseling and infant growth monitoring, infant feeding counseling, immunizations, and micronutrient supplementation at child welfare clinics. The district administration runs a community-based health insurance scheme as part of the National health insurance scheme. In 2005, HIV prevalence in Manya Krobo declined from 7.4 to 5.0%. Voluntary counseling and testing services and prevention of MTCT for HIV are provided at the three hospitals where mothers receive standard ART as indicated above. Counseling services are also provided to help HIV positive mothers make an informed infant feeding decision as recommended by the World Health Organization.[271] Earlier studies of HIV positive mothers indicate that most choose to breastfeed their babies after counseling on the risks of transmission during breastfeeding.[272] The study also found that perceived stigma was associated with the decision to breastfeed. Mothers were concerned that refusal to breastfeed will cause family and neighbors to suspect their HIV status. There are also indications that mothers unduly postpone their need to seek medical care. This delay may be due to the common use of traditional remedies.
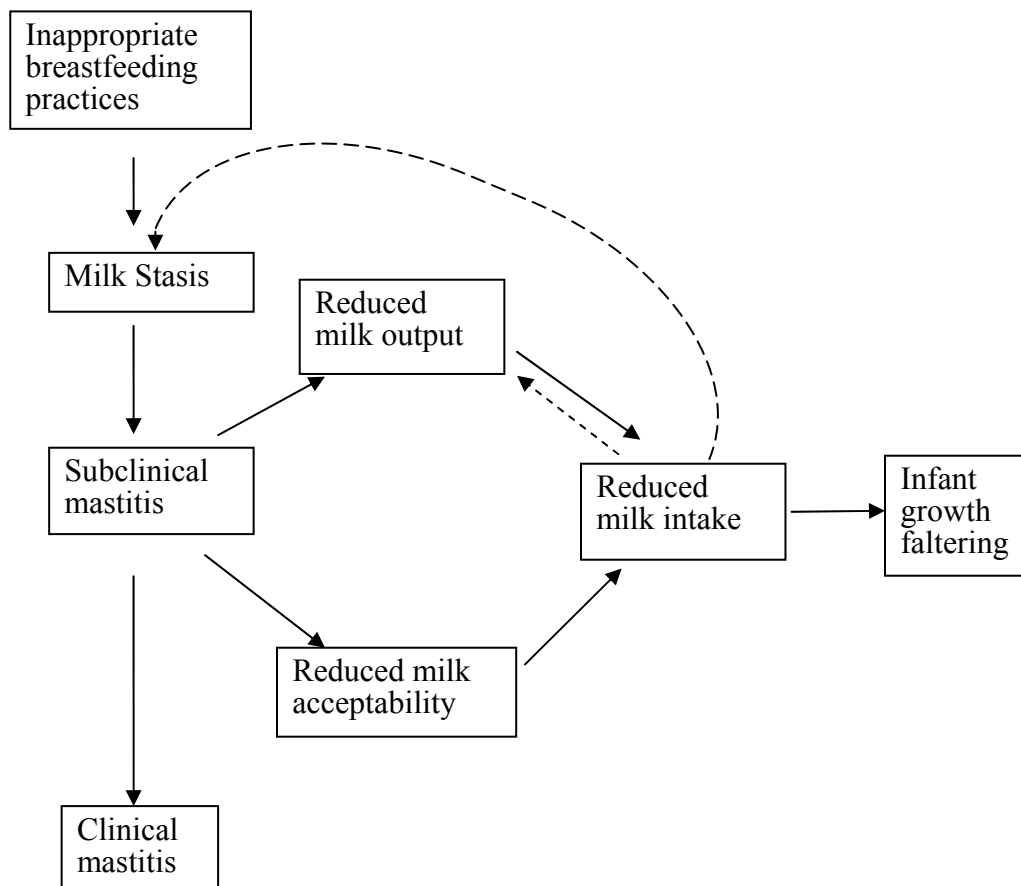
Figure 2.1. Proposed pathways by which Subclinical mastitis (SCM) affects infant growth
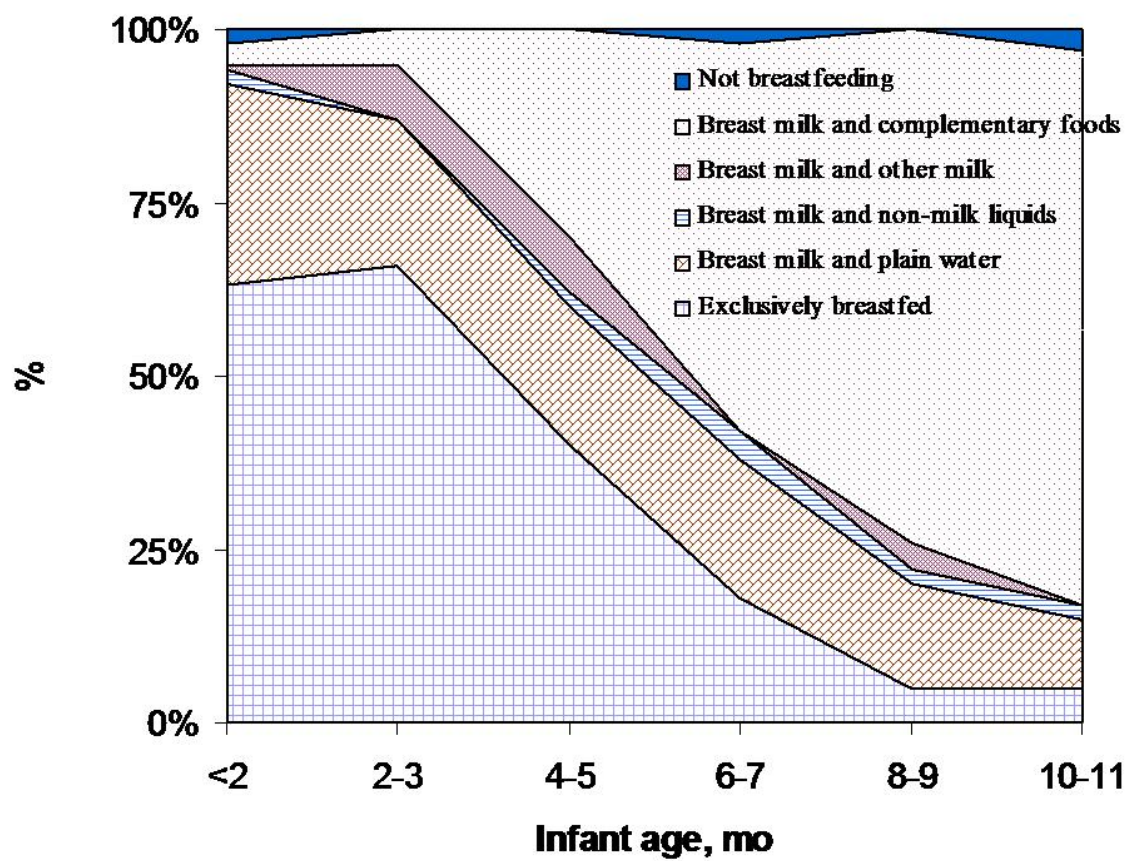
Figure 2.2. Infant feeding behavior of Ghanainan infants (modified from GSS/ORC Macro Int, 2004)
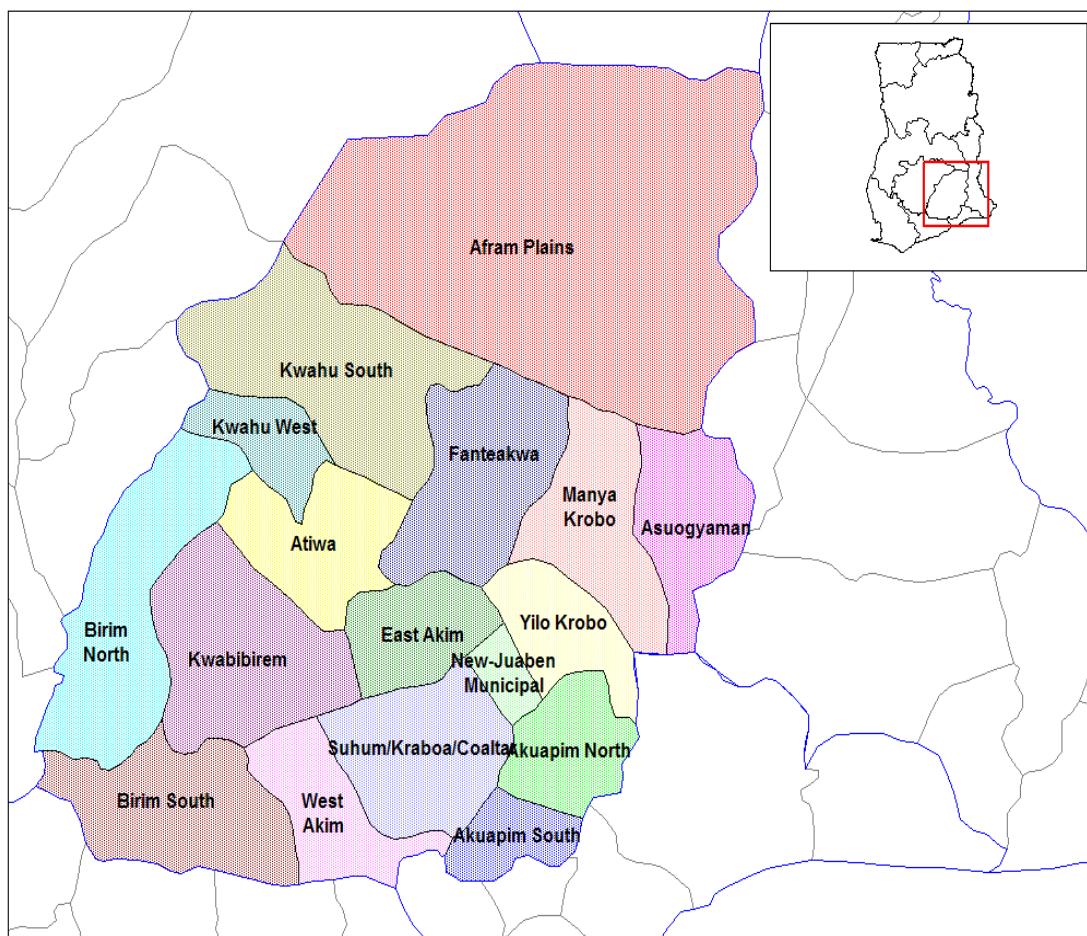
Figure 2.3. Map of Ghana showing location of Manya Krobo

Chapter 3. General Methods

<u>Study design</u>

The three studies reported in this dissertation were conducted in the same district in Ghana. Each study involved the recruitment of a different set of mothers. There had previously been no study on SCM or any other breast health problems among lactating women in Ghana.

The first study investigated the prevalence of SCM among a community of Ghanaian lactating women. We hypothesized that SCM will be detected in at least one quarter of lactating women at three or four months postpartum. Knowledge of SCM prevalence was necessary for two reasons: 1) to determine whether SCM was of public health importance in the Ghanaian setting and thus worth pursuing as a research project, and 2) to decide what sample size would be appropriate for the subsequent studies included in the project.

A longitudinal study was subsequently carried out to determine the association between SCM and infant growth and feeding practices beyond the third month postpartum. A cross-sectional study of infant breast milk intake was carried out with a separate group of mother-infant pairs to investigate the effect of SCM on infant breast milk intake.

<u>Ethical approval and informed consent</u>

Ethical approval was obtained from the Institutional Review Boards of Iowa State University (ISU) and the Noguchi Memorial Institute for Medical Research (NMIMR) at the University of Ghana. Additionally, a formal letter of approval and support was obtained from the Manya Krobo District health administration. Written informed consent was obtained from all participants in each study.

<u>Study area and participants</u>

All three studies were carried out in the Manya Krobo district of the Eastern region of Ghana. The district is situated at Longitude 0° and Latitudes 6°15′ north of the equator and is about 45 minutes driving from Accra, the capital city. Manya Krobo is mostly rural with a

population of 165,409 persons.[273] The hilly nature in parts of the district creates two main ecological zones for the district. Lower Manya is a low lying zone consisting of both peri-urban and rural communities with some access to social and utility services. Upper Manya consists of largely rural farming communities located in the hilly zone of the district (about 500 to 660 m above sea level). The upper Manya communities have less access to social and utility services.

The ecology of the district is suitable for vegetable and fruit farming which constitute the main occupation of the district. On the north-eastern boundary of the district is the Volta lake from which the men harvest freshwater fish to be sold by women. In peri-urban communities, however, women are mostly artisans or traders who sell food and household commodities.

Most communities in the district have access to pipe-borne water, primary education, and health services that include prenatal counseling, infant growth monitoring, infant feeding counseling, immunizations, and micronutrient supplementation, all of which are accessible from, often, mobile child welfare clinics (CWC). The district and indeed the entire Eastern region have a high HIV prevalence rate. The 2004 sentinel surveillance revealed that HIV prevalence surveyed at the Atua Hospital in the district was 7.4%, and at the time, that rate was higher than anywhere else in the country.[269] The rate has since declined to 5.0% in 2005.[274] Due to the relatively high prevalence of HIV, the district was among the first to benefit from services and support for voluntary counseling and testing of HIV in two of its hospitals. In addition to these services, infant feeding counseling and support is currently provided to pregnant mothers to reduce the risk of mother-to-child transmission of HIV. However, these services are centralized and are mostly accessible to women living in the lower Manya area.

Rapid survey

Study design

The rapid survey was designed to obtain a quick appraisal of the occurrence of breast health problems among lactating women at three and four months postpartum in a Ghanaian community.

Sample size determination

A sample size of 110 mothers was estimated for the rapid survey. This sample size was determined based on the prevalence of SCM reported in previous studies[24,37] and was expected to give the study a power of 80% to detect at least a 25% prevalence of SCM in the study population. The prevalence of SCM among lactating women in Manya Krobo at 3 months postpartum was assumed to be similar to that of lactating women in other African countries.[24,37] The confidence limit was set at 95%.

Study participants

One hundred and seventeen lactating women were recruited from 10 CWC in this study. Women who have had singleton births and were attending CWC during the recruitment period (November 2005 to January 2006) were invited to participate if they were three to four months postpartum, at least 18 years old, and resident in the Manya Krobo district.

Data collected from participants

Study mothers completed a brief survey on recent health history including self-reported overall health perception including questions like, "How would you describe your health today"? and "Have you had any other health problems in the last week"? Breast health questions included: "Have you had breast pain/swelling since you had this child and if the answer is yes, when did it occur? Data on maternal age and number of live births were also collected. Maternal mid-upper arm circumference (MUAC) was measured to the nearest 0.1 cm on the left arm using a non-stretchable tape measure (Chasmors Ltd, London). No

information on infant health, feeding, and growth were collected. A spot breast milk sample of at least 10 mL was obtained from each breast of each mother by manual expression.

Breast milk collection and storage

Before expressing breast milk, the women washed both hands thoroughly with disinfectant liquid hand soap and running water, rinsed with deionised water and dried their hands with clean paper towels. The first drops of expressed breast milk were discarded after which the nipple and surrounding areola was cleaned with cotton gauze soaked with 70% ethyl alcohol. Breast milk expressed thereafter was collected in 60 mL plastic vials with snap-on caps. Upon completion of expression from one side, hand rinsing was repeated with deionised water and breast surface cleaning with ethyl alcohol was carried out before continuing to express milk from the other breast. All breast milk samples collected were immediately stowed in a cold chest for transportation to the field laboratory where it was stored at -20 ^{0}C. Samples were subsequently transported to Accra, for storage at -32 ^{0}C until analyses.

Laboratory methods

Sodium and potassium content analyses were performed using standard methods that employ ion-selective electrodes.[141] Briefly, all analyses were performed on whole milk without any sample pre-treatment. Samples were thawed to room temperature (25 ^{0}C). Thawed milk was then homogenized using a vortex mixer to allow the aqueous and lipid compartments to mix uniformly and 0.1 mL of sample was aspirated for analyses of sodium and potassium using ATAC 8000 ion-selective electrode analyzer (Clinical Data Inc, Smithfield, USA). In addition to the internal saline standards used by the ATAC 8000, analytical quality was monitored by simultaneous analyses of milk and a saline standard with known electrolyte concentration.

Statistical analyses

All data were analyzed using SPSS 15.0 (SPSS Inc, Chicago). Descriptive statistics including arithmetic mean, median, and standard deviations were computed for maternal age and MUAC data. Our MUAC data were compared with a MUAC reference threshold of 18.5 cm for screening moderate undernutrition.[200] Categorical maternal health data were summarized into frequencies. Results of breast milk analyses were expressed as Na/K ratio for each sample. Geometric mean was computed to describe the asymmetric distribution of Na/K data. SCM status groups were defined based on Na/K ratio in either one (unilateral) or both breasts (bilateral). Analysis of variance was used to test group differences in log transformed Na/K data. Differences were considered significant in this study at $p < 0.05$. The prevalence of SCM was computed as the percentage of women with Na/K > 1.0.[24,37]

Longitudinal study

Study design

The longitudinal study was conducted between February and October, 2006. The mother-infant pairs recruited into this study were observed during regularly scheduled home visits carried out every two weeks over a total duration of three consecutive months between the third and sixth month postpartum. With the exception of those who were lost to follow-up, each mother-infant pair was observed seven times.

Sample size determination

Sample size estimation for this study was based on SCM prevalence reported in previous studies[24,37] as well as observed infant growth characteristics in Ghana and elsewhere.[266,275] A total of 110 women and their singleton infants were estimated to be recruited into the study. This number of mother-infant pairs was required to test the hypotheses relating SCM to reduced infant growth and infant feeding as indicated in Table 3.1. All estimations were based on a power of 80%, a confidence limit of 95% and expected attrition of 20%.

Study participants

Most mothers included in the study were from peri-urban communities in the Manya Krobo district. One hundred and sixty three lactating women were invited from nine CWC to participate in the longitudinal study. However, only 115 women were available at baseline while 108 women remained in the study until the seventh observation. Inclusion criteria for women in the study were: residence in the Manya Krobo district, less than 3 months postpartum, at least 18 years, and willingness to continue participation with their infants through the entire duration of the study. Women with twin births were excluded from participating in the study.

Data collection procedures and tools

All recruitments were done prior to the infant attaining three months old; observations were initiated on the day the infant turned three months old and subsequent home visits were scheduled two weeks ahead of each previous home visit. In some cases, the mothers were not available for observation on the scheduled date of home visit. When that situation occurred, arrangements were made for observation to be carried out on a day as close to the scheduled visit day as possible. More often than not, such visits were carried out one day before or after the planned date of observation.

At recruitment, a brief demographic survey was completed by trained field workers. Field workers also recorded the home addresses of mothers using popular land marks, and community names. Where possible, maps were drawn by field assistants, with the assistance of the mother to enable easy access to mother's home for observations. At recruitment, a date, time and place for the first home visit was arranged with each mother. Subsequent home visit appointments were made after each home visit.

During the home visits, field workers collected maternal and infant health histories covering the seven days prior to the day of the home visit. This information was recorded in a questionnaire designed specifically for that purpose (appendix 3b). The questionnaire

collected information on the occurrence of common disease symptoms for both mother and infant as well as the frequency of occurrence of these conditions in the last seven days.

A food frequency questionnaire was used to record all breast milk, and other liquid and solid foods taken by the infant during the last seven days. The frequency of water and multivitamin intake during the same period was also recorded. The food frequency tool used was an abridged version of one that has been tested and is currently being used by other on-going studies in the district.

Maternal weight and standing height were measured at baseline by trained field workers. Maternal weight measurements were taken a second time among some but not all mothers on the seventh visit. Infant weight, head circumference, mid-upper arm circumference and length were measured and recorded at baseline. All infant measurements were carried out during each home visit.

All anthropometric measurements were carried out using standard procedures. Infant weight was measured by tarring using the Tanita BWB800S digital floor scale (Tanita Corporation; Tokyo, Japan). Most infants were measured without clothing; in some cases, infants were measured with clothing and their net weight was determined as the difference between the clothing and the weight with clothing. Maternal weight was also measured using the Tanita BWB800S digital floor scale. Head circumference was measured to the nearest 0.1 cm using a non-stretchable tape measure (Chasmors Ltd.; London, United Kingdom). The same tape was also used to measure infant mid-upper arm circumference. Infant recumbent length and maternal height were measured to the nearest 0.1 cm using the Shorr infant/child/adult/height/length measuring board (Shorr Productions; Maryland, USA).

About 5 mL of breast milk expressed manually from each breast were obtained from each mother at each home visit. Breast milk samples were stored in clean plastic vials with screw top caps for transportation to the field laboratory. The same breast milk collection protocol described for the rapid survey was observed in the collection of breast milk samples

for the longitudinal study. All breast milk samples were transported on ice to the Noguchi Memorial Institute for Medical Research laboratories in Accra to be stored at -32 ^{0}C until analysis.

Laboratory analyses

Sodium and potassium content analyses were performed using standard methods that employ ion selective electrode analyzer.[141] Briefly, all analyses were performed on whole milk without any sample pre-treatment. Samples were thawed to room temperature (25 ^{0}C). Thawed milk was then mixed using a vortex mixer to allow the aqueous and lipid compartments to mix uniformly and 0.1 mL of sample was aspirated for analysis of sodium and potassium using Medica Easylyte ion-selective electrode analyzer (Medica Corporation; Bedford, USA). In addition to the 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

All data were analyzed using SPSS 15.0 (SPSS Inc, Chicago). Descriptive statistics including arithmetic mean, median, and standard deviations were computed for continuous maternal and infant data. Categorical maternal and infant data were summarized into frequencies. Breast milk sodium and potassium data were expressed as Na/K. The prevalence of SCM was computed as the percentage of women with Na/K > 1.0. Multiple linear regression analysis was performed to determine the predictors of infant growth. Differences between groups were considered significant study at $p < 0.05$.

Cross-sectional study

Study design

This cross-sectional study was designed to compare the breast milk intake of infants whose mothers had SCM with those whose mothers did not.

Study participants

Data for the cross-sectional study were collected between July 2006 and February 2007. A total of 72 mother-infant pairs were recruited from 7 CWC in the Manya Krobo district to participate in the study. Inclusion criteria for women recruited for the cross-sectional study were: mother aged at least 18 years, breastfeeding infant at recruitment, has singleton birth of infant aged between 3 and 6 months and willing to participate in a 12- hour breast milk intake observation.

Data collection procedures and tools

At recruitment, a field worker explained the protocol of the study to the mother. Upon acceptance of study procedures and completion of the informed consent document, women were then recruited to participate in the study. Mothers were asked to provide detailed directions to their homes. Where necessary, maps were sketched by field workers to enable ease of access to mother's address.

At a scheduled date during the 3rd and 6th months postpartum, each mother was visited at her home to collect at least 5 mL of breast milk from each breast using plastic vials rinsed with de-ionized water. All breast milk samples were collected by manual expression using the protocol described earlier in the description of the rapid survey. A 12-hour home visit was then scheduled for the next day.

Breast milk analyses protocol

About 2 mL of the breast milk sample collected was tested for subclinical mastitis immediately after milk collection using the California mastitis test (CMT). The CMT procedure involved mixing 2 mL of breast milk with an equal amount of the CMT reagent in a test cup and swirling the cup in counter clockwise manner. Thickening or gelatinization of the mixture after about 10 seconds was indication for SCM. The severity of SCM was related to the degree of thickening observed.[147]

The rest of the breast milk was kept on ice in a sealed container and transported to storage in a freezer at -32 ^{0}C until it was analyzed for sodium and potassium content. Sodium and potassium analyses were carried out with Easylyte (Medica Corporation, Bedford, USA), an electrolyte analyzer that utilizes ion-selective electrode technology. Electrolyte analyses involved thawing of milk to room temperature and then drawing 0.01 mL of milk into the analyzer for analyses.[141]

Breast milk intake

During the 12-hour home visit, breast milk intake by the infant was estimated for each breastfeed using the test weighing procedure.[189,276] Briefly, the process involved recording the weight of the infant just before and then immediately after the infant is breastfed without a change of clothing or diapers in between the process.

Infant health and dietary intake

During the home visits, field workers collected maternal and infant health histories covering the seven days prior to the day of the home visit. This information was recorded in a questionnaire designed specifically for that purpose (appendix 4c). The questionnaire collected information on the occurrence of common disease symptoms for both mother and infant as well as the frequency of occurrence of these conditions in the last seven days.

A food frequency questionnaire was used to record all breast milk, and other liquid and solid foods taken by the infant during the last seven days. The frequency of water and multivitamin intake during the same period was also recorded. The food frequency tool used was an abridged version of one that has been tested and is in current use by other on-going studies in the district (appendix 4b).

Anthropometry

All anthropometric measurements were carried out using standard procedures. Infant weight was measured using the Tanita BWB800S digital floor scale (Tanita Corporation; Tokyo, Japan). All infants were measured with clothing. Maternal weight was measured also

using the Tanita BWB800S digital floor scale (Tanita Corporation; Tokyo, Japan). Head

circumference was measured to the nearest 0.1 cm using a non-stretchable tape measure

(Chasmors Ltd.; London). The same tape was used to measure infant mid-upper arm

circumference. Infant recumbent length and maternal standing height were measured to the

nearest 0.1 cm using the Shorr infant/child/adult/height/length measuring board (Shorr

Productions; Maryland, USA).

<u>Statistical analyses</u>

All data were analyzed using SPSS 15.0 (SPSS Inc; Chicago). Descriptive statistics

including arithmetic mean, median, and standard deviations were computed for maternal age,

parity, and infant and maternal anthropometry data. Categorical maternal health data were

summarized using frequencies. SCM was diagnosed using CMT > 1 and Na/K > 1.0.

Differences in breast milk intake across groups were described using 95% confidence

intervals. Group differences were considered significant in this study at $p < 0.05$.

Table 3.1. Estimates used in computing sample sizes

Outcome	D*	p*	SD	Sample size
Incidence of high Na/K[‡a]	15 %	19 %		107
3-6 months non-exclusive breastfeeding rate[‡b]	25 %	60 %		60
Growth[†c]	70 g/month		0.1	44

[a] Gomo et. al.[37]   [b] GSS,[267]   [c] Baumgartner et. al.[275]

[1] Sample size equation:

$$^{‡}n = \frac{(Z_{\alpha/2}+Z_{\beta})^2 \, p^*(1-p^*) \, (r+1)}{(d^*)^2 \, r}$$

$$^{†}n = \frac{(Z_{\alpha/2}+Z_{\beta})^2 \, SD^2 \, (r+1)}{(d^*)^2 \, r}$$

$Z_{\alpha/2}$=standard normal distribution of d^* at α=5 %
Z_{β}=standard normal distribution of d* for 80 % power.
SD= observed standard deviation associated with d*
P*= estimated incidence of SCM
D^*= expected difference in proportions/means
r = ratio of women with SCM to those without SCM

Chapter 4. Subclinical mastitis is common among Ghanaian lactating women, 3 to 4 months postpartum

Richmond N.O. Aryeetey, PhD; Grace S. Marquis; PhD, Leo Timms, PhD; Anna Lartey, PhD; Lucy Brakohiapa, PhD.

Abstract

Subclinical mastitis (SCM) is an asymptomatic inflammation of mammary tissue and has been associated with lactation failure, sub-optimal growth in early infancy, and increased risk of mother-to-child transmission of HIV via breast milk. We carried out a rapid survey to determine the prevalence of SCM among lactating Ghanaian women between 3 and 4 months postpartum. Bilateral breast milk samples were obtained from 117 lactating women in Manya Krobo, Ghana and analyzed for sodium (Na) and potassium (K). Additionally, we measured maternal mid-upper arm circumference and recorded recent maternal health history. An elevated sodium-potassium ratio (Na/K) above 1.0 was considered indicative of SCM. Overall, SCM prevalence was 45.3% of which 29.9% was unilateral. Na/K was not associated with maternal health or nutritional status. The rate of SCM in this community is high; there is the need for lactation support to reduce SCM, thereby reducing the risk of adverse infant outcomes.

Keywords: breastfeeding, infant growth, subclinical mastitis, sodium:potassium ratio, Ghana

Introduction

Suboptimal breast health may compromise maternal ability to breastfeed and the well-being of a breastfeeding infant. Subclinical mastitis (SCM) is an asymptomatic inflammatory condition of the lactating breast.[1] The condition is associated with a number of adverse outcomes including lactation failure,[2] infant growth faltering during the early

postpartum period,[2,3] and increased risk of mother-to-child transmission of the Human Immunodeficiency virus (HIV).[4]

SCM is thought to be caused primarily by milk stasis,[5] which results from inadequate milk removal from the breasts. Milk stasis can increase the risk of bacterial infection of stagnated milk in the breast, occasionally leading to infectious mastitis.[6] During an episode of SCM, the tight epithelial junctions separating milk and plasma become compromised, leading to the leakage of plasma components, including sodium and chloride ions into milk.[7] An elevated sodium-potassium ratio (Na/K) above 1.0 is considered indicative of SCM.[4,5]

The present study was carried out to determine the prevalence of SCM among lactating Ghanaian women during the three to four month postpartum period. This study was conducted in Ghana as part of a larger study to determine the mechanism(s) linking SCM with infant growth faltering.

Methods

<u>Study area and participants</u>

The study was conducted in the Manya Krobo district in the Eastern region of Ghana between November 2005 and February 2006. Most communities in the district have access to potable water and health services that include prenatal clinics and infant growth monitoring, immunizations, and vitamin A supplementation that are accessible at child welfare clinics. Manya Krobo has a population of 165,409 living mostly in rural communities.[8] The majority of the women recruited into the current study, however, were residing in peri-urban communities where CWC were most accessible. In 2004, HIV prevalence in Manya Krobo was 7.4%.[9] During the study period, there were three health institutions in the district that provided HIV voluntary counseling and testing services and infant feeding counseling to reduce the risk of mother-to-child transmission of HIV.

We hypothesized that SCM would be detected in at least one quarter of lactating women at 3 to 4 months postpartum based on the findings from a study in South Africa in

which the authors reported that 25% of lactating women had Na/K > 1.0 at 3 months.[1,4] A sample size of 110 women was thus estimated, based on a statistical power of 80% to demonstrate the suggested prevalence in this population of lactating Ghanaian women.[1,4] Confidence limits were set at 95%. One hundred and seventeen lactating women were recruited from 10 CWC for this study. Women were invited to participate if they were 3 to 4 months postpartum, at least 18 years old, and resided in the Manya Krobo district. Written informed consent was obtained from all participants.

Approval was obtained from the Institutional Review Boards of Iowa State University (ISU) and the Noguchi Memorial Institute for Medical Research (NMIMR) at the University of Ghana. Additionally, a formal letter of approval and support was obtained from the Manya Krobo district health administration.

Data collected from participants

Study participants completed a brief survey on the following questions on self-reported health indicators, to determine their associations with the development of SCM: overall health evaluation ('How would you describe your health today?'); breast health ('Have you had breast pain since you had this child?' and 'If yes, when did it occur?'); and recent disease symptoms ('Have you had any other health problems in the last week?'). Maternal age and number of live births were collected also. Mid-upper arm circumference (MUAC), a crude indicator of nutritional status, was measured on each participant to the nearest 0.1 cm on the left arm using a non-stretchable tape measure (Chasmors Ltd.; London, United Kingdom).

Milk collection protocol and storage

A spot milk sample of at least 10 mL was obtained by manual expression from each breast of each participant. Before expressing breast milk, the women washed both hands thoroughly with disinfectant liquid hand soap and running water, rinsed with deionised 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 was repeated with deionised water and breast surface cleaning with ethyl alcohol before milk was expressed from the other breast. This hand washing protocol was observed by the women as well as the field staff who assisted the women to express the milk. The reason for the hand washing protocol was to reduce contamination of the milk by perspiration on the hands and breast of the woman. The alcohol swab was used to reduce microbial contamination of the sample as a portion of milk was cultured for bacterial infection. Data for bacterial culture is not reported in this article. All milk samples collected were immediately stored in a cold chest for transport to the field laboratory and stored at -20 ^{0}C. Samples were subsequently transported to Accra, for storage at -32 ^{0}C until analysis.

Laboratory methods

Milk sodium and potassium content analyses of breast milk samples were performed using standard methods that employ ion selective electrode analyzer.[10] Briefly, all analyses were performed on whole milk without any sample pre-treatment. Samples were thawed to room temperature (25 ^{0}C). Thawed milk was then homogenized using a vortex mixer to allow the aqueous and lipid compartments to mix uniformly and 0.1 mL of sample was aspirated for analyses of sodium and potassium using ATAC 8000 ion-selective electrode analyzer (Clinical Data Inc; Smithfield, USA). In addition to the internal saline standards used by the ATAC 8000, analytical quality was monitored by simultaneous analyses of milk and a saline standard with known electrolyte concentration. All milk analyses were carried out once. Duplicates were only performed when unusually high sodium concentrations (> 10 mmol/L) were obtained.

<u>Data analyses</u>

All data were analyzed using SPSS 15.0 (SPSS Inc; Chicago). Descriptive statistics including arithmetic mean, median, and standard deviations were computed for maternal age and MUAC data. MUAC data were compared with a MUAC reference threshold of 18.5 cm for screening moderate undernutrition.[11] Categorical maternal health data were summarized into frequencies. Results of the milk analyses were expressed as Na/K ratio for each sample. Geometric mean was computed to describe the asymmetric distribution of Na/K data. SCM status groups were defined based on Na/K ratio in either one (unilateral) or both (bilateral) breasts. Analysis of variance was used to test group differences in natural log transformed Na/K data. Differences were considered significant in this study at $p<0.05$. The prevalence of SCM was computed as the percentage of women with Na/K> 1.0.[4,5]

Results

<u>Population Characteristics</u>

Over a period of three months, we screened 1296 women who attended the selected CWC of whom 154 women were 3-4 months postpartum. Of these, 6 women refused to participate while 31 were excluded because the mother was either not available at the clinic (n=21), under 18 years of age (n=3), had a twin birth (n=1), or was previously enrolled into the study at an earlier visit (n=6). In total, 117 women met the inclusion criteria and were enrolled in the study (Figure 4.1).

About 42% (n=49) of women in our study were between 18 and 25 years of age while 23% (n=27) were 30 years or older (Table 4.1). The median MUAC of the women was 26.6 cm. More than 81% (n=95) of the women had 3 or fewer live birth experiences; 30% were primiparous (n=35). Although most of the women (92%; n=108) reported being in good health, a few complained of general body pains (5.1%), waist pain (3.4%), fever (3.4%) and headaches (2.6%) within the last seven days. Sixteen women reported postpartum breast health problems including engorgement (11.9%) and breast pain (6.7%) with 5.1% reporting

both engorgement and pain. One woman each reported sore nipples and a boil on the breast. Only four of the reported symptoms occurred within the two weeks prior to enrollment into the study.

A total of 229 breast milk samples were collected constituting 112 bilateral samples and 5 unilateral samples (participants were unable or unwilling to provide milk from the second breast). The median duration of milk expression was 5 to 10 minutes for each breast.

Na/K and SCM prevalence

The sodium and potassium levels in milk from right and left breasts are presented in Table 4.2. A wide range in milk sodium and potassium levels from both right and left breasts was observed. We found no significant differences between left and right breast milk samples for sodium (p=0.80) or potassium (p=0.89).

The geometric mean breast milk Na/K was 0.84; there was no difference in Na/K between right and left breasts. The Na/K in breast milk from women with unilateral SCM was significantly greater than Na/K in women without elevated Na/K in both breasts (p<0.01). There was no significant difference between Na/K in milk from women with bilateral and those with unilateral SCM. Na/K was not associated with parity, reported maternal health perception, and reported breast health problems at anytime after delivery. However, maternal MUAC below 29 cm (p<0.003) and age below 34 years (p<0.017) were both associated with higher Na/K.

Using a Na/K ratio of 1.0 as a threshold, 45.3% of participants had elevated breast milk Na/K indicative of SCM. Unilaterally elevated Na/K was observed in 29.9% of the study population while 15.4% had bilaterally elevated Na/K. There was a significant between-breast difference in Na/K (p<0.01) among women with unilaterally elevated Na/K (Figure 4.2).

Discussion

This study documented very high rates of elevated Na/K among lactating women reporting excellent health and no indication of malnutrition. Previously published reports of the prevalence of Na/K > 1.0 have ranged between 9 and 25% at three months postpartum.[1,4] We focused on women who were between three and four months postpartum for two reasons: firstly, to provide data beyond the third month postpartum period since most of the available data on SCM reports data up to three months postpartum, and secondly, the one month interval between 3 and 4 months was to reduce a dilution effect of the prevalence estimate since SCM prevalence typically reduces over time postpartum.[1,17] Our results therefore confirm previously reported sustained high SCM prevalence of 23%[4] and 25%[1] at 14 weeks postpartum, suggesting that SCM may occur beyond the third month postpartum.

It has been reported that healthy women at one month postpartum had breast milk Na/K ≤ 0.6.[10] Some studies have reported moderate SCM, diagnosed as Na/K > 0.6 but ≤ 1.0.[1,3,12] Moderate SCM may therefore have a lower predictive power for outcomes associated with SCM, compared to Na/K > 1.0. In their study of HIV infected women, Willumsen et al.[4] reported significant elevation of breast milk viral load in women with breast milk Na/K > 1.0 compared to those with 0.6 < Na/K ≤ 1.0. Both lactogenesis and weaning are associated with increased mammary permeability and leakage of plasma electrolytes into milk spaces, thereby increasing breast milk sodium concentration.[5,7]

Average breast milk sodium and potassium concentration ranges between 5 and 6 mmol/L and 13 and 14 mmol/L, respectively, beyond the lactogenesis and weaning periods.[10] In our study, a wide range in both sodium and potassium concentration were observed. The observed wide range in concentration was not surprising because among women without SCM, breast milk electrolyte concentration is tightly regulated. However, leakage of the alveolar epithelium leads to a spike in the breast milk concentration, far above that which is observed in the absence of mammary inflammation.

It is not known whether SCM at this later stage in lactation (i.e., 3-4 months postpartum) continues to affect weight gain and other infant outcomes. If it does, there will be need for interventions to reduce SCM rates beyond early lactation period. A trial in Bangladesh has indicated a protective effect of lactation counseling before or after birth on the rate of SCM during the early postpartum period.[12] The effect of a similar intervention on the rate of SCM occurring at a later period in lactation remains untested.

Detailed information on infant feeding was not collected in this rapid survey. Results from other studies,[4] however, lead us to assume that early introduction of complementary feeding may partly explain the high SCM prevalence observed in this community. Typically, complementary feeding starts at three months or earlier in Ghana.[13] Early introduction of complementary feeds has been associated with infrequent breastfeeding and reduced breast emptying, both of which can lead to milk stasis and subsequent elevation of breast milk sodium.[14]

MUAC ≤ 18.5 is considered an indicator of severe malnourishment among adults.[11] Women in our study who had MUAC below 29 cm had higher Na/K. Filteau et al.[3] have demonstrated that maternal supplementation with vitamin E reduced breast milk Na/K. Low maternal MUAC may suggest underlying poor nutritional status although the link between low MUAC and poor micronutrient status at this stage may only be implied since there is no data clearly establishing a relationship. The lower Na/K among women aged 34 years and above may be a function of higher parity and lactation experience. Earlier studies have reported that primiparous[15] and women between 21 and 35 years[16] had a higher risk of mastitis.

While SCM has been found to be high among populations with high HIV prevalence,[4,17] the effect of HIV infection on SCM in this community remains unknown because we did not test for HIV. At the current HIV prevalence of 5%, its effect on SCM prevalence among our study participants is likely to be small. We, however, recommend that

future studies assess HIV status to allow determination of the contribution of HIV infection to the incidence of SCM.

Conclusion

The high prevalence of SCM observed in this community suggests the need for further investigation to determine the factors that promote elevated Na/K among these women and immediate interventions to decrease the prevalence of SCM and the risk of adverse outcomes to the infants. Interventions should be targeted at younger and malnourished mothers who are at a higher risk of elevated Na/K.

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Table 4.1. Characteristics of lactating Ghanaian women, 3-4 mo postpartum (N=117)

Maternal characteristics	Mean	SD	Median	Range
Age, y	27.0	5.8	27.0	18.0 – 46.0
Live births, #	2.5	1.4	2.0	1.0 – 9.0
Mid-upper arm circumference, cm	27.3	3.3	26.6	19.3 – 36.5

Table 4.2. Sodium and potassium levels in milk of lactating Ghanaian women, 3-4 mo postpartum

Milk characteristics[1]	Geometric Mean	SD	Range			N
Right breast						116
Sodium, mmol/L	11.23	16.78	4.56	-	115.90	
Potassium, mmol/L	13.53	12.57	4.56	-	91.50	
Sodium/Potassium ratio	0.83	2.25	0.05	-	16.98	
Left breast						113
Sodium, mmol/L	11.43	12.11	5.00	-	116.70	
Potassium, mmol/L	13.43	14.53	7.14	-	90.40	
Sodium/Potassium ratio	0.85	1.57	0.06	-	16.34	

[1]Normal values: sodium, 5-6 mmol/L; potassium, 13-14 mmol/L; sodium:potassium ratio, ≤ 0.6 .

Women visiting child welfare clinic (n=1,296)

Women with children 3-4 months old (n=154)

Women with children outside 3-4 months range (n=1,142)

Women included n=(117)

Women excluded (n=37)

Mother unavailable (n=21)
Previously included in study (n=6)
Refusal to participate (n=6)
Mother's age < 18 y (n=3)
Twin births (n=1)
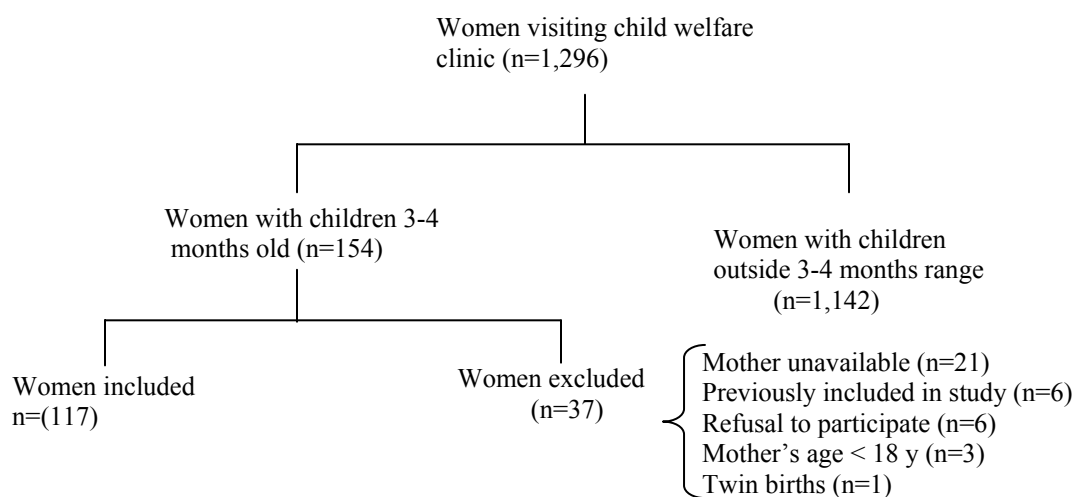
Figure 4.1. Flow diagram of sample selection
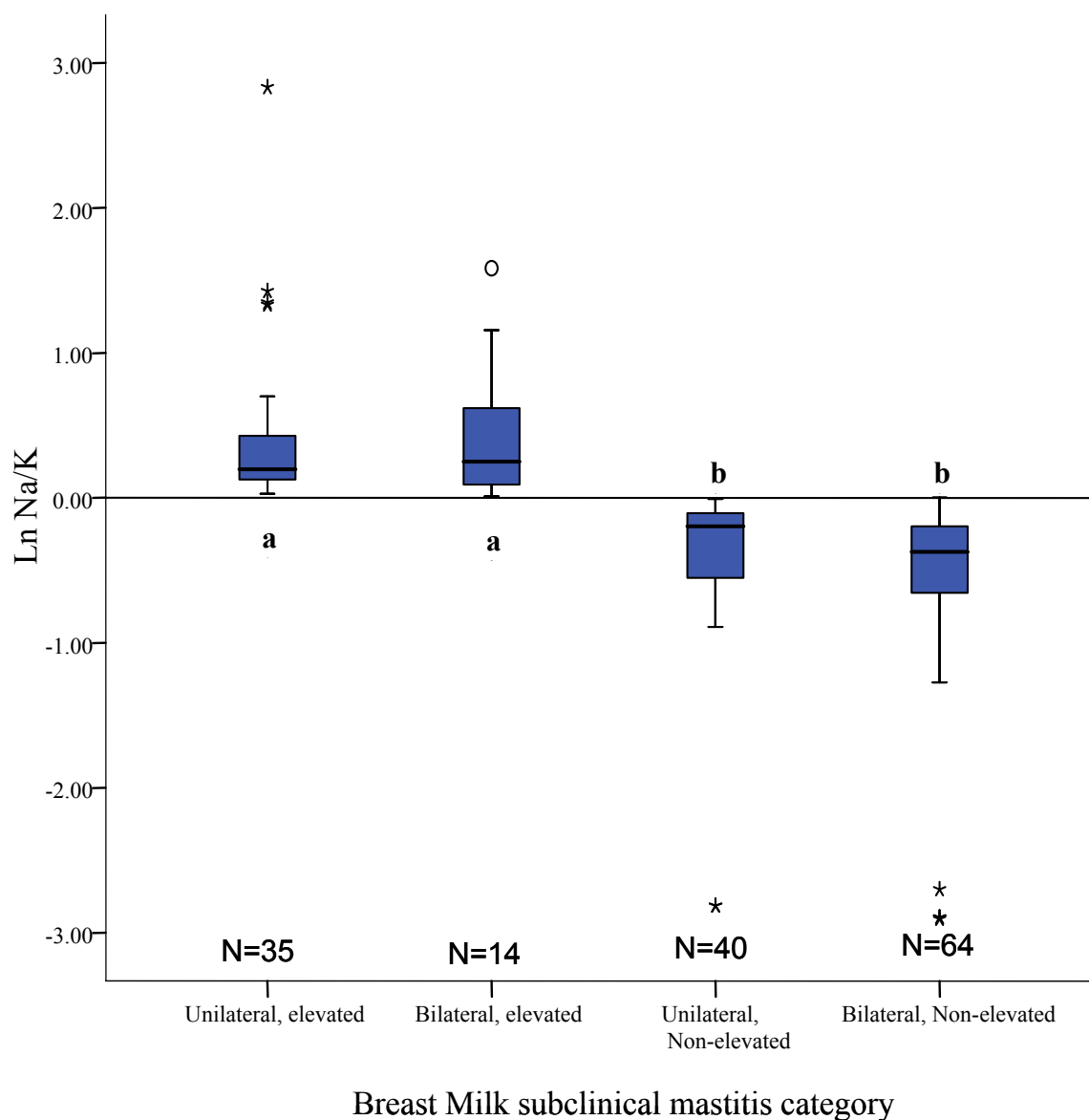
Figure 4.2. Sodium:potassium ratios of breast milk from lactating Ghanaian women, 3-4 months postpartum, by subclinical mastitis (SCM) status

Legend:
Box plot categories represents women who had elevated Na/K in one breast (Unilateral, elevated), elevated Na/K in both breasts (Bilateral, elevated), normal Na/K in one breast(Unilateral, Non-elevated) and normal Na/K in both breasts (Bilateral, Non-elevated). The box plot includes: the 25th and 75th percentiles delineated by the box, the median is represented by the dark horizontal line, the whiskers represent 1.5 x interquartile range, outlier values (o) are 1.5-3 times the interquartile range, and extreme values (*) are more than 3 times the interquartile range. Dissimilar superscript letters represent significant group differences.

Chapter 5. Is subclinical mastitis associated with reduced breast milk intake?

A paper to be submitted to *Pediatrics*

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Abstract

OBJECTIVE: Subclinical mastitis (SCM) has been associated with infant growth faltering. The mechanism explaining this association remains unknown. We hypothesized that SCM is associated with reduced infant breast milk intake.

METHODS: We compared 12-hour breast milk intake of infants of two groups of Ghanaian lactating mothers who either tested positive or negative for SCM. Only mothers above 18 years and between 3 and 6 months postpartum were included in this study. Breast milk intake was measured using test weighing. SCM positive mothers (N=37) were selected using the California mastitis test (CMT) score ≥ 1.0. Twenty three mothers had negative CMT scores. SCM diagnosis was confirmed using elevated breast milk sodium-potassium ratio (Na/K) > 1.0.

RESULTS: Breast milk intake tended to be lower for infants whose mothers were diagnosed for SCM using 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 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 multiple linear regression, infant weight (p<0.01) and frequency of feeding (p<0.01) but not maternal SCM status were associated with milk intake.

CONCLUSION: The major predictors of milk intake were infant weight and feeding frequency. The observed effect SCM status on milk intake is minimal and disappears when infant weight and feeding frequency are considered.

Key words: breast milk intake, subclinical mastitis, infant, feeding frequency, Ghana

Introduction

There is compelling evidence that breastfeeding is associated with optimal infant growth and protection from diarrhea, respiratory infections, and other illnesses.[1,2,3] However, some lactating women experience breastfeeding difficulties that may eventually lead to early cessation of breastfeeding or introduction of complementary feeding before the recommended six months.[4,5]

A common problem that has been associated with lactation failure is subclinical mastitis (SCM).[5] 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.[5,6] 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 is considered to be elevated above 1.0.[5,6] Although SCM is most prevalent during early lactation, SCM rates of 12% to 23% at 14 weeks postpartum have been reported.[7,8]

It is common knowledge in the dairy industry that cows diagnosed with SCM produce less milk.[9] 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.[9,10] Kitchen[11] 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.[12] Milk output of the unaffected breast is capable of compensating for the affected breast without noticeable changes in common breastfeeding indicators. Conner[13] has reported a single case in which an infant accepted milk from one breast with reluctance and nursed normally from the other. Tasting of the milk by the infant's father showed that milk from the rejected breast had a salty taste. Later analyses showed that the sodium content of the affected breast was 103 mmol/L compared to a typical breast milk sodium concentration of < 10 mmol/L in non-weaning lactating women.[14]

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 an explanatory variable linking infant growth faltering with maternal SCM.

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 165,409.[15] 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 of about 5% has been observed in Manya Krobo[16] compared to the national HIV prevalence that is currently 2.7%.[17] Previous studies in Manya Krobo suggest a high exclusive breastfeeding rate of 83.1% among three to six month old infants.[18]

Participants

A total of 72 mother-infant pairs were recruited from 7 child welfare clinics in the Manya Krobo district. Women were included in the study if they satisfied the following

criteria: at least 18 years old, singleton birth, less than three months postpartum and intending to breastfeed beyond three months postpartum.

Data collection procedures

At recruitment, field workers explained the protocol of the study to each eligible woman. Upon accepting the study procedures and signing the informed consent document, mothers were asked to provide their home addresses and an appointment was scheduled for a 12-hour home observation visit on a date that was to occur after the infant turned three months old but was less than six months.

Measurement of breast milk intake

On the scheduled day of the home visit, field workers spent twelve consecutive hours at the residence of the mother to observe all infant breast milk intakes during the period 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.[19] 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 (The Sartorius Group; Goettingen, Germany). The scale was calibrated weekly using standard weight blocks. Milk volume was calculated assuming 1g/ml.

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, still 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.[20]

Morbidity and infant feeding

During the home visit, maternal and infant health histories over the previous seven

days 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). Any treatment for these symptoms and

the source of treatment was also recorded.

A food frequency questionnaire was used to record all dietary intake of the infant

including breast milk and other liquid and solid foods. The questionnaire had a list of foods,

including liquid, semi-solid and solid foods, as well as breast milk. Mothers were asked to

recall intake of any of the listed foods or drinks taken by the infant and the frequency of

intake of these items in the last 7 days. Mothers were also asked to indicate if the infant had

any other foods that were not listed on the questionnaire.

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 content. Before expressing

breast milk, the women washed both hands thoroughly with disinfectant liquid hand soap and

running water, rinsed with deionised 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 was repeated with deionised water and cleaning the breast surface with ethyl alcohol before milk was expressed from the other breast.

About 2 mL of the milk sample were tested for SCM immediately after collection using the California mastitis test (CMT).[21] 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.[21] The severity of SCM inflammation was observed 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 a score of zero.

The remaining milk sample was kept on ice in a sealed container and transported to temporary storage in the field laboratory at -20 ^{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

All data analyses were performed using SPSS 15.0 (SPSS Inc; Chicago, USA). Descriptive statistics including arithmetic means and standard deviations were computed for

continuous maternal and infant descriptive variables. Categorical descriptive 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 among SCM groups as well as the association with other maternal and infant factors. A multiple linear regression model was used to identify other infant and maternal factors associated with 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 CMT scores while 7 others had CMT scores but not sodium and potassium data. We only had complete data on sodium and potassium as well as CMT for 60 out of the 72 mother-infant pairs who participated in the study. Thus, only these 60 women were included in bivariate and multivariate analyses described below. About 62% of the 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 5.1 compares the characteristics of study participants across SCM groups based on Na/K. Women who tested positive for SCM tended to be younger (p=0.02) and were most likely to be first time mothers (p=0.02). There were no other significant differences in maternal characteristics between the SCM groups. Infant length was lower among infants whose mothers had elevated Na/K. There was, also, a tendency for lower infant weight (p=0.05) and head circumference (p=0.05) among infants whose mothers tested positive for SCM (Na/K > 1.0).

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 who 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 5.1 displays the distribution of milk intake among 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 5.2) 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 stepwise multiple linear regression analyses, 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 multiple linear regression model but which failed to predict milk intake included maternal SCM status, age, primiparity, and body mass index as well as infant gender, length, head circumference, and arm circumference.

Discussion

The objective of this study was to determine whether SCM status was associated with a decrease in infant milk intake and thereby serves as a mechanistic pathway by which SCM contributes to infant growth faltering.[5,6] Our bivariate 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). Multivariate analyses, however, revealed that infant weight and frequency of feeding, but not maternal SCM status, were the factors

that explained the variation in infant milk intake rather than SCM status. This outcome suggests that the difference in milk intake attributed to SCM was captured in multivariate analyses by either infant weight, feeding frequency or both.

To our knowledge, this is the first study investigating the relationship between SCM and infant intake of breast milk beyond early lactation. Manganaro and collegues[22] have recently reported an inverse relationship between breast milk sodium and infant milk intake during the first week postpartum. The results were also consistent with findings in dairy cattle in which SCM is known to reduce milk output and permanently impair lactational performance.[11,23]

SCM typically occurs unilaterally and hence it is possible for milk output from a healthy breast to compensate for the adverse effect of SCM on an affected breast.[24] As reported by Connor[13] infants may be capable of differentiating between normal and breast milk with elevated sodium and thus exhibit a preference for the former. An ideal design for a study of the effect of SCM on lactational performance, therefore, would be to measure milk output 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.[25,26] have demonstrated the capacity to measure milk output using a computerized breast measurement system that allows measurement of changes in breast volume. 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.[27] These findings have implications for interventions to support 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.[19] Costs and logistics constrained us to use 12-hour test weighing sessions rather than a preferable 24-hour test weighing session. Matheny and Picciano[28] 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, insensible water loss was not estimated. Arthur et al.[19] have demonstrated significant underestimation of infant test weighing without consideration for insensible water loss. Although our comparisons across groups are not affected by not controlling for insensible water loss, it is probable that there was underestimation of 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.[21] In an earlier study, we had observed that the CMT overestimates the prevalence of SCM with the inclusion of the Trace score.[18] Na/K which is commonly used to diagnose SCM[6,7] was therefore used to confirm CMT scores. Using Na/K diagnosis, SCM rate was 27.7%. This was not an unexpected outcome based on what we had learned in other earlier studies we conducted in the same community.[18]

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 typically higher in cows than humans.[29] 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. Na/K diagnosis is typically made using a threshold of 1.0, which is considered to be equivalent to a sodium concentration of 18 mmol/L.[30] This level of milk sodium is typically observed in breast milk during mastitis in the absence of

weaning. However, it is not known whether the fluctuation in milk electrolytes observed during the onset of lactation or weaning follows the same pattern as the acute changes occurring in the milk of non-weaning lactating women.[14]

Conclusions

Our results indicate that infant weight and feeding frequency are the major determinants of infant milk intake. Maternal subclinical mastitis did not predict infant breast milk intake. Further studies are needed among infants less than three months to determine whether subclinical mastitis is associated with infant breast milk intake.

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Table 5.1. Characteristics of Ghanaian lactating women and their infants by subclinical mastitis (SCM) status

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 defined as Na/K > 1.0

[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
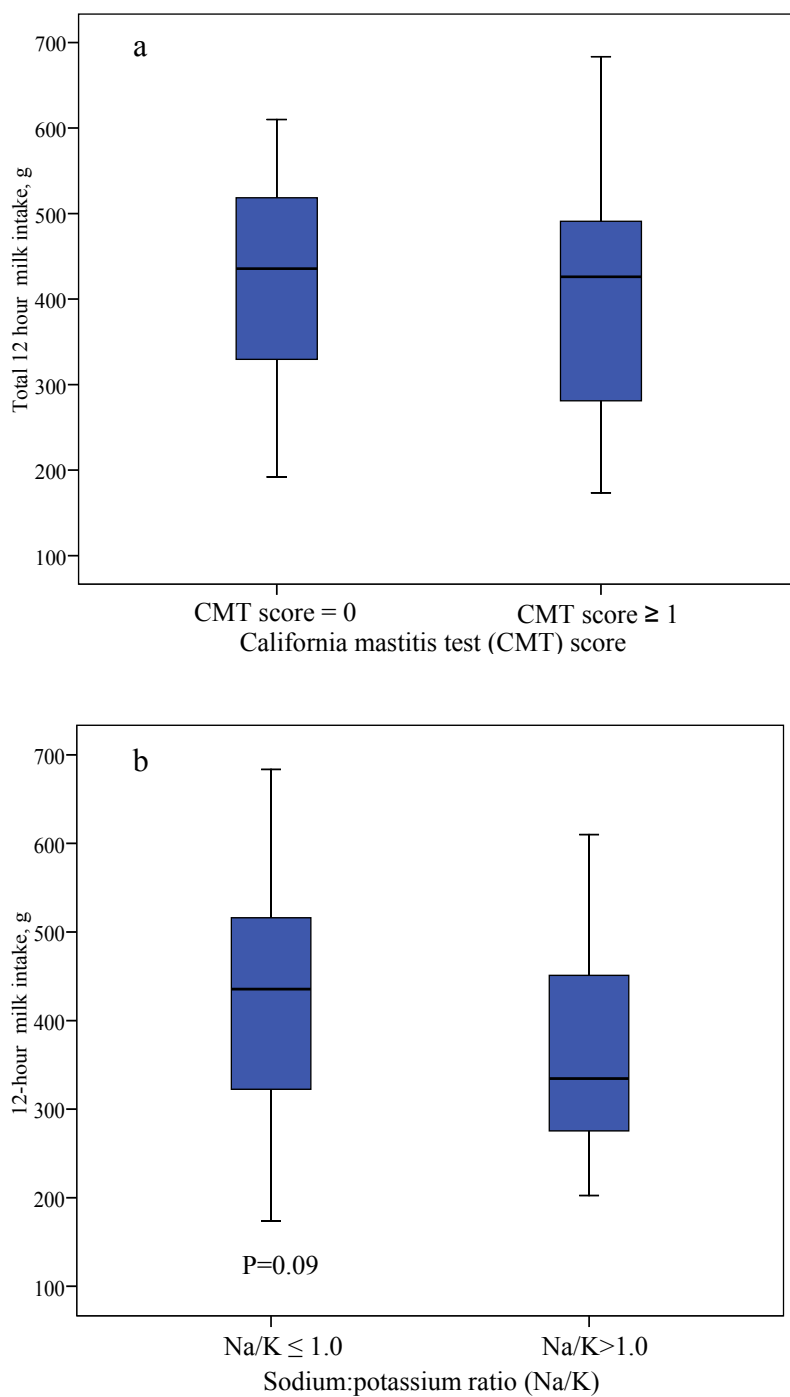
Figure 5.1. 12-hour breast milk inake of Ghanaian infants by maternal subclinical mastitis (SCM) test

Legend:
(a) intake compared among California mastitis test score groups; (b) intake compared among sodium:potassium ratio groups.
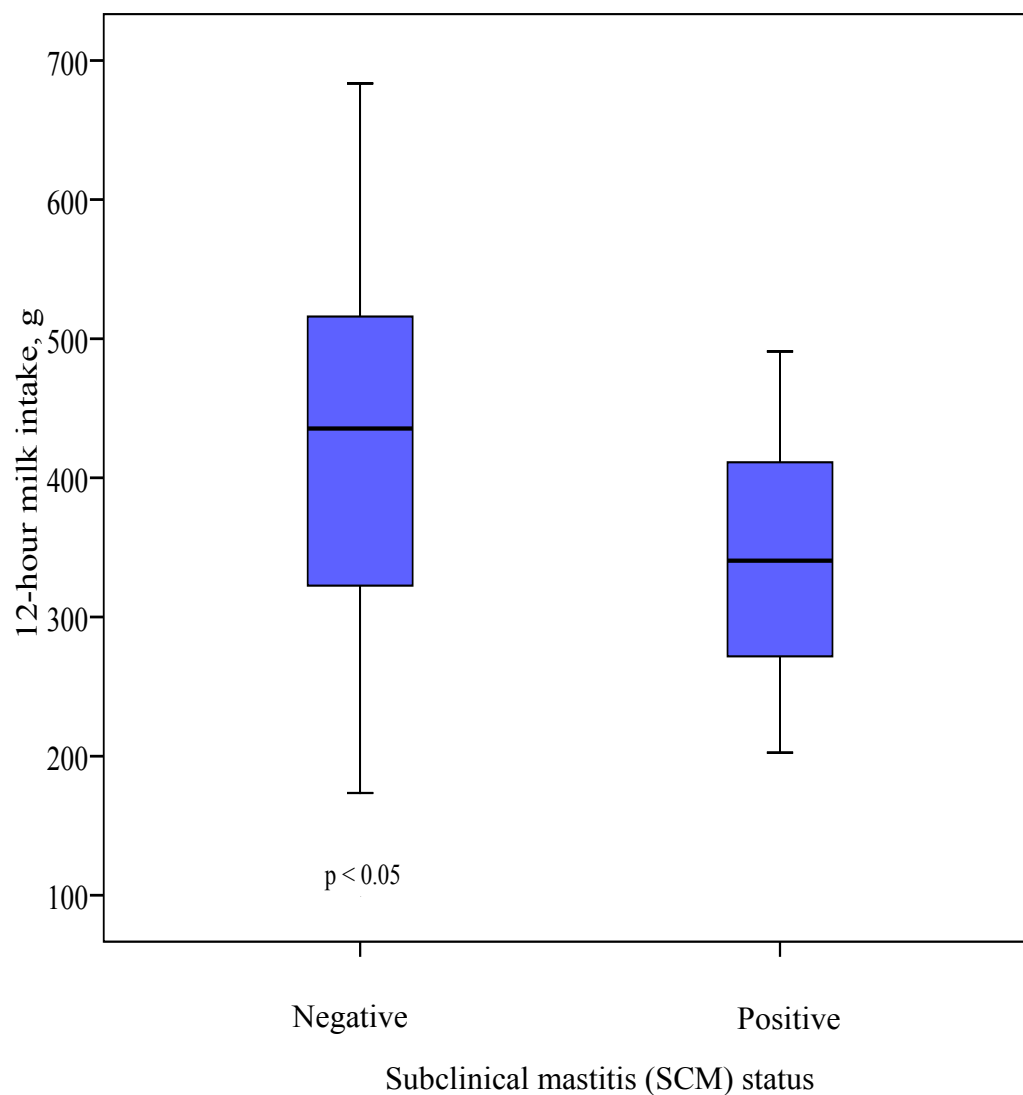
Figure 5.2. 12-hour infant breast milk intake by maternal subclinical mastitis (SCM) status.

Legend:
Positive: Women whose breast milk had Na/K > 1.0 as well as CMT test score ≥ 1.
Negative: Women whose breast milk had Na/K ≤ 1.0 and CMT score=0, or Na/K>1 and CMT score=0, or Na/K ≤ 1.0 and CMT≥1.

Chapter 6. Subclinical mastitis occurring between the third and sixth month postpartum does not predict growth of Ghanaian infants

A paper to be submitted to *Pediatrics*

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Abstract

INTRODUCTION. Subclinical mastitis (SCM) is a common inflammatory condition of the lactating breast that has been associated with growth faltering among infants during early lactation. We hypothesized that SCM continues to occur at a high rate beyond the third month postpartum and adversely affect infant growth.

METHODS. A cohort of 115 lactating Ghanaian mothers and their infants were recruited and observed in seven consecutive home visits every two weeks between the third and sixth month postpartum. Infant feeding, anthropometric, and morbidity data as well as maternal socio-demographic, morbidity, and anthropometric data, and bilateral breast milk samples were collected at baseline and subsequent visits. Breast milk samples were analyzed for sodium and potassium to determine SCM status, defined as: No SCM (Na/K $\leq$ 0.6); moderate SCM (0.6 < Na/K $\leq$ 1.0); severe SCM (Na/K > 1.0). Outcome measures included in analyses were infant weight, length, head circumference, and mid-upper arm circumference at six months.

RESULTS. At least 16% and 25% of mothers had severe and moderately elevated Na/K, respectively, in one or both breasts between the third and sixth month postpartum. There were no associations between SCM and any of the growth outcomes. The factors that predicted infant growth between the third and sixth month included infant gender and fever as well as maternal education, illness, nipple health, occupation, and parity.

CONCLUSION. Severe SCM occurring between the third and sixth month postpartum was common but not associated with infant growth. Intervention is needed in this community to reduce incidence of SCM, especially during the first six months postpartum.

Key words: subclinical mastitis, growth, breastfeeding, Ghana

Introduction

Subclinical mastitis (SCM) is a common asymptomatic inflammation of the lactating breast and is thought to be caused primarily by milk stasis.[1-4] Underlying factors that predispose women to SCM include poor lactation practice, poor anti-oxidant micronutrient status and localized or systemic infection.[5-7] SCM is commonly diagnosed as elevated milk sodium or sodium-potassium ratio. Although SCM is most common during early lactation, the condition may occur beyond the third month postpartum.[8,9]

SCM has been associated with growth faltering among infants during the first three months of lactation.[3,10,11] The mechanism(s) by which SCM affects infant growth remains unknown. Morton[11] has reported that elevated sodium is predictive of lactation failure. A recent study among Italian infants reported that elevated breast milk sodium is associated with reduced breast milk intake on the third day postpartum.[12] Evidence from dairy cattle suggests a mechanism that involves reduced milk production.[13] A case study reported by Connor[14] suggested in 1979, that elevated breast milk sodium may be associated with reduced acceptability of breast milk by the infant. Although there are only few published studies on the association between SCM and infant outcomes, the ones outlined above provide important clues to the mechanistic pathway between SCM and infant growth faltering.

Although there is a dearth of evidence to support a mechanistic pathway between SCM and infant growth faltering, a determination of the extent of growth faltering attributable to SCM is needed as growth is an important public health index of infant survival

and well-being and is listed as one of the indicators of the Millennium Development Goals.[15] A poor growth pattern during infancy and early childhood is associated with increased risk of infant mortality and severe morbidity linked with increased case fatality.[16,17] Infants whose growth pattern becomes impaired experience more frequent episodes of gastrointestinal and respiratory tract infections.[18,19] There is also evidence showing that growth impairment during infancy could delay mental development, reduce intellectual achievement, and subsequently lead to reduced work capacity in adulthood.[16,20,21] Currently, one out of three children below five years are either underweight or stunted, globally.[22] In sub-Saharan Africa, 38% of children below five years have low stature for their age.

Earlier studies reporting an adverse effect of SCM on infant growth have focused mostly on infants up to three months old because SCM is most prevalent during early lactation.[3,10] It is not known, however, whether the observed effect of SCM on infant growth persists beyond the first 3 months. The period between three to six months is important to observe because it is associated with the introduction of mixed feeding.[23] Mixed feeding, in turn, is associated with elevated Na/K.[1]

We observed a cohort of Ghanaian mother-infant pairs to determine the association between SCM and growth of children between the third and sixth month in a primarily peri-urban Ghanaian community. We hypothesized that SCM occurs at a high rate beyond the third month postpartum and may affect infant growth during the three to six months period.

Methods

This prospective cohort study was conducted between February and October, 2006. The mother-infant pairs recruited into this study were observed during seven regularly scheduled home visits carried out approximately every two weeks over a total duration of three consecutive months between the 3rd and 6th month postpartum. All participants signed written informed consent forms that were approved by Iowa State University and the Noguchi Memorial Institute for Medical Research at the University of Ghana.

Study participants and procedures

The study was carried out in the Manya Krobo district of the Eastern region of Ghana. The district has a population of 165,409 persons; mostly living in rural communities.[24] More than 80% of residents are from the native Krobo ethnic group. The primary occupation is subsistence farming of vegetables and livestock. Most participants included in the study were recruited from peri-urban communities.

Out of 2,718 lactating mothers attending the nine government child welfare clinics, 163 satisfied all inclusion criteria: residence in the Manya Krobo district, less than 3 months postpartum, at least 18 years old, intention to breastfeed through the 6th month postpartum, and singleton birth. However, only 135 mothers agreed to participate in the study through six months postpartum.

At recruitment, a brief demographic survey was collected together with the home addresses of participants using easy-to-identify land marks and community names and where possible, maps to the home were drawn. An appointment was scheduled with the mother for the day and time for the first home visit.

Maternal and infant morbidity

During each of the seven home visits, trained field workers collected maternal and infant morbidity data covering the seven days prior to the day of home visit. The mother was asked to recall the occurrence and frequency of occurrence of specific symptoms. Maternal symptoms included fever, breast pain, breast engorgement and sore/cracked nipples. Infant symptoms included diarrhea, cough, fever, breathing difficulty (unusual or labored breathing pattern), mouth sores and vomiting. Diarrhea was diagnosed as three or more liquid stools reported by the mother within a period of 24 hours.

Dietary intake

During each home visit, mothers and other caregivers were interviewed by field workers using a food frequency questionnaire to record all dietary intake of the infant,

including breast milk and other liquid, semi-solid and solid foods consumed by the infant during the last seven days. The questionnaire had a list of foods, including liquid, semi-solid and solid foods, as well as breast milk from which field workers asked the mothers to recall intake of any of the listed foods or drinks taken by the infant and the frequency of intake of these items in the last 7 days. Mothers were also asked to indicate if the infant had any other foods that were not listed on the questionnaire.

Anthropometry

Field workers were trained and regularly standardized to perform maternal and infant anthropometric measurements using standard methods.[25] Maternal weight and standing height as well as infant weight, recumbent length, head circumference, and mid-upper arm circumference were measured and recorded at baseline. During subsequent visits, however, only infant anthropometric measurements were taken.

Both maternal and infant weights were measured to the nearest 0.1 kg using the Tanita BWB800S digital floor scale (Tanita Corporation; Tokyo, Japan). Infant head circumference and mid-upper arm circumference were measured to the nearest 0.1 cm using a non-stretchable tape measure (Chasmors Ltd.; London, United Kingdom). Infant recumbent length and maternal standing height were both measured to the nearest 0.1 cm using the Shorr infant/child/adult height/length measuring board (Shorr Productions; Maryland, USA). All anthropometric measurements were taken in duplicate by a pair of field workers. The questionnaires were designed to ensure that field workers observed and recorded measurements independently of each other. After each measurement was recorded, field workers compared their measurements and if the difference exceeded maximum allowed differences, the particular measurement was repeated only once.

Breast milk collection

At each visit, a sample of 5 mL of breast milk was expressed manually from each breast. Breast milk samples were stored on ice in clean plastic vials with screw tops for

transportation to the field laboratory. An elaborate milk collection protocol was used to prevent contamination of the milk. Before expressing breast milk, the women washed both hands thoroughly with disinfectant liquid hand soap and running water, rinsed with deionised 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 using 60 mL plastic vials. Upon completion of expression from one side, hand rinsing was repeated with deionised water and breast surface cleaning with ethyl alcohol before milk was expressed from the other breast. All milk samples were then transferred into the screw top storage vials and transported on ice to the Noguchi Memorial Institute for Medical Research laboratories in Accra to be stored at -32 ^{0}C until analysis.

Laboratory procedures

Breast milk sodium and potassium analyses were performed using standard methods that employ ion selective electrode analyzer.[26] Briefly, all analyses were performed on whole milk without any sample pre-treatment. 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 (Medica Corporation, Bedford, USA) for the analyses of sodium and potassium. 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. Milk samples from left and right breasts were analyzed separately.

Statistical analyses

All data analyses were performed using SPSS 15.0 software (SPSS Inc; Chicago, Illinois, USA). Data obtained during the first home visit were considered as baseline. Maternal, infant and household characteristics were described using frequencies and 95% confidence intervals. Sodium:potassium ratios (Na/K) were computed and used for SCM

diagnoses as follows: no SCM (Na/K ≤ 0.6); moderate SCM (0.6 > Na/K ≤1.0); severe SCM (Na/K > 1.0).[1,11] Food frequency data were used to categorize infants into three feeding pattern groups: exclusive breastfeeding (given breast milk alone), predominant breastfeeding (breast milk plus water and other non-dietary fluids), and mixed feeding (breast milk plus other liquid and solid foods). Maternal weight and height obtained at baseline were used to compute maternal body mass index (BMI). Growth outcomes were reported as the difference between measurements taken at the 6th month and baseline as well as the attained growth measured at the sixth month. Infant growth measures were summarized using arithmetic means and standard deviations. Bivariate analyses of relationships between growth and categorical and continuous independent variables were tested using one-way analysis of variance and simple linear regression, respectively. Variables that were significant as well as had *a priori* theoretical associations with infant growth were included in the multiple linear regression models to determine the relationships between maternal and infant characteristics and infant growth (Table 6.5). Partial-F tests were then used to determine whether maternal SCM accounted for unexplained variance in the earlier model. Statistical significance was set at p<0.05, unless otherwise indicated.

Results

A total of 135 lactating mothers and their infants met the inclusion criteria and agreed to participate in the study (Figure 6.1). However, some mothers moved out of the study area or withdrew from the study prior to commencement of home visits. Baseline data were obtained from 115 mothers. Subsequently, more than 75% (n=87) of the mothers completed all the six remaining home visits while almost 89% (n=102) completed at least five out of six remaining home visits. Overall, a total of 747 out of 805 home visits were completed. A majority of visits (85%) were completed within ± 1 day of the scheduled visit dates. Emigration out of study area was the main reason for loss to follow-up; most of the emigrants had come to live temporarily with their family either prior to or immediately after delivery.

Eighty percent of study participants were between 18 and 30 years old and about 37% were primiparous. The median number of live births among the study participants was 2, with a range between one and six. Almost 90% of mothers had at least one year of education but only 16% had high school education. More than half of the mothers were petty traders and most lived in households with no pipe-borne water (83.5%), refrigerator (67.8%) or telephone (70.4%).

A comparison of maternal and infant as well as household characteristics across baseline SCM groups is shown in Table 6.1. Maternal BMI was significantly higher (p<0.01) among mothers with severe SCM at baseline. All other maternal characteristics were not different across SCM groups. Overall, mothers were healthy as evidenced by the low incidence of reported disease symptoms during the entire study period. Overt breast health problems were uncommon among these mothers; only 1% of mothers reported either breast pain or cracked nipples during the study.

At least 16% of mothers had severe SCM in one or both breasts at each visit (Figure 6.2). The highest prevalence of severe SCM in one or both breasts, was observed at baseline (23.3%). Moderate SCM in at least one breast ranged between 25.2 and 37.5%. Therefore at each time during the study, about half of the mothers had moderate or severely elevated Na/K in one or both breasts. About 22% of mothers tested positive for severe SCM only once during the study period. Only 7% of mothers tested positive for severe SCM seven out of seven observation times during the study.

The baseline sample included 52 male and 63 female infants. Most infants were born at home, thus, birth weight was unavailable for 70% of them. Compared to mothers, infants had a greater incidence of disease symptoms. For instance, reported coughing and difficult breathing episodes were documented in more than 20% of all home visits. Overall, diarrhea episodes were reported during 11% of home visits but only 4% of mothers reported blood in infant stools. There were no differences in the occurrence of any infant disease symptoms

across SCM groups. No deaths were reported among any of the mother-infant pairs during the study period.

Over 88% of infants were breastfeeding exclusively at baseline and the rate of exclusive breastfeeding remained above 80% until the sixth month of age when it reduced to 61% (Table 6.2). Mixed feeding was observed among 4% of infants at baseline and increased gradually through the 5th month. There was almost a 200% increase (n=10 to n=27) in the number of infants who were reported to be receiving liquid and solid foods in addition to breast milk between the $5^1/_2$ and 6th month home visit. The overall rate of mixed feeding during the study period was just above 11%. Infant feeding patterns did not differ across SCM groups at baseline (Table 6.1).

On average, each infant gained 1.3 ± 0.4 kg in weight and added 5.5 ± 1.2 cm to their length between baseline and the seventh home visit. Tables 6.3 and 6.4 compare infant growth between baseline and the 6th month as well as attained growth measurement at all observed times during the study by maternal baseline SCM status. No consistent pattern was observed in the anthropometric indicators at any time point across baseline SCM groups. At all observed times, none of the group differences in weight, length, head circumference or mid-upper arm circumference was statistically significant. Episodes of severe maternal SCM during the 3rd and 6th months were not related to any of the growth measurements at any time point.

In bivariate analyses, the strongest predictors of growth measurements at six months were the respective growth measurements at 3 months. Boys had greater growth measurements than girls (p<0.001). There was a tendency for head circumference (p=0.079) and recumbent length (p=0.067) to be associated with infant feeding during the 3rd month and 4th months, respectively. Maternal BMI was directly associated with infant weight (p=0.017) but not length, head circumference, or mid-upper arm circumference at 6 months. No other maternal and infant characteristics were significantly associated with infant growth.

The outcomes of multiple linear regression analyses are shown in Tables 6.6. There was no effect of episodes of severe maternal SCM on infant growth measurements at 6 months. Infant weight was directly associated with nipple soreness, and inversely with maternal education, and parity, after controlling for infant weight at 3 months, and maternal age. Length at 6 months was directly associated with maternal illness and inversely with infant fever episodes when length at 3 months as well as gender and parity were included in the model. Head circumference at 6 months favored boys and was lower in infants whose mothers were farmers. Maternal education was inversely associated with mid-upper arm circumference at 6 months.

Discussion

Elevated Na/K is considered an indicator of SCM and has been associated with infant growth faltering during early lactation, particularly during the first 3 months postpartum.[3,10,11,27] Our study of Ghanaian infants did not observe an association between infant growth and SCM during this later period. Rather, in four out of seven measurements over time, we observed non-significantly greater average weight and head circumference among infants whose mothers had SCM at baseline. Infant mid-upper arm circumference measurements did not follow any consistent pattern when compared across baseline SCM status of the mother. Neither was any relationship observed between maternal episodes of severe SCM and infant growth. The observed outcomes were not altered with the inclusion of moderate SCM as a predictor of growth. With the exception of mid-upper arm circumference, more than 80% of the variation was explained by the variables included in the multiple linear regression models to explain growth measurements at 6 months.

There are not many published studies that were designed to assess the relationship between SCM and infant growth. Most of the few published studies reporting an adverse impact of SCM on growth examined SCM and growth data collected during the first three months postpartum. One study from Zimbabwe included data up to four and half months of

age.[10] To the best of our knowledge, this current study is the first to report the effect of SCM on growth between the third and sixth month postpartum. This study contributes to the existing literature on predictors of growth in Ghana where infant growth faltering often becomes evident early around the third month postpartum.[23]

SCM is most common during the early postpartum period.[8,9] We were unable to capture information on maternal SCM occurring earlier than the third month postpartum because our data collection was restricted to the period between the third and sixth month postpartum. Future studies of the cumulative effect of SCM on growth should include data from early lactation when SCM is most frequent. Also, a majority of infant birth weights were not available because most mothers delivered at home. Birth weight data, if available would have provided clues about the growth rate of infants during the first three months. In this current study, growth that occurred prior to the 3rd month was an important determinant of growth between the 3rd and 6th month postpartum. Therefore, the inability to control for SCM during the early postpartum period and lack of birth weight data may partly explain why we did not find any association between growth and SCM.[10] It is necessary to also indicate the effect that low instrument precision may have had on the outcomes of this study. The Tanita BWB800S weighing scale used to measure infant weights only had a precision of 0.1 kg. Mullany et al.[28] have reported underestimation of poor infant growth performance using low precision measuring instrument.

A third explanation for the observed outcomes may be drawn from the fact that the inflammation occurring during SCM appears to be an acute process.[6] It is possible that SCM may become resolved, depending on maternal immune status, micronutrient status and maternal breastfeeding practices, before it influences infant growth. The effect of SCM on growth may therefore have a lesser impact during the third and sixth month when the infant is growing at a slower rate.

The high prevalence of severe SCM (20.7%) observed at the third month in our study is consistent with earlier publications: at 3 months postpartum, severe SCM ranges between 9 and 25%.[1,3,5,11] The wide range in the literature may partly be explained by whether milk samples used for analyses were obtained from only one breast at random, pooled from both breasts or analyzed separately and the results averaged between breasts. Several studies have shown that Na/K ratio is often different between breasts.[5,8] Consistent with previous studies, breast health problems were rare among these women.[7,29] Our results therefore show that subclinical mammary inflammation is common among Ghanaian lactating mothers from the third to the sixth month postpartum in the absence of overt symptoms.

Maternal factors that were associated with infant growth included years of education, parity, illness, and fever. In contrast with previously published studies,[30] maternal education was negatively associated with weight gain. We did not collect any data that could explain this outcome. However, based solely on the observations made during home visits, it appears that less educated women were less likely to be employed outside their home and/or more likely to be self employed. As a result, they may have more control of the time they needed to care and feed the infant. Reed et al.[31] have demonstrated that higher maternal education was beneficial only when it correlated with a high socio-economic environment.

The positive association between maternal illness and growth is opposite to what is expected; that is, maternal illness may adversely affect infant growth by reducing effectiveness of infant care and breastfeeding as reported in previous studies.[32,33] The low incidence of maternal illness may have contributed to this outcome. As expected, length at 6 months was lower among infants whose mothers had fever.

Infant characteristics that were associated with growth were gender and fever. Boys had significantly greater weight, length, head circumference and mid-upper arm circumference at 6 months. This gender difference is a reflection of the observation that during the first 10 years of life, boys have greater average growth measurement compared to

girls.[34] Infant fever was inversely associated with length at 6 months. Studies in similar settings have reported associations between history of fever and stunting among infants at six months and beyond.[35,36] Infant illness may affect growth by increasing energy and nutrient requirements, reducing appetite for complementary foods, and limiting nutrient utilization.[37-39]

Conclusion

We observed high rates of SCM among lactating Ghanaian women in the absence of overt breast health problems. Our study did not find an association between SCM and infant growth between the third and sixth month postpartum. Attained growth measures at six months were also associated with infant gender and fever as well as maternal illness, cracked/sore nipple, parity, and occupation. Our results have implications for targeting support for lactating mothers during the early postpartum period and to reduce the incidence of infant morbidity.

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Table 6.1. Characteristics of Ghanaian lactating mothers and their infants at baseline (N=115)

	Subclinical mastitis status at baseline			
	None/moderate (Na/K ≤ 1.0)		Severe (Na/K > 1.0)	
Mother				
Age, y	25.8	(24.6, 27.0)[1]	26.0	(23.7, 28.2)
Education, y	7.2	(6.4, 7.9)	6.5	(5.1, 7.1)
Parity, #	2.2	(1.9, 2.4)	2.0	(1.5, 2.4)
Body mass index, kg/m^2	22.3	(21.6, 23.0)	25.0	(23.8, 26.1)[a]
Married	96.6		100.0	
Earning income	90.6		92.9	
Fever	5.7		10.7	
Respiratory symptoms	9.3		15.4	
Infant				
Exclusively breastfed	88.5		78.6	
Weight, kg	5.9	(5.7, 6.0)	5.9	(5.6, 6.3)
Length, cm	59.5	(59.0, 60.0)	59.7	(58.7, 60.7)
Age, y	2.9	(2.9, 3.0)	3.0	(3.0, 3.0)
Female	52.9		60.7	
Reported fever	12.7		10.7	
Household				
Electricity	71.3		85.7	
No running water at home	82.8		85.7	
Own radio	64.4		71.4	
Own refrigerator	28.7		42.9	

[1] Arithmetic mean (95% CI) or %
[a] Significant group difference at $p<0.01$

Table 6.2. Bimonthly feeding behavior of Ghanaian infants, 3-6 months old

Infant feeding behavior	Infant age, mo							Overall
	3.0	3.5	4.0	4.5	5.0	5.5	6.0	
Exclusive breastfeeding, %	86.1	90.1	89.6	86.1	84.2	84.8	61.1	83.1
Predominant Breastfeeding, %	9.6	4.5	2.8	4.0	4.0	4.8	9.3	5.6
Mixed feeding, %	4.3	5.4	7.5	9.9	11.9	10.5	29.6	11.2
N	115	111	106	101	101	105	108	747

Exclusive breastfeeding: only breast milk fed to infant;
Predominant breastfeeding: breast milk is major source of dietary intake, water and other non-dietary fluids may be given;
Mixed feeding: liquid and solid foods given in addition to breast milk.

Table 6.3. Growth indicators of Ghanaian infants 3-6 mo, by maternal baseline subclinical mastitis (SCM) status

| Growth measure | Infant age, mo | SCM status[1] | | | | | N |
		No SCM[2]			Severe		
Weight, kg	3.0	5.8	±	0.8	5.9 ±	0.9	115
	3.5	6.2	±	0.8	6.2 ±	0.8	111
	4.0	6.4	±	0.9	6.4 ±	0.4	106
	4.5	6.5	±	0.9	6.8 ±	0.9	101
	5.0	6.8	±	0.9	6.7 ±	0.7	101
	5.5	6.9	±	0.9	7.1 ±	0.8	105
	6.0	7.1	±	1.0	7.3 ±	0.6	107
Length, cm	3.0	59.4	±	2.1	59.7 ±	2.7	114
	3.5	60.7	±	2.3	60.3 ±	2.1	111
	4.0	61.7	±	2.3	61.4 ±	1.4	105
	4.5	62.4	±	2.2	63.0 ±	2.5	100
	5.0	63.3	±	2.3	63.1 ±	2.0	101
	5.5	64.2	±	2.3	63.7 ±	1.9	105
	6.0	65.1	±	2.4	64.7 ±	1.9	105
Mid-upper arm circumference, cm	3.0	13.5	±	1.0	13.6 ±	1.0	115
	3.5	13.8	±	1.0	13.8 ±	1.0	111
	4.0	13.8	±	1.1	13.8 ±	0.5	106
	4.5	13.9	±	1.1	14.1 ±	1.0	101
	5.0	14.1	±	1.1	14.2 ±	0.9	101
	5.5	14.0	±	1.1	14.3 ±	1.0	105
	6.0	14.1	±	1.1	14.4 ±	0.8	106
Head circumference, cm	3.0	39.7	±	1.3	39.6 ±	1.5	115
	3.5	40.3	±	1.4	40.1 ±	1.1	111
	4.0	40.7	±	1.3	40.6 ±	1.3	106
	4.5	41.0	±	1.3	41.3 ±	1.3	101
	5.0	41.4	±	1.3	41.5 ±	1.1	101
	5.5	41.9	±	1.3	41.7 ±	1.2	105
	6.0	42.2	±	1.3	42.0 ±	1.0	106

[1] No SCM: Na/K ≤ 1; Severe SCM : Na/K>1
[2] Mean ± SD

Table 6.4. Change in growth measurements of Ghanaian infants 3-6 mo, by maternal baseline subclinical mastitis (SCM) status

| Changes[1] in growth measurements | SCM status[2] | | | | N |
	No SCM		Severe		
Weight gained, kg	1.3 ±	0.4[3]	1.4 ±	0.3	108
Length gained, cm	5.6 ±	1.2	5.4 ±	1.2	106
Head circumference gained, cm	2.5 ±	0.5	2.6 ±	0.6	107
Mid-upper arm circumference gained, cm	0.5 ±	0.6	0.7 ±	0.5	107

[1] Difference in dependent variable measured at 6 months and at baseline (3 months)
[2] No SCM: Na/K ≤ 1; Severe SCM : Na/K>1
[3] Mean ± SD

Table 6.5. Variables from bivariate analyses that were included in multiple linear regression models of attained weight, length, head circumference, and mid-upper arm circumference at 6 months

Dependent Variables	Infant weight, kg	Infant length, cm	Head circumference, cm	Mid-upper arm circumference, cm
Independent variables	Education Parity Infant age Breast pain[2] Breast engorgement[3] Cracked/sore nipples[4] Blood in stool[6] Weight at 3 months[*] SCM[8]	Parity Infant gender[*] Maternal illness[1] Length at 3 months[*] Infant mouth sore[5] Infant fever[7] SCM[8]	Occupation (farming) Infant gender[*] Head circumference at 3 months[*] SCM[8]	Maternal education Occupation (office worker) Ethnicity (Akan) Mid-upper arm circumference[*] SCM[8]

[1] Reported maternal disease symptoms during 3rd – 6th month
[2] Reported breast pain during 3rd - 6th
[3] Reported breast engorgement/swelling during 3rd - 6th month
[4] Reported cracked/sore nipple during 3rd - 6th month
[5] Reported infant mouth sores during 3rd - 6th month
[6] Reported liquid stool with blood during 3rd - 6th month
[7] Reported infant fever during 3rd - 6th month
[8] Episodes of subclinical mastitis (SCM) during 3rd - 6th month; SCM defined as Na/K > 1
[*] Statistically significant association with dependent variable at $p<0.05$ observed in bivariate analyses

Table 6.6. Factors associated with attained size in Ghanaian infants, 3-6 mo

Explanatory variables	Regression coefficient	p-value	F value	R^2
Weight at 6 months, kg			71.18	0.84
Infant weight at 3 months, kg	0.97	<0.01		
Education, y	-0.02	0.03		
Parity, #	-0.09	0.03		
Maternal age, y	0.01	0.07		
Breast pain[1]	-0.19	0.12		
Cracked/sore nipple[2]	0.26	0.02		
Severe SCM episodes[3]	0.02	0.12		
Length at 6 months, cm			63.83	0.80
Gender (female)	-0.42	0.03		
Length at 3 months, cm	0.85	<0.01		
Parity, #	-0.15	0.04		
Maternal illness score[4]	0.23	0.01		
Infant fever[5]	-0.22	0.01		
Severe SCM episodes[3]	-0.06	0.11		
Head circumference at 6 months, cm			219.44	0.87
Head circumference at 3 months, cm	0.84	<0.01		
Gender(female)	-0.25	0.06		
Severe SCM episodes[3]	0.02	0.13		
Mid-upper arm circumference at 6 months, cm			78.02	0.70
Mid-upper arm circumference at 3 months, cm	0.86	<0.01		
Maternal education, y	-0.03	0.02		
Severe SCM episodes[3]	0.03	0.25		

[1] Reported breast pain during 3rd - 6th month
[2] Reported cracked/sore nipple during 3rd - 6th month
[3] Episodes of subclinical mastitis (SCM) during 3rd - 6th month; SCM defined as Na/K > 1
[4] Reported maternal disease symptoms during 3rd - 6th month
[5] Reported infant fever during 3rd – 6th month

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┌─────────────────────────────┐
│ Mothers visiting Child Welfare │
│ Clinic (n=2698)              │
└─────────────────────────────┘
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┌──────────────────┐        ┌──────────────────┐
│ Ineligible (n=2535) │      │ Eligible (n=163)  │
└──────────────────┘        └──────────────────┘
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18years +
Resident in Manya Krobo
< 3 months postpartum
Intends to breastfeed through
6th month

21U; 7R

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┌──────────────────┐
│ Recruited (n=135) │
└──────────────────┘
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12R; 7F; 1V

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┌─────────────────────────┐
│ 3 mo (baseline) (n=115)  │
└─────────────────────────┘
```

4U

```
┌──────────────────┐
│ 3.5 mo (n=111)    │
└──────────────────┘
```

6U; (1)

```
┌──────────────────┐
│ 4.0 mo (n=106)    │
└──────────────────┘
```

8U; (3)

```
┌──────────────────┐
│ 4.5 mo (n=101)    │
└──────────────────┘
```

4U; (4)

```
┌──────────────────┐
│ 5.0 mo (n=101)    │
└──────────────────┘
```

5U; (9)

```
┌──────────────────┐
│ 5.5 mo (n=105)    │
└──────────────────┘
```

1T; (4)

```
┌──────────────────┐
│ 6.0 mo (n=108)    │
└──────────────────┘
```
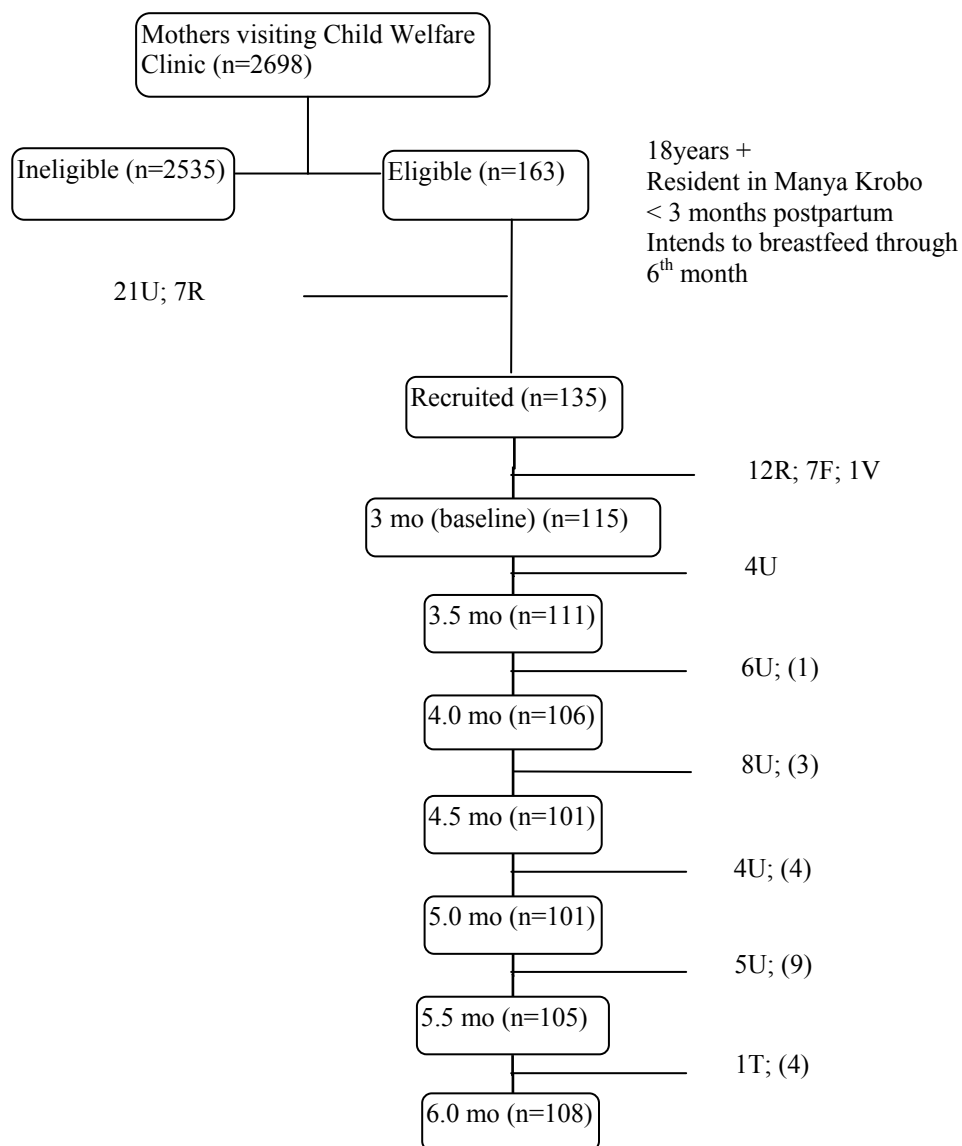
Figure 6.1. Flow diagram of mother-infant pairs included in the study

Legend:
F: false address; R: relocated from study area; T: traveled; U: unavailable at home visit period; V: voluntary withdrawal. Number in parenthesis reflects pairs that were available for home visit after being unavailable during a previous home visit
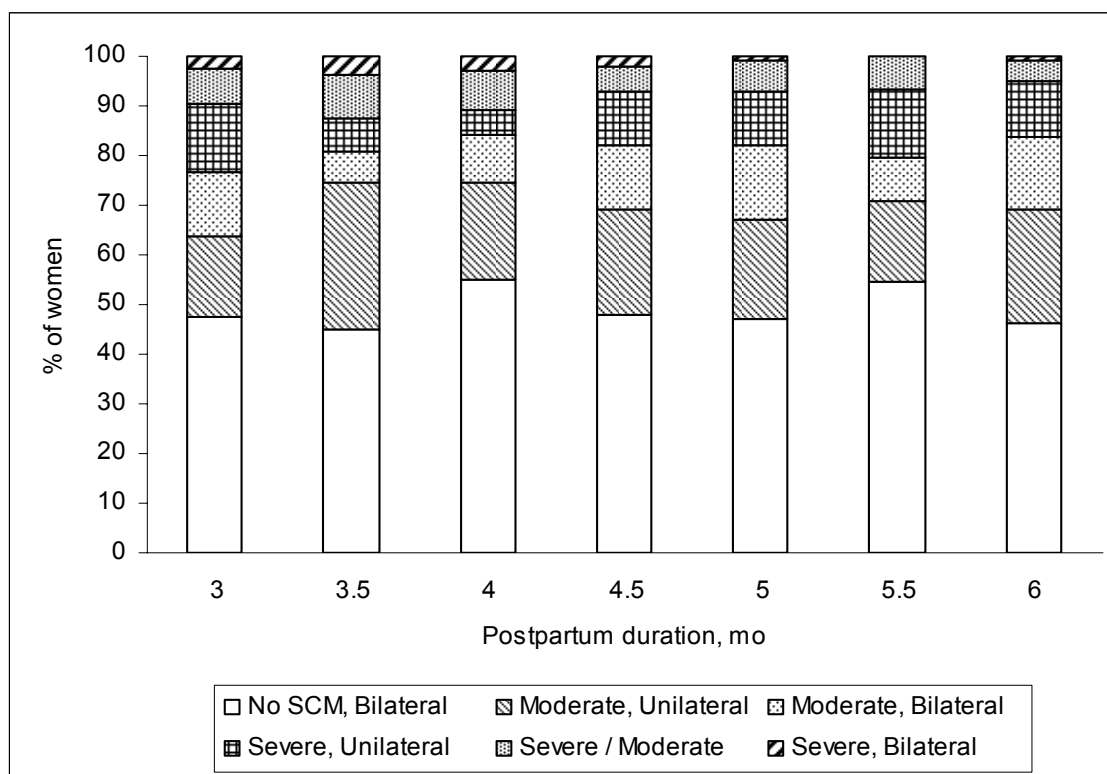
Figure 6.2. Prevalence of subclinical mastitis (SCM) among Ghanaian lactating mothers , 3-6 mo postpartum

Legend:
No SCM: Na/K ≤ 0.6; Moderate SCM: 0.6 < Na/K ≤ 1.0; Severe SCM: Na/K > 1.0.
Unilateral and bilateral refers to SCM diagnosis in one or both breasts, respectively.
Severe/Moderate refers to a diagnosis of moderate SCM in one breast and severe SCM in the other breast.

Chapter 7. General Conclusions

Subclinical mastitis (SCM) is an inflammatory condition of the lactating breast that affects many women. Studies on SCM have recently gained momentum because of the reported association between SCM and increased risk of HIV transfer from mother to infant via breast milk. In addition, the condition is known to affect lactation performance as well as have adverse effects on the growth of infants up to the third month of life. The mechanism for the effect of SCM on infant growth is unknown.

The results of the studies carried out in Manya Krobo district indicate that SCM occurrence is high in the Manya Krobo district and probably elsewhere in Ghana. As reported in other studies, the majority of the observed SCM was unilateral. There is need, therefore, for interventions to reduce prevalence of SCM in this district. These interventions could be beneficial to both mothers and infants by reducing infant outcomes associated with SCM as reported in other studies.

There was not sufficient evidence to support the hypothesis that SCM was associated with reduced infant breast milk intake in our study. Although significant differences were observed in breast milk intake across maternal SCM status in bivariate analyses, these differences disappeared in multivariate analyses. Infant feeding frequency, which is associated with both SCM and milk intake, and infant weight were the factors that explained the variation in breast milk intake. The causal pathway(s) relating SCM and infant growth faltering remains unknown. Earlier studies reporting the association between SCM and infant growth faltering had involved infants at 3 months of age or less. In our study, all infants whose breast milk intake was assessed were between three and six months old. There is the need for future studies, therefore, to repeat the mechanistic study among infants below 3 months to further examine the effect of SCM on milk intake.

The outcome of the longitudinal study indicated that factors including infant gender and fever as well as maternal education, illness, nipple health, occupation, and parity

explained most of the variation in infant growth between the third and sixth month. No association was observed between episodes of SCM and infant growth during the same period. This outcome may partly be explained by the fact that SCM is most common during early lactation and therefore its effects on growth may be greatest during this early stage of growth. The early postpartum period is also associated with a faster infant growth rate which may explain why in the longitudinal study, we did not find any association between SCM and infant growth. Future studies on the effect of SCM on infant growth beyond 3 months postpartum may benefit from data on SCM and infant growth occurring during the early postpartum period.

Altogether, these studies indicate that SCM is a common condition among lactating women. The reported maternal and infant outcomes may be limited to the early postpartum period prior to the third month. Simple interventions that reduced SCM prevalence using a single lactation counseling session either at birth or soon after birth have been demonstrated among Bangladeshi women. This approach may be useful among Ghanaian women because it is simple and inexpensive.

In another study that has not been reported in this dissertation, we explored the use of simple SCM diagnostic tests from the dairy industry for use among lactating women. The simple tests investigated in that study were not sensitive compared to Na/K. There is therefore the need for future studies to continue to search for simple and inexpensive diagnostic tests for SCM among lactating women as this will be beneficial for identifying mothers with SCM, at least during the early postpartum period.

It is thought that clinical mastitis results from and is a severe expression of SCM. Reducing SCM among lactating women is therefore important for reducing the risk of clinical mastitis. As reported in other studies, reducing SCM may reduce the risk of lactation failure and probably increase the duration of exclusive breastfeeding. Although our results do not show any association between SCM and either milk intake and growth of infants older

than 3 months, it is important to remember that SCM has been associated with both milk intake and growth faltering among infants below 3 months. For the infant, reducing subclinical mastitis may result in reduced risk of HIV transmission.

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Appendices

Appendix 1: Informed consent documents

<u>Appendix 1a: Rapid study informed consent document</u>

Title of Study: Is subclinical mastitis a determinant of infants' milk intake and growth: a rapid survey

Investigators: Grace S Marquis, PhD, Marcus Kehrli, PhD, Anna Lartey, PhD, Richmond Aryeetey, MPH

This is a research study. Please take your time in deciding if you would like to participate. Please feel free to ask questions at any time.

INTRODUCTION
The purpose of this study is to obtain information about breast health among Ghanaian lactating women. Breast problems can affect the health of both lactating mothers and their children. There is currently no available information on breast health among Ghanaian women. Results from this study will be useful for improving recommendations for successful breastfeeding.
You are being invited to participate in this study because you visited the child welfare clinic/ hospital today.

DESCRIPTION OF PROCEDURES
If you agree to participate in this study, you will be asked to answer questions in a brief interview. You will also be asked to provide a one-time breast milk sample of about one spoonful of milk from each breast. These procedures will take approximately ten minutes.

RISKS
There are no foreseeable risks at this time from participating in this study.

BENEFITS
The results of the test that will be performed on your sample will be made available to you through the clinic within one week. It is hoped that the information gained in this study will benefit society by providing information that can be used to improve interventions aimed at improving maternal and child health.

COSTS AND COMPENSATION
You will not have any costs from participating in this study. You will receive a small token as compensation for your participation in this study.

PARTICIPANT RIGHTS
Your participation in this study is completely voluntary and you may refuse to participate or leave the study at any time. If you decide to not participate in the study, it will not result in any penalty or loss of benefits to which you are otherwise entitled.

CONFIDENTIALITY
Records identifying participants will be kept confidential to the extent permitted by applicable laws and regulations and will not be made publicly available. To ensure confidentiality to the extent permitted by law, the following measures will be taken. All records will be kept under lock and key with access only by the principal investigator or data management staff authorized by the principal investigator. Data stored on a computer will be managed by principal investigator with participants identified with codes. Names of participants will not be stored on the computer. If the results are published, your identity will remain confidential.

QUESTIONS OR PROBLEMS
You are encouraged to ask questions at any time during this study. For further information about the study contact Dr Grace S Marquis, 1127 HNSB, Iowa State University, Ames, IA (USA); telephone number 515-294-9231 or Dr Anna Lartey, Dept. of Nutrition and Food Science, University of Ghana, Legon; telephone number 021-513-294. If you have any questions about the rights of research subjects or research-related injury, please contact Ginny Austin Eason, IRB Administrator, (515) 294-4566, austingr@iastate.edu, or Diane Ament, Research Compliance Officer (515) 294-3115, dament@iastate.edu.
****
SUBJECT SIGNATURE
Your signature indicates that you voluntarily agree to participate in this study, that the study has been explained to you, that you have been given the time to read the document and that your questions have been satisfactorily answered. You will receive a copy of the signed and dated written informed consent prior to your participation in the study.

Subject's Name (printed) _____

_____   _____
(Subject's Signature)   (Date)

INVESTIGATOR STATEMENT
I certify that the participant has been given adequate time to read and learn about the study and all of their questions have been answered. It is my opinion that the participant understands the purpose, risks, benefits and the procedures that will be followed in this study and has voluntarily agreed to participate.

_____   _____
(Signature of Person Obtaining Informed Consent)   (Date)

Appendix 1b: Informed consent document for longitudinal study

Title of Study: Is subclinical mastitis a determinant of infants' milk intake and growth: longitudinal study

Investigators: Grace S Marquis, PhD, Marcus Kehrli, PhD, Anna Lartey, PhD, Richmond Aryeetey, MPH

This is a research study. Please take your time in deciding if you would like to participate. Please feel free to ask questions at any time.

INTRODUCTION
The purpose of this study is to obtain information about breast health among Ghanaian lactating women. One common problem, subclinical mastitis, is a condition of the breast that cannot be seen but has health consequences for both lactating mothers and their children. There is currently no available information on subclinical mastitis among Ghanaian women. Results from this study will be useful in improving our recommendations for maintaining breast health among women.
You and your child are being invited to participate in this study because you attended the child welfare clinic at this hospital.

DESCRIPTION OF PROCEDURES
If you agree to participate in this study with your child, your participation will last for a total of three months, starting from when your child is three months old. During each of the 7 bi-monthly follow-up visits, you will be asked to answer questions on the health of you and your child, and your child's feeding pattern. At each visit, breast milk samples (of about one tablespoonful) will be collected from each breast by our trained field staff who would also take measurement of your child's growth. At the time of recruitment and also at the last follow-up visit, measurements of your weight and height will be taken. Each visit is expected to last for at least one hour at a venue of your choice (either your home or the hospital).

RISKS
There are no foreseeable risks at this time from participating in this study.

BENEFITS
It is hoped that the information gained in this study will benefit society by providing information that can be used to improve interventions aimed at improving maternal and child health.

COSTS AND COMPENSATION
You will not have any costs from participating in this study. You will receive a small token as compensation for your participation in this study.

PARTICIPANT RIGHTS
Your participation in this study is completely voluntary and you may refuse to participate or leave the study at any time. If you decide not to participate in the study, it will not result in any penalty or loss of benefits to which you are otherwise entitled.

CONFIDENTIALITY
Records identifying participants will be kept confidential to the extent permitted by applicable laws and regulations and will not be made publicly available. To ensure confidentiality to the extent permitted by law, the following measures will be taken. All records will be kept under lock and key with access only by the principal investigator or data management staff authorized by the principal investigator. Data stored on a computer will be managed by principal investigator with participants identified with codes. Names of participants will not be stored on the computer. If the results are published, your identity will remain confidential.

QUESTIONS OR PROBLEMS
You are encouraged to ask questions at any time during this study. For further information about the study contact Dr Grace S Marquis, 1127 HNSB, Iowa State University, Ames, IA (USA); telephone number 515-294-9231 or Dr Anna Lartey, Dept. of Nutrition and Food Science, University of Ghana, Legon; telephone number 021-513-294. If you have any questions about the rights of research subjects or research-related injury, please contact Ginny Austin Eason, IRB Administrator, (515) 294-4566, austingr@iastate.edu, or Diane Ament, Research Compliance Officer (515) 294-3115, dament@iastate.edu.
****

SUBJECT SIGNATURE
Your signature indicates that you voluntarily agree to participate in this study, that the study has been explained to you, that you have been given the time to read the document and that your questions have been satisfactorily answered. You will receive a copy of the signed and dated written informed consent prior to your participation in the study.
Subject's Name (printed)

_____   _____
(Subject's Signature)   (Date)

_____   _____ _____
(Signature of Parent/Guardian or   (Date)
Legally Authorized Representative)

INVESTIGATOR STATEMENT
I certify that the participant has been given adequate time to read and learn about the study and all of their questions have been answered. It is my opinion that the participant understands the purpose, risks, benefits and the procedures that will be followed in this study and has voluntarily agreed to participate.

_____   _____
(Signature of Person Obtaining   (Date)
Informed Consent)

Appendix 1c: Informed consent document for cross-sectional study

Title of Study: Is Subclinical mastitis a determinant of infants' milk intake and growth: a cross-sectional study of breast milk intake

Investigators: Grace S Marquis, PhD, Marcus Kehrli, PhD, Anna Lartey, PhD, Richmond Aryeetey, MPH

This is a research study. Please take your time in deciding if you would like to participate. Please feel free to ask questions at any time.

INTRODUCTION
The purpose of this study is to obtain information about breast health among Ghanaian lactating women. Breast problems can affect the health of both lactating mothers and their children. There is currently no available information on breast health among Ghanaian women. Results from this study will be useful in improving our recommendations for successful breastfeeding.
You and your child are being invited to participate in this study because you attended the child welfare clinic at this hospital.

DESCRIPTION OF PROCEDURES
If you agree to participate in this study with your child, your participation will involve providing a breast milk sample collected at the clinic and a brief questionnaire. Thereafter a visit will be arranged to your home. During this home visit, which will last a trained field staff will measure the weight of your child before and after every time he/she is put to the breast to feed in order to know how much milk he/she drinks.

RISKS
There are no foreseeable risks at this time from participating in this study.

BENEFITS
At the end of the home visit, recommendations on infant feeding will be given to you. The information gained in this study will benefit society by providing information that can be used to improve interventions aimed at improving maternal and child health.

COSTS AND COMPENSATION
You will not have any costs from participating in this study. You will receive a small token as compensation for your participation in this study.

PARTICIPANT RIGHTS
Your participation in this study is completely voluntary and you may refuse to participate or leave the study at any time. If you decide not to participate in the study, it will not result in any penalty or loss of benefits to which you are otherwise entitled.

CONFIDENTIALITY
Records identifying participants will be kept confidential to the extent permitted by applicable laws and regulations and will not be made publicly available. To ensure confidentiality to the extent permitted by law, the following measures will be taken. All records will be kept under lock and key with access only by the principal investigator or data management staff authorized by the principal investigator. Data stored on a computer will be managed by principal investigator with participants identified with codes. Names of participants will not be stored on the computer. If the results are published, your identity will remain confidential.

QUESTIONS OR PROBLEMS
You are encouraged to ask questions at any time during this study. For further information about the study contact Dr Grace S Marquis, 1127 HNSB, Iowa State University, Ames, IA (USA); telephone number 515-294-9231 or Dr Anna Lartey, Dept. of Nutrition and Food Science, University of Ghana, Legon; telephone number 021-513-294. If you have any questions about the rights of research subjects or research-related injury, please contact Ginny Austin Eason, IRB Administrator, (515) 294-4566, austingr@iastate.edu, or Diane Ament, Research Compliance Officer (515) 294-3115, dament@iastate.edu.
****

SUBJECT SIGNATURE
Your signature indicates that you voluntarily agree to participate in this study with your child, that the study has been explained to you, that you have been given the time to read the document and that your questions have been satisfactorily answered. You will receive a copy of the signed and dated written informed consent prior to your participation in the study.

Subject's Name (printed) _____

_____   _____
(Subject's Signature)   (Date)

Child's Name (printed) _____

_____   _____
(Signature of Parent/Guardian or   (Date)
Legally Authorized Representative of child)

INVESTIGATOR STATEMENT
I certify that the participant has been given adequate time to read and learn about the study and all of their questions have been answered. It is my opinion that the participant understands the purpose, risks, benefits and the procedures that will be followed in this study and has voluntarily agreed to participate.

_____   _____

(Signature of Person Obtaining   (Date)
Informed Consent)

Appendix 2: Rapid survey instruments

Appendix 2a: Rapid survey questionnaire

A Rapid survey of subclinical mastitis among Ghanaian breastfeeding women between the 3rd and 4th month postpartum

Participant ID   Date of Interview (dd/mm/yy)   Interviewer Identifier
_____   ___ / ___ / ___   _____

1. Mother's Age (yrs) _____   2.Community_____

3. Date of Delivery (dd/mm/yy) ____/____/____

4. Number of live births_____

5. Reason for Visiting Hospital   1. Child Welfare Clinic   2. Child Sick
        3. Mother/Caregiver Sick   4. Other_____

6. How would you describe your health today? 1. Excellent/Good   2. As usual/Normal
                                              3. Not Good/Bad   4. Cannot tell

7. Indicate breast-related health problems:
                Yes   No   If Yes, Indicate when:
                             [1=Today; 2=In the last7 days; 3= in the last 7-14 days;
                             4= Later than 14 days ago]
Pain in the breast(s)
Swollen breast/
Engorgement
Cracked nipples
Sore nipples
Other(s), describe

8. Have you had any other health problems in the last week?   1. Yes   2. No (If yes, go to 9)

9. Indicate the health problem(s)   1. Fever   2. Body Pains   3. Diarrhea
                                      4. Waist Pains   5. Headache
                            6. Other _____

10.   1st   2nd
Maternal MUAC (cm)   ___ ___ . ___   ___ ___ . ___

11.	Breast milk sample taken? (Y or N)	Time Sample was collected	Duration of milk collection 1= <5 Min 2= 5-10 Min 3= 10-15 Min 4> 15 Min

Left breast
Right breast
If No, reason:

_____

12.Comments_____

_____

Appendix 3: Longitudinal study instruments.

Appendix 3a. Longitudinal study recruitment questionnaire

A longitudinal study of breast health among Ghanaian breastfeeding women

Participant ID   Date of Interview (dd/mm/yy)   Interviewer Identifier
____   ___ / ___ / ___   _____

Mother's Common Name_____
Community_____

Infant Characteristics
1. Sex   1. Male   2. Female

2. Child Date of Birth (dd/mm/yy) ____/_____/_____

3. Weight at birth (kg)   ____. ____ ____

Maternal Information
4. Age (yrs)_____

5. Education (yrs) _____

(Circle one)   Post-Secondary
None = 0   Voc/Tech – started = 13
Class 1–6 (Primary) = 1-6   Voc/Tech – completed = 14
Junior Secondary School   JSS 1 = 7, Prof. Diploma – started = 15
JSS 2 = 8, JSS 3/Middle school completed = 9   Prof. Diploma – completed = 16
Senior Secondary School   University – started = 17
SSS 1 = 10, SSS 2 = 11, SSS 3 = 12   University – completed = 18
                                     Other/informal =19

6. Ethnicity (Circle one) 1. Krobo   2. Ga/Adangbe   3. Ewe   4. Akan
                   5. Northerner   6. Other_____

7. Marital status (Circle one)   1. Married   2. Cohabitant   3. Single   4. Separated

8. Number of Live Births_____

9. Primary Occupation _____

10. Place of Work   1. Home   2. Outside home   3. Home + Outside Home
                     4. Not working currently

11. If mother works outside home, do you take child with you?   1. Yes, all the time
       2. Yes, sometimes   3. No

12. Your current residence is: 1. Primary/Marital home   2. Parents' home
                          3. Other Relative's home   4.Other _____

13. How soon do you intend to move residence?   1. One Week or less
              2. Between One week and One Month   3. After one month or more
              4. After Three months   5. Other _____

15. Do you have the following in your home (Y/N):

Electricity			Television	
Access to pipe-borne water			Radio/cassette player	
Material of housing	Mud		Telephone/cellular Phone	
	Mud and Cement		Bicycle	
	Blocks/Cement		Gas cooker	
	Aluminum Roofing		Refrigerator/Freezer	
			VCD/DVD	

Describe directions leading to household where mother presently resides (draw map at back!)
_____
_____
_____
_____
_____

Contact information (Relative/Neighbour/Friend)
Name _____
Address_____
_____
_____
Tel.no._____

Name _____

Address_____
_____
_____
_____
_____

Tel.no._____

Describe directions leading to mother's "Second" household

_____

_____

_____

_____

_____

Appendix 3b. Longitudinal study maternal and infant health questionnaire

A Longitudinal study of breast health among Ghanaian breastfeeding women

PartID   Date Completed (dd/mm/yy)   Interviewer Identifier
_____   ___ / ___ / ___   _____

Complete this health record starting from today(1) and track back to the last 7 days

Time of interview: ____ :____(am/pm)   Visit #_____

*Informant _____

Maternal and Child health record

Day of recall	1	2	3	4	5	6	7
Child health							
1. # Total Stools							
2a. # Liq Stools							
2b. Mother thinks has diarrea (Y/N)							
3. Blood (Y/N)							
4. Vomit (#/day)							
* 5. Mouth sores							
* 6. Fever							
* 7. Cough							
8. Difficulty breathing (Y/N)							
9. Other _____							
* 10.Treatment							
Maternal health							
* 11. Fever							
* 12. Illness							
13. Pain in the breast (Y/N)							
14. Swolleness in breast/engorgement (Y/N)							
15. Sore/cracked Nipples (Y/N)							
* 16. Treatment							

* Refer to code list.

Code list

Informant	Mouth	Fever	Cough	Treatment
0=no one available	0=none	0=no	0=no	0=none
1=mother or guardian of child	1=oral thrush	1=reported fever	1=reported	1=health professional
2=family < 12 y who lives in house	2=ulcers	2=fever observed	2= observed	2=Traditional healer/TBA
3=family ≥ 12 y who lives in house	3=both	8=DK		3=home remedies
4=neighbor	4=other			

Illness
0=none
1=respiratory
2=gastrointestinal
3=skin
4=mouth lesions
5=other specify

Appendix 3c. Longitudinal study home follow-up questionnaire

A Longitudinal Study of breast health among Ghanaian breastfeeding women: follow-up

PartID   Date Completed (dd/mm/yy)   Interviewer Identifier
_____   ___ / ___ / ___   _____

Visit #_____

1. Please tell me how often [child name] has received the following foods and/or liquids during last 7 days:

Food item	1. Everyday 2. 5-6 times a week 3. 3-4 times a week 4. 1-2 times a week 5. none
Breast milk	
Cow's milk	
Infant Formula	
Water	
Tea	
Porridge eg Koko, rice water	
Fruit juice, eg orange juice	
Soda eg fanta, Coke	
Soup	
Bread	
Fruit	
Biscuit/Cookies	
Stew	
Solid foods eg rice, yam, cassava	
Animal foods eg fish, egg, beef	
Multivitamins/ codliver oil	
Ice Cream eg yoghurt, FanIce	
Other (write in) _____	
Other (write in) _____	
Other (write in) _____	

2a. Did [child name] receive breast milk from anyone else apart from you (mother) in the last 7 days?
        1. Yes   2. No   3. Don't know
2b. If Yes to question 3, What is relationship of person to you?
_____

3. Breast milk sample taken? (Y/N)
Left Breast
Right Breast

If No, reason_____

Child Measurement

	1A	1B	2A	2B
Weight (kg)	__ __ . __	__ __ . __	__ __ . __	__ __ . __
Length (cm)	__ __ . __	__ __ . __	__ __ . __	__ __ . __
Head circumference (cm)	__ __ . __	__ __ . __	__ __ . __	__ __ . __
Arm circumference (cm)	__ __ . __	__ __ . __	__ __ . __	__ __ . __

Maternal Measurements

	1A	1B	2A	2B
Weight (kg)	__ __ . __	__ __ . __	__ __ . __	__ __ . __
Height (cm)	__ __ __ . __	__ __ __ . __	__ __ __ . __	__ __ __ . __

Appendix 4. Cross-sectional study instruments

Appendix 4a: Cross-sectional study recruitment questionnaire

A study of breast milk intake by infants among Ghanaian breastfeeding women

1. Date_____

2. Name (include house name)_____

3. Age (yrs)_____

4. Is your child Breastfeeding?   1. Yes   2. No

5. Child date of birth ____/_____/_____   6. Clinic_____

House address (Describe address with detail)
a._____
_____
_____

b._____
_____
_____
_____

Telephone Number (if any)_____

Draw directions to address

Appendix 4b. Cross-sectional study household questionnaire

A cross-sectional study of breast milk intake among Ghanaian breastfeeding women

PartID   Date completed (dd/mm/yy)   Interviewer identifier
_____   ___/_____/_____   _____

Place of interview _____

Maternal Information
1. Age (yrs)_____

2. Education (yrs) _____
    (Circle one)   Post-Secondary
None = 0   Voc/Tech – started = 13
Class 1–6 (Primary) = 1-6   Voc/Tech – completed = 14
Junior Secondary School   JSS 1 = 7,   Prof. Diploma – started = 15
JSS 2 = 8, JSS 3/Middle school completed = 9   Prof. Diploma – completed = 16
Senior Secondary School   University – started = 17
SSS 1 = 10, SSS 2 = 11, SSS 3 = 12   University – completed = 18
                                                     Other/informal =19

3. Ethnicity (Circle one) 1. Krobo   2. Ga/Adangbe   3. Ewe   4. Akan   5. Northerners
                           6. Other_____

4. Marital status (Circle one)   1. Single   . Cohabitants 3. Married   4. Divorced

5. Number of Live births_____

6. Primary Occupation _____

7. Place of Work   1. Home   2. Outside home   3. Home + Outside Home

8. If works outside home, do you take child with you?   1. Yes , all the time
                           2. Yes, sometimes   3. No

Mother   1st   2nd
9. Weight (kg)   ___ ___ ___ . ___   ___ ___ ___ . ___

10. Height (cm)   ___ ___ ___ . ___   ___ ___ ___ . ___

Infant Characteristics
11. Sex   1. Male   2. Female

12. Weight at birth (kg) ____ . ____ ____

	1st	2nd
13. Weight (kg)	___ ___ . ___	___ ___ . ___
14. Length (cm)	___ ___ ___ . ___	___ ___ ___ . ___
15. Head circumference (cm)	___ ___ . ___	___ ___ . ___
16. Arm circumference (cm)	___ ___ . ___	___ ___ . ___

17. Please tell me how often [child name] has received the following foods and/or liquids during last 7 days:

Food item	1. Everyday 2. 5-6 times a week 3. 3-4 times a week 4. 1-2 times a week 5. none
Breast milk	
Cow's milk	
Infant Formula	
Water	
Tea	
Porridge eg Koko, rice water	
Fruit juice, eg orange juice	
Soda eg fanta, Coke	
Soup	
Bread	
Fruit	
Biscuit/Cookies	
Stew	
Solid foods eg rice, yam, cassava	
Animal foods eg fish, egg, beef	
Multivitamins/ codliver oil	
Ice Cream eg yoghurt, FanIce	
Other (write in) _____	
Other (write in) _____	
Other (write in) _____	

Appendix 4c. Longitudinal study maternal and infant health questionnaire

A Longitudinal study of breast health among Ghanaian breastfeeding women

PartID _____   Date Completed (dd/mm/yy) ___ / ___ / ___   Interviewer Identifier _____

Complete this health record starting from today(1) and track back to the last 7 days

Time of interview: ____ :____(am/pm)

*Informant _____

Maternal and Child health record

	Day of recall	1	2	3	4	5	6	7
	Child health							
	1. # Total Stools							
	2a. # Liq Stools							
	2b. Mother thinks has diarrea (Y/N)							
	3. Blood (Y/N)							
	4. Vomit (#/day)							
*	5. Mouth sores							
*	6. Fever							
*	7. Cough							
	8. Difficulty breathing (Y/N)							
	9. Other _____							
*	10.Treatment							
	Maternal health							
*	11. Fever							
*	12. Illness							
	13. Pain in the breast (Y/N)							
	14. Swolleness in breast/engorgement (Y/N)							
	15. Sore/cracked Nipples (Y/N)							
*	16. Treatment							

* Refer to code list.

Code list

Informant	Mouth	Fever	Cough	Treatment
0=no one available	0=none	0=no	0=no	0=none
1=mother or guardian of child	1=oral thrush	1=reported fever	1=reported	1=health professional
2=family < 12 y who lives in house	2=ulcers	2=fever observed	2= observed	2=Traditional healer/TBA
3=family ≥ 12 y who lives in house	3=both	8=DK		3=home remedies
4=neighbor	4=other			

Illness
0=none
1=respiratory
2=gastrointestinal
3=skin
4=mouth lesions
5=other specify

Appendix 4d. Breast milk intake assessment questionnaire

A longitudinal Study of breast health among Ghanaian breastfeeding women: follow-up

Household identifier __/__/__   Date completed __/__/__
Interviewer identifier __ __ __

Complete this table during breast milk intake assessment

	NUMBER OF BREASTFEEDS									
	1	2	3	4	5	6	7	8	9	10
Weight before feed (g)										
Weight after feed (g)										
Total weight of feed (g)										
HOURS	TIME of FEED									
Hour began feed										
Hour finished feed										
Duration of feed (minutes)										
Total minutes on breast that didn't feed										
Total minutes breastfeed										
Observations:										