ORIGINAL RESEARCH

CURRENT DEVELOPMENTS IN NUTRITION

Food and Nutrition of Indigenous Peoples

Infant Anthropometry and Growth Velocity Before 6 Months are Associated with Breastfeeding Practices and the Presence of Subclinical Mastitis and Maternal Intestinal Protozoa in Indigenous Communities in Guatemala

Hilary M Wren-Atilola,[1] Noel W Solomons,[2] Marilyn E Scott,[3] and Kristine G Koski[1]

[1]School of Human Nutrition, McGill University (Macdonald Campus), Ste-Anne de Bellevue, Quebec, Canada; [2]Center for Studies of Sensory Impairment, Aging and Metabolism (CeSSIAM), Guatemala City, Guatemala; and [3]Institute of Parasitology, McGill University (Macdonald Campus), Ste-Anne de Bellevue, Quebec, Canada

ABSTRACT

Background: The possibility that maternal health status and breastfeeding practices contribute to growth faltering before 6 mo is underexplored.

Objectives: This longitudinal study investigated whether indicators of subclinical mastitis (SCM) and breast inflammation, maternal fecal-oral contamination, and/or breastfeeding practices were associated with infant anthropometry or growth velocity before 6 mo.

Methods: Indigenous *Mam*-Mayan mother-infant dyads ($n = 140$) were recruited. Breast milk was collected at early (<6 wk) and established (4–6 mo) lactation when maternal and infant anthropometry were measured. Milk Na:K ratio as an indicator of SCM and concentrations of 4 proinflammatory cytokines were measured. Maternal stool samples were examined for the presence of intestinal parasites including nonpathogenic protozoa (*Endolimax nana*, *Iodamoeba bütschlii*, *Entamoeba coli*, *Blastocystis hominis*). Questionnaires characterized breastfeeding and hygiene practices. Multiple linear regression identified factors associated with infant growth attainment [weight-for-age *z*-score (WAZ), length-for-age *z*-score (LAZ), and head circumference-for-age *z*-score (HCAZ)] and growth velocity (expressed as change per day from early to established lactation). Multiple logistic regression identified factors associated with increased odds of underweight, stunting, and low head circumference at both lactation stages.

Results: A higher Na:K ratio, individual nonpathogenic protozoa, and specific breastfeeding and hygiene practices were associated with impaired growth before 6 wk and at 4–6 mo in exclusively breastfed infants. Growth velocity for weight was inversely associated with *Entamoeba coli* but cranial growth was associated positively with *Iodamoeba bütschlii* whereas feeding colostrum in early lactation was protective and decreased the odds of an HCAZ < -2 SD. Finally, the presence of SCM in early lactation increased the likelihood of both WAZ < -2 SD and LAZ < -2 SD by 6 wk.

Conclusions: Prevention of SCM can improve early infant weight, but measures that promote the feeding of colostrum and reduce exposure to fecal-oral contamination might be required to minimize infant growth faltering in breastfed infants. *Curr Dev Nutr* 2021;5:nzab086.

Keywords: breastfeeding, subclinical mastitis, nonpathogenic protozoa, infant anthropometry, growth velocity, colostrum, hygiene practices

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Address correspondence to KGK (e-mail: kristine.koski@mcgill.ca).

Abbreviations used: BBB, blood–brain barrier; CeSSIAM, Center for Studies of Sensory Impairment, Aging and Metabolism; CHW, community healthcare worker; HCAZ, head circumference-for-age *z*-score; LAZ, length-for-age *z*-score; LIMIC, low- and middle-income countries; SCM, subclinical mastitis; WASH, water, sanitation, and hygiene; WAZ, weight-for-age *z*-score; WLZ, weight-for-length *z*-score.

Introduction

Globally, it is estimated that 149 million children are stunted before the age of 5 y in low-and middle-income countries (LMICs) (1). Maternal stunting in adulthood has been causally linked to a higher risk of dystocia or difficult labor and with poor birth outcomes (2), and its early-life presentation is inextricably related to poor child development including delayed cognitive achievement, lower school achievement, and higher probability of adult noncommunicable chronic diseases (3). The World Health Assembly has called for a 40% reduction in stunting by 2025 (4), but current evidence shows that improved nutrition alone has a modest effect on early child growth (5). Others have suggested that the unexplained variability in early infant growth faltering could relate to a more prominent role of maternal health than previously recognized (6).

One understudied maternal condition is subclinical mastitis (SCM), an asymptomatic inflammatory condition of the mammary gland, usually diagnosed by an elevated milk Na:K molar ratio (7). SCM has been associated with lower infant weight in Bangladesh (8) and Zimbabwe

(9). In Zambia, SCM was negatively associated with infant weight-for-age *z*-score (WAZ) at 6 and 16 wk, and with infant length-for-age *z*-score (LAZ) at 6 wk, but not at 16 wk (10, 11). In Ghana, a study of 60 lactating mother-infant dyads between 3 and 6 mo postpartum found that mothers with SCM had infants with lower length, and a nonstatistical trend was also observed for lower infant weight and lower infant head circumference (12). A European study reported that infants born to mothers with SCM had smaller head circumferences, lower weight, lower weight-for-length *z*-score (WLZ), and lower BMI, but that differences disappeared as lactation progressed from 2 to 120 d postpartum (13).

It is believed that milk stasis underscores SCM and that stasis occurs when milk is not efficiently removed from the breast because of restricted flow due to poor infant feeding practices or because reduced feeding frequency or duration lowers volume thereby creating an ideal condition for bacterial overgrowth (7). This leads to a cytokine-mediated inflammatory response, and the release of cytokines into milk is thought to trigger physical damage to mammary epithelial tissue and to increase its permeability (8). SCM has been associated with higher concentrations of milk cytokines including IL-6, IL-8, and TNF-α (14) and inversely with WLZ before 46 d postpartum (15).

Another understudied factor is higher exposure of mother-infant dyads to unhygienic environments in developing countries. It has been suggested that improving water, sanitation, and hygiene (WASH) interventions can improve growth by disrupting pathways through which fecal-oral transmission of intestinal parasites, including nonpathogenic protozoa, occurs (16). Lack of proper sanitation, poor hygiene, and close animal contact are considered risk factors for parasites such as *Blastocystis* spp. (17, 18); in rural Bangladesh, handwashing and hygienic sanitation interventions significantly reduced childhood *Giardia* infections (19). However, recently, 3 large, cluster-based randomized controlled trials have found no effect of basic WASH interventions on childhood linear growth and only mixed effects on childhood diarrhea (20). The authors stated that the biological plausibility of WASH as an intervention is not challenged by these findings but that the household-level elementary WASH interventions employed in these trials were not effective enough in reducing enteropathogen exposure to facilitate linear growth (20).

The WHO recommends optimal breastfeeding practices such as exclusive breastfeeding before 6 mo of age, early initiation within 1 h postpartum, the feeding of colostrum, and adequacy of feeding frequency as effective strategies to optimize infant growth (21). It is well accepted that these ideal breastfeeding practices support nutrient absorption and reduce the susceptibility of infants to infections (22). Colostrum is considered the primary source of IgA and has been associated with benefits including the protection against microorganisms (23), favorable microbiota selection (24), and reduced risk of chronic inflammatory diseases (25).

Guatemala has Latin America's highest prevalence of stunting, and rural indigenous Mayan children in Guatemala are among the most stunted in the world (26, 27). Previously our cross-sectional study in these communities revealed that 52% of infants aged <6 mo were stunted, 15.5% were underweight, and 22.8% had low head circumference (28), and that SCM occurred in 14% of mothers and was associated with increased odds of infant stunting, underweight, and low head circumference (29). We also reported that maternal nonpathogenic intestinal protozoa were common, and that *Entamoeba coli* increased the likelihood of low head circumference, whereas higher breastfeeding frequency lowered its odds (29). The possibility that SCM and breast inflammation, breastfeeding practices, and indicators of fecal-oral contamination might contribute to growth faltering in breastfed infants has not yet been explored in a longitudinal study.

The objectives of this longitudinal study were to assess if indicators consistent with SCM (milk Na:K ratio >0.6) and breast inflammation (milk proinflammatory cytokines IL-1β, IL-6, IL-8, TNF-α), indicators of breastfeeding practices, and/or indicators of fecal-oral contamination *1*) were associated with growth attainment for WAZ, LAZ, and head-circumference-for-age *z*-score (HCAZ) at early and established lactation; *2*) were associated with growth velocity (expressed as change per day from early to established lactation) for infant weight, length, and head circumference; and/or *3*) emerged as determinants of stunting (LAZ < −2 SD), underweight (WAZ < −2 SD), and low head circumference (HCAZ < −2 SD) at early and established lactation in breastfed infants before 6 mo of age.

Methods

Ethics, study site selection, and study design

Ethical approval for this study was obtained from the McGill University Institutional Review Board and the Center for Studies of Sensory Impairment, Aging and Metabolism (CeSSIAM) Human Subjects Committee in Guatemala. This longitudinal study goes beyond our earlier cross-sectional study (29) and aimed to undercover relations with infant growth using both static (attainment) and kinetic (velocity) parameters. For this study, indigenous *Mam*-Mayan mother-infant dyads (n = 140) were recruited during pregnancy (6–9 mo postpartum) as part of a larger study (28). Mothers were recruited with the support of *comadronas* (traditional midwives) and community healthcare workers (CHWs). All participating mothers provided written informed consent before participation. Exclusion criteria included any missing data due to loss of follow-up (n = 7), resulting in final samples of 140 mother-infant dyads during early lactation and 133 during established lactation.

Field studies were conducted from June 2012 through January 2013 in 8 rural *Mam*-speaking communities of the San Juan Ostuncalco region in Guatemala. Questionnaires, maternal and infant anthropometric measurements, and maternal breast milk, urine, and feces samples were collected at both early (before 6 wk) and established (4–6 mo) lactation. Subjects were informed of their own and child's results via the study's lead physician. In brief, participants were referred to the public health system whenever medically indicated. Laboratory results were communicated to participants directly, and treatment was provided free of charge for any diagnosed infection. Local midwives provided breastfeeding support. All data were locked in a secure office during the study collection period. Data were anonymized at study completion. Findings were shared with community partners at CeSSIAM. Details of the study site, design, and methods have been published previously (28).

SCM

A single unilateral breast milk sample was collected from each lactating mother at 2 time points, early and established lactation. Details of breast milk sample collection, transportation, storage, and analysis of

milk minerals, Na and K, have been described previously (29). In brief, a single milk sample, from the breast not recently used for feeding, was collected under the supervision of a trained *comadrona*. In this study, milk samples were analyzed in triplicate for Na and K using a Varian ICP-820MS (Analytik Jena) equipped with a Collision Reaction Interface including calibration standards, internal, and external controls as described previously (30).

Mothers were classified based on the presence or absence of SCM, defined using a cutoff of a sodium:potassium molar ratio (Na:K ratio) >0.6. This cutoff was established according to previously published categories in human milk: Na:K ratio ≤0.6 is considered normal; >0.6 to 1.0 is considered moderately elevated with subclinical inflammation; and >1.0 is considered highly elevated and indicative of severe SCM (31, 32). The ratio is advantageous because it permits the use of milk samples without consideration of time of sampling or time since the infant was last fed; it controls for the distribution of Na and K between aqueous and lipid phases of milk and declining concentrations of both Na and K throughout lactation (8). Researchers have also shown a <5% discordance in SCM between breasts (10). A higher milk Na:K ratio (continuous variable) was used as an indicator consistent with SCM.

Milk cytokines

The concentrations of 4 proinflammatory cytokines, IL-1β, IL-6, IL-8, and TNF-α, were measured using the MILLIPLEX MAP Human high-sensitivity T-cell magnetic bead panel (HSTCMAG-28SK; EMD Millipore) as previously described (30). SCM is a common, asymptomatic inflammatory condition, where cytokines are transiently released by mammary gland epithelial and immune cells into milk, enhancing permeability and altering milk composition (13, 14). Milk proinflammatory cytokines (continuous variables) were used as indicators of breast inflammation.

Questionnaires

Details of the questionnaire have been previously described (33). Briefly, the study questionnaire was developed in June 2011 and pilot tested for cultural appropriateness in 50 nonparticipant mothers with similar characteristics to the intended population. The following year, trained *comadronas* and CHWs administered the questionnaires orally in either Spanish or *Mam* to participants during a 30-min interview. Questionnaires were conducted at both early and established lactation. Information on sociodemographics (household assets, electricity), maternal factors (age, parity, age at first pregnancy), infant factors (sex, diarrhea in the past week), sanitation infrastructure (home faucet, toilet), and self-reported breastfeeding practices (feeding of colostrum, feeding initiation within 1 h postpartum, breastfeeding [exclusive/predominant, or mixed feeding] (21)) and breastfeeding frequency were recorded.

Maternal fecal and urine analysis

Maternal urine and stool sample collection and analyses were described previously (28). For this study, a single stool sample was analyzed using direct smear by an experienced laboratory technician. Presence of intestinal nematodes (*Ascaris*, *Trichuris*, hookworm) and both pathogenic (*Giardia* spp., *Entamoeba histolytica*, and *Entamoeba dispar*) and nonpathogenic (*Blastocystis hominis*, *Entamoeba coli*, *Endolimax nana*, and *Iodamoeba bütschlii*) protozoa were recorded. A pilot study using the more sensitive Kato Katz technique had detected no nematode infections in a sample of 30 women. An experienced laboratory technician analyzed the urine samples using dipstick analysis and urine microscopy to yield pyuria, defined as >5 neutrophils per high-power field of uncentrifuged urine (28).

Anthropometry

Infant recumbent length, weight, and head circumference were measured in duplicate as described previously (28). All infant measurements were collected during early and established lactation. Infant WAZ, LAZ, and HCAZ were calculated using WHO Growth Reference Standards (34) and WHO Anthro Software 3.1. In addition, growth velocity (expressed as change in infant growth per day) between early and established lactation was calculated for absolute weight (grams), length (centimeters), and head circumference (centimeters).

Statistical analyses

Data analyses were performed using SPSS version 22.0 (SPSS, Inc.). Prior to analyses, data were checked for normality of residuals using the Shapiro–Wilk test and homogeneity of variances using the Levene test and [ln(y)] transformed. Proinflammatory cytokines IL-1β, IL-6, IL-8, and TNF-α were natural log transformed to achieve normality. Nontransformed means and SDs, percentages (95% CI), or standardized β coefficients are reported in the tables.

Multivariate linear regression models were used to examine hypothesized associations of indicators of SCM and breast inflammation, fecal-oral contamination, and breastfeeding practices with growth attainment for WAZ, LAZ, and HCAZ at both stages of lactation and with growth velocity (expressed as change in infant growth per day) for weight, length, and head circumference from early to established lactation. With regards to sanitation infrastructure, a home faucet and toilet were individually explored in each of the statistical models and only those that entered the model and/or were significant were reported. To build anthropometric models at early lactation, only measurements at early lactation were entered. To build anthropometric models at established lactation and models for daily growth velocity, explanatory variables from both early and established lactation were included along with infants' early WAZ, LAZ, or HCAZ as appropriate. Forward stepwise regression was used to obtain variables for each linear model. Variables that were significant ($P < 0.05$) were selected and at each step, variables that increased the R^2 were added. A maximum number of predictor variables were included as determined by sample size. Highly correlated variables ($P > 0.07$, variance inflation factor > 10) were noted to avoid multicollinearity; all variables entering the models are included in tables. For all linear regression models, the Nagelkerke R^2 was reported.

Multiple logistic regression models were used to examine hypothesized associations of indicators of SCM and breast inflammation, fecal-oral contamination, and breastfeeding practices with stunting, underweight, and low head circumference. χ^2 and Fisher exact tests (categorical variables) and Student t tests (continuous variables) were used to compare variables between infants with normal anthropometric scores and those that were stunted, underweight, or had low head circumference. To build anthropometric models, only significant variables from univariate analysis at both stages of lactation (early and established) were included. Variables in the final model with $P \leq 0.05$ were considered significant.

TABLE 1 Characteristics of indigenous Guatemalan mother-infant dyads[1]

	Early lactation, <6 wk (n = 140)	Established lactation, 4–6 mo (n = 133)
Maternal factors		
Height, cm	146.5 ± 0.5	146.5 ± 0.5
Weight, kg	51.1 ± 0.7	50.5 ± 0.7
SCM (% yes)	27.1 (20.2, 35.2)	9.2 (5.2, 15.7)
Milk Na:K ratio	0.63 ± 0.05	0.43 ± 0.02
Milk composition[2]		
IL-1β, pg/mL	4.93 ± 3.95	1.21 ± 0.48
IL-6, pg/mL	5.17 ± 1.97	2.20 ± 0.58
IL-8, pg/mL	101.3 ± 35.8	82.0 ± 13.8
TNFα, pg/mL	6.26 ± 1.25	5.28 ± 1.04
Urine and stool exam[3]		
Urine pyuria (%)	41.5 (33.4, 50.1)	19.3 (13.0, 27.7)
Stool *Entamoeba coli* (%)	42.2 (33.7, 51.1)	38.7 (30.0, 48.2)
Stool *Blastocystis hominis* (%)	22.3 (15.8, 30.5)	19.8 (13.3, 28.4)
Stool *Endolimax nana* (%)	21.5 (15.1, 29.6)	18.9 (12.6, 27.4)
Stool *Iodamoeba bütschlii* (%)	20.7 (14.4, 28.7)	18.9 (12.6, 27.4)
Infant factors		
WAZ	−0.79 ± 0.10	−0.95 ± 0.10
Underweight[4] (%)	12.9 (8.4, 19.5)	15.8 (10.6, 22.9)
LAZ	−1.54 ± 0.11	−1.77 ± 0.11
Stunting[4] (%)	31.7 (24.5, 39.8)	40.6 (32.6, 49.1)
WLZ	0.57 ± 0.09	0.48 ± 0.08
HCAZ	−0.51 ± 0.13	−0.52 ± 0.13
Low head circumference[4] (%)	15.8 (10.7, 22.8)	11.5 (7.1, 18.0)
Household factors		
Own faucet for water (%)	81.4 (74.2, 87.0)	—
Toilet (%)	15.7 (10.6, 22.6)	—
Electricity (%)	81.4 (74.2, 87.0)	—

[1]Values are arithmetic means ± SDs or percentages (95% CI). HCAZ, head circumference-for-age *z*-score; LAZ, length-for-age *z*-score; SCM, subclinical mastitis; WAZ, weight-for-age *z*-score; WLZ, weight-for-length *z*-score.
[2]n = 121 for early lactation, n = 111 for established lactation.
[3]n = 121 for early lactation, n = 106 for established lactation.
[4]Underweight was defined as WAZ < −2 SD, stunting was defined as LAZ < −2 SD, and low head circumference was defined as HCAZ < −2 SD.

Results

Population characteristics

Mothers' average age was 24.2 ± 0.6 y, parity was 2.8 ± 0.2, and age at first pregnancy was 18 ± 0.2 y (**Table 1**). More than half of mothers (53%) reported that they initiated breastfeeding within 1 h of delivery, 92% reported feeding colostrum, and all stated they breastfed their infants on average 11.4 ± 0.4 times per day in early and 12.0 ± 0.4 times per day during established lactation. During early lactation 93.4% exclusively breastfed with only 6.6% offering complementary foods, but during established lactation 43.6% were offering complementary foods in addition to breast milk.

Two maternal health conditions were examined. SCM was present in 27.1% of mothers during early lactation whereas only 9.2% of these mothers had SCM during established lactation. As for maternal intestinal parasitic infections, mothers had a very low prevalence of *Ascaris lumbricoides* (2%), *Giardia* spp. (3%), and *Entamoeba histolytica* and *Entamoeba dispar* (both 1%), and no *Trichuris* or hookworm were detected; because of their low prevalence these infections were not further considered. In contrast, nonpathogenic intestinal protozoa (protist parasites) were more common (18.9–42.2%) in mothers: *Entamoeba coli*, *Blastocystis hominis*, *Endolimax nana*, and *Iodamoeba bütschlii*. Urine analysis showed that almost half (42%) of mothers had pyuria. Most mothers (81%) had electricity and a home faucet as a water source but few (16%) had a toilet.

Linear regression models for WAZ, LAZ, and HCAZ at early and established lactation

Growth attainment for WAZ and growth velocity for weight.

In early lactation, higher maternal weight was positively associated with WAZ (standardized $\beta = 0.293$, $P = 0.004$), whereas maternal stool *Endolimax nana* was negatively associated with WAZ (standardized $\beta = -0.080$, $P = 0.026$) before 6 wk (**Table 2**).

In established lactation, WAZ was positively associated with higher WAZ before 6 wk (standardized $\beta = 0.412$, $P < 0.0001$) and higher maternal height (standardized $\beta = 0.229$, $P = 0.025$) whereas maternal stool *Entamoeba coli* measured during established lactation was negatively associated with WAZ (standardized $\beta = -0.241$, $P = 0.010$) (Table 2).

The daily rate of increase in infant weight from early to established lactation was negatively associated with maternal stool *Entamoeba coli* measured during established lactation (standardized $\beta = -0.182$, $P = 0.047$) (Table 2).

TABLE 2 Multiple linear regression models associating indicators of SCM and breast inflammation, breastfeeding practices, and indicators of fecal-oral contamination with growth attainment for WAZ and growth velocity[1]

	Standardized β coefficient		*P*
Growth attainment: WAZ early lactation			
Maternal height, cm	0.023		0.82
Maternal weight, kg $_{early}$	0.293		0.004
Initiated breastfeeding <1 h (% yes)	0.132		0.15
Fed colostrum (% yes)	0.113		0.26
Na:K ratio $_{early}$	−0.126		0.20
Stool *Endolimax nana* $_{early}$ (% yes)	−0.080		0.026
R^2_{adj}		0.179	
Growth attainment: WAZ established lactation			
WAZ $_{early}$	0.412		<0.0001
Maternal height, cm	0.229		0.025
Maternal weight, kg $_{established}$	0.070		0.48
Fed colostrum (% yes)	0.070		0.47
Na:K ratio $_{early}$	0.186		0.05
Stool *Entamoeba coli* $_{established}$ (% yes)	−0.241		0.010
R^2_{adj}		0.303	
Growth velocity: rate of increase in infant weight, kg/d			
Maternal height, cm	0.087		0.38
Maternal weight, kg $_{established}$	0.130		0.18
Infant sex (% male)	−0.131		0.16
Stool *Entamoeba coli* $_{established}$ (% yes)	−0.182		0.047
R^2_{adj}		0.070	

[1]Linear models considering growth attainment for WAZ and growth velocity for weight as the dependent variables and the factors listed below as the independent variables. $n = 109$ for early lactation, $n = 106$ for established lactation, and $n = 116$ for growth velocity. SCM, subclinical mastitis; WAZ, weight-for-age *z*-score.

Growth attainment LAZ and growth velocity for length.

In early lactation, maternal urine pyuria was positively associated with LAZ (standardized $\beta = 0.263$, $P = 0.006$) whereas higher milk IL-1β was negatively associated with LAZ (standardized $\beta = -0.201$, $P = 0.040$) before 6 wk (**Table 3**).

In established lactation, LAZ was positively associated with higher LAZ before 6 wk (standardized $\beta = 0.276$, $P = 0.005$), higher maternal height (standardized $\beta = 0.223$, $P = 0.036$), higher milk Na:K ratio (standardized $\beta = 0.224$, $P = 0.021$) measured during early lactation, and having fed colostrum (standardized $\beta = 0.201$, $P = 0.042$) (Table 3).

The daily rate of increase in infant length from early to established lactation was negatively associated with higher infant length before 6 wk (standardized $\beta = -0.266$, $P = 0.006$) and positively associated with older maternal age at first pregnancy (standardized $\beta = 0.296$, $P = 0.002$), higher maternal weight (standardized $\beta = 0.283$, $P = 0.003$), and higher milk IL-1β measured during early lactation (standardized $\beta = 0.281$, $P = 0.010$) (Table 3).

Growth attainment for HCAZ and growth velocity for head circumference.

In early lactation, higher maternal weight (standardized $\beta = 0.206$, $P = 0.023$) and having fed colostrum (standardized $\beta = 0.260$, $P = 0.004$) were positively associated with HCAZ, whereas higher milk Na:K ratio was negatively associated with HCAZ (standardized $\beta = -0.185$, $P = 0.035$) before 6 wk (**Table 4**).

In established lactation, HCAZ was positively associated with higher HCAZ before 6 wk (standardized $\beta = 0.308$, $P = 0.002$) and higher milk IL-8 (standardized $\beta = 0.255$, $P = 0.014$), whereas higher breastfeeding frequency (standardized $\beta = -0.249$, $P = 0.012$) and maternal stool *Endolimax nana* measured during established lactation (standardized $\beta = -0.204$, $P = 0.035$) were negatively associated with HCAZ (Table 4).

The daily rate of increase in infant head circumference from early to established lactation was positively associated with maternal stool *Iodamoeba bütschlii* (standardized $\beta = 0.217$, $P = 0.033$) and negatively associated with higher infant head circumference (standardized $\beta = -0.396$, $P = <0.0001$), and higher breastfeeding frequency (standardized $\beta = -0.195$, $P = 0.040$) (Table 4).

Logistic regression models for underweight, stunting, and low head circumference at early and established lactation

Underweight (WAZ < −2 SD).

In early lactation, higher milk Na:K ratio was associated with increased odds (OR = 2.064, $P = 0.034$) of underweight, whereas in established lactation, maternal stool *Entamoeba coli* was associated with increased odds (OR = 5.094, $P = 0.045$) of underweight (WAZ < −2 SD) (**Table 5**).

Stunting (LAZ < −2 SD).

In early lactation, a higher milk Na:K ratio was associated with an increased odds (OR = 1.899, $P = 0.05$) of stunting whereas maternal urine pyuria was associated with decreased odds (OR = 0.312, $P = 0.010$) of stunting. Importantly, in established lactation, the presence of a toilet in the home was associated with decreased odds (OR = 0.053, $P = 0.003$) of stunting (Table 5).

TABLE 3 Multiple linear regression models associating indicators of SCM and breast inflammation, breastfeeding practices, and indicators of fecal-oral contamination with growth attainment for LAZ and growth velocity[1]

	Standardized β coefficient		*P*
Growth attainment: LAZ early lactation			
Maternal height, cm	0.058		0.58
Maternal weight, kg	0.113		0.28
Na:K ratio $_{early}$	−0.105		0.27
IL-1β, pg/mL $_{early}$	−0.201		0.040
Urine pyuria $_{early}$ (% yes)	0.263		0.006
Stool *Endolimax nana* $_{early}$ (% yes)	0.156		0.10
R^2_{adj}		0.150	
Growth attainment: LAZ established lactation			
LAZ $_{early}$	0.276		0.005
Maternal height, cm	0.223		0.036
Maternal weight, kg $_{established}$	0.198		0.05
Fed colostrum (% yes)	0.201		0.042
Na:K ratio $_{early}$	0.224		0.021
Urine pyuria $_{established}$ (% yes)	−0.113		0.23
R^2_{adj}		0.263	
Growth velocity: rate of increase in infant length, cm/d			
Infant length $_{early}$	−0.266		0.006
Age at first pregnancy, y	0.296		0.002
Maternal weight, kg $_{established}$	0.283		0.003
Na:K ratio $_{established}$	−0.045		0.62
IL-1β, pg/mL $_{early}$	0.281		0.010
TNFα, pg/mL $_{early}$	0.073		0.48
Urine pyuria $_{early}$ (% yes)	0.004		0.97
R^2_{adj}		0.269	

[1] Linear models considering growth attainment for LAZ and growth velocity for length as the dependent variables and the factors listed below as the independent variables. $n = 103$ for early lactation, $n = 106$ for established lactation, and $n = 97$ for growth velocity. LAZ, length-for-age *z*-score; SCM, subclinical mastitis.

Low head circumference (HCAZ < −2 SD).
In early lactation, having fed colostrum was associated with decreased odds (OR = 0.161, $P = 0.048$) of low head circumference. Hypothesized variables did not emerge in statistical modeling for low head circumference in established lactation (Table 5).

Discussion

Infant growth faltering is known to occur in breastfed infants. In a previous cross-sectional study in indigenous *Mam*-Mayan mothers in the Western Highlands of Guatemala that measured Na and K using inductively coupled plasma mass spectrometry, we showed that the presence of SCM was associated with increased odds of infant stunting, underweight, and low head circumference and that maternal nonpathogenic protozoa increased the likelihood of low head circumference (29). Previous studies, using flame atomic absorption spectroscopy to measure Na and K in milk, had reported SCM was associated with lower infant weight (8) and lower infant WAZ and LAZ at 6 wk postpartum (10, 11).

In this longitudinal study conducted in the same population—but with different mothers—we investigated if differences in exposure to SCM or nonpathogenic protozoa during early or established lactation impacted infant growth attainment at each stage of lactation or interval growth velocity from early to established lactation. Our results revealed several notable observations. First, a higher milk Na:K ratio in early lactation, consistent with the presence of SCM, was associated with increased odds of infants being underweight and stunted by 6 wk. Second, feeding colostrum was associated with improved linear and cranial growth and importantly protected against low head circumference (HCAZ < −2 SD) occurring before 6 wk. Third, indicators of maternal fecal-oral contamination showed that the presence of a toilet lowered the odds of stunting at 4–6 mo. However, our findings identified that 2 putative nonpathogenic protozoa, *Endolimax nana* and *Entamoeba coli*, negatively impacted WAZ attainment by 6 wk and 4–6 mo, respectively. Moreover, *Entamoeba coli* was associated with lowered growth velocity and with increased odds of the infant being underweight at 4–6 mo. In contrast, *Iodamoeba bütschlii* was associated with increased cranial growth velocity, further demonstrating differential responses of infant anthropometric indices to protozoa. Collectively these results provide important evidence that 2 largely understudied maternal conditions, SCM and putative nonpathogenic protozoa, can compromise growth of breastfed infants before 6 mo of age.

Weight and underweight

This longitudinal study showed that 2 factors, namely nonpathogenic protozoans and a higher milk Na:K ratio, had negative impacts on infant weight. Three explanations for a negative association of nonpathogenic protozoans with infant weight are possible. First, it is possible that in addition to breast milk, mothers fed their infants with milk powder mixed with contaminated water. If this were the case, water infected

TABLE 4 Multiple linear regression models associating indicators of SCM and breast inflammation, breastfeeding practices, and indicators of fecal-oral contamination with growth attainment for HCAZ and growth velocity[1]

	Standardized β coefficient		*P*
Growth attainment: HCAZ early lactation			
Maternal height, cm	0.155		0.09
Maternal weight, kg $_{early}$	0.206		0.023
Fed colostrum (% yes)	0.260		0.004
Na:K ratio $_{early}$	−0.185		0.035
R^2_{adj}		0.185	
Growth attainment: HCAZ established lactation			
HCAZ $_{early}$	0.308		0.002
Maternal height (cm)	0.177		0.08
Breastfeeding frequency (#/24 h) $_{established}$	−0.249		0.012
IL-8, pg/mL $_{established}$	0.255		0.014
Stool *Endolimax nana* $_{established}$ (% yes)	−0.204		0.035
Own faucet (% yes)	−0.114		0.25
R^2_{adj}		0.241	
Growth velocity: rate of increase in infant head circumference, cm/d			
Infant head circumference, cm $_{early}$	−0.396		<0.0001
Breastfeeding frequency (#/24 h) $_{established}$	−0.195		0.040
Na:K ratio $_{early}$	0.094		0.37
IL-8, pg/mL $_{established}$	0.098		0.43
TNFα, pg/mL $_{established}$	0.073		0.54
Stool *Iodamoeba bütschlii* $_{early}$ (% yes)	0.217		0.033
Stool *Endolimax nana* $_{established}$ (% yes)	−0.097		0.31
R^2_{adj}		0.338	

[1] Linear models considering growth attainment for HCAZ and growth velocity for head circumference as the dependent variables and the factors listed below as the independent variables. $n = 132$ for early lactation, $n = 106$ for established, and $n = 80$ for growth velocity. HCAZ, head circumference-for-age *z*-score; SCM, subclinical mastitis.

by animal or human excreta could transmit both nonpathogenic and pathogenic microbes (35). However, this is not the case because almost all mothers in this study exclusively breastfed their infants and predominantly breastfed infants were only given *agüitas* made with boiled water. Second, mothers and infants are living in the same environment and therefore it is possible that the breastfed infant can also harbor or be infected with the same or similar nonpathogenic protozoans as the mother. This study did not evaluate the stool of infants, though previous work in this population during only established lactation found that approximately one-third of infants had fecal leukocytosis, indicative of chronic exposure to fecal contamination (28). However, work from Mexico in older children found that intestinal protozoa, particularly *Entamoeba coli*, were associated with a higher percentage of body fat and food intake (36, 37) suggesting the insult of fecal-oral contamination can be modified by child age. Third, it is conceivable that mothers with protist parasites might influence their infants' microbiome and affect growth. Research now proposes that the maternal microbiome is intergenerational, perpetuating growth impairments into successive generations with gut microbial translocation during pregnancy and/or via the "milk–microbiota" interaction (38). It has been estimated that 25–30% of the infant bacterial microbiota originates from breast milk, highlighting an essential role for milk in a normal infant microbiota ecosystem (39). The relation to poor infant weight could also be the result of other factors such as pathogenic protozoans; however, our study found rates of <3% for *Giardia* spp., *Entamoeba histolytica*, and *Entamoeba dispar* and therefore these were likely not a factor in our study.

Our study also found that a higher milk Na:K ratio, consistent with SCM, increased the odds of being underweight in early lactation. This finding confirms previous work in Bangledesh (8), Zimbabwe (9), Zambia (10, 11), and most recently Europe (13), where SCM has been associated with reduced infant weight. On the one hand, SCM might lower milk volume, although previous research found that milk intake among infants did not differ for mothers with SCM (12). On the other hand, SCM has been shown to alter milk nutrient (13, 15) composition and younger infants might thus be more susceptible to these compositional changes.

Linear growth and stunting

In contrast to weight, nonpathogenic protozoans did not emerge in statistical models for linear growth. Rather, indicators consistent with SCM and breast inflammation, the protective nature of sanitation, and breastfeeding practices were observed. In early lactation, higher milk IL-1β was negatively associated with LAZ attainment. Previously, a murine study associated higher IL-1β in maternal serum with reduced neonatal linear growth (40). It has been proposed that higher IL-1β acts on growth plate cartilage to induce growth-suppressive effects by disrupting the insulin-like growth factor axis (41, 42). In comparison with early lactation, higher milk IL-1β was positively associated with infant length velocity, which does not align with the prevailing theory that

TABLE 5 Multiple logistic regression models associating indicators of SCM and breast inflammation, breastfeeding practices, and indicators of fecal-oral contamination with infant stunting, underweight, and low head circumference[1]

	Early lactation			Established lactation		
	Unadjusted OR	OR	*P*	Unadjusted OR	OR	*P*
Underweight				Underweight		
Na:K ratio $_{early}$	2.237	2.064	0.034	WAZ $_{early}$	0.389	0.353	0.009
Age at first pregnancy, y	0.79	0.807	0.11	Maternal height, cm	0.866	0.840	0.011
				Na:K ratio $_{early}$	1.799	0.654	0.52
				Entamoeba coli $_{established}$ (% yes)	3.172	5.094	0.045
				Iodamoeba bütschlii $_{established}$ (%, yes)	3.257	1.447	0.66
Stunting				Stunting		
Maternal height, cm	0.902	0.895	0.007	LAZ $_{early}$	0.451	0.402	<0.0001
Na:K ratio $_{early}$	1.853	1.899	0.05	Maternal height, cm	0.891	0.893	0.028
Urine pyuria $_{early}$ (% yes)	0.352	0.312	0.010	Maternal weight, kg $_{established}$	0.921	0.921	0.009
Electricity (% yes)	4.119	3.163	0.09	Infant sex (% male)	2.647	8.333	<0.0001
				Toilet (% yes)	0.151	0.053	0.003
Low head circumference				Low head circumference		
Age at first pregnancy, y	0.805	0.793	0.10	HCAZ $_{early}$	0.721	0.793	0.35
Maternal height, cm	0.909	0.887	0.023	Maternal height, cm	0.898	0.841	0.019
Fed colostrum (% yes)	0.243	0.161	0.048	Na:K ratio $_{early}$	1.796	1.370	0.53
Na:K ratio $_{early}$	2.511	1.913	0.10				

[1]Early lactation: $n = 115$ for stunting (LAZ < −2 SD) and $n = 133$ for underweight (WAZ < −2 SD) and low head circumference (HCAZ < −2 SD). Established lactation: $n = 131$ for stunting, $n = 101$ for underweight, and $n = 104$ for low head circumference. All unadjusted OR values have $P \leq 0.05$. HCAZ, head circumference-for-age *z*-score; LAZ, length-for-age *z*-score; SCM, subclinical mastitis; WAZ, weight-for-age *z*-score.

proinflammatory cytokines limit growth during lactation. This was similar to our unexpected study finding that maternal pyuria was protective against stunting in early lactation. However, it may be that the accumulation of cytokines in milk and leukocytes in urine are important defense mechanisms against pathogens, and that mothers who are therefore able to effectively mount immunological responses against pathogens could have infants with "better" growth compared with mothers less able to fight infection.

A higher milk Na:K ratio was associated with increased odds of stunting in early lactation. This parallels our previous research findings among these indigenous communities (29) and is similar to work in Zambia where milk Na:K ratio was negatively associated with LAZ (10, 11). However, the negative association between a higher milk Na:K ratio with early LAZ attainment was reversed in established lactation, suggesting the importance of considering stage of lactation. It could be hypothesized that infants require a higher concentration of Na as lactation progresses, and research in these communities has suggested an inadequacy of Na in infants' breast milk intake from mothers without SCM (14). Future studies examining this relation should build upon this research by taking into account lactation stages.

This study showed that having fed colostrum was positively associated with LAZ attainment in established lactation. The importance of feeding colostrum is well established (43). Interestingly, a recent study supports the preventative nature of human colostrum against parasitic infections, stating that phagocytosis of *Giardia lamblia* by macrophages in milk is one of the defense mechanisms that can eliminate this parasite (44). Despite our study having low rates of *G. lamblia* and other pathogenic organisms, it could be that feeding colostrum has a long-lasting benefit that outweighs the harmful impact of both parasitic and nonpathogenic protozoans, although further research is needed.

The protective nature of sanitation was evident in our study because having a toilet at the home was associated with decreased odds of stunting. It is long believed that the presence of toilets could reduce fecal contact and its adverse effects on growth (45) and thus global efforts should continue to accelerate provision of toilets to people who currently lack them as a modifiable public health intervention. In our study, the absence of a home faucet (20%) was not associated with a known benefit, whereas the presence of a toilet (16%) did decrease the odds of stunting. Unfortunately, this study did not collect water samples from the home, which limits the understanding of how the detection of nonpathogenic protozoa in maternal stool might be a surrogate marker for poor hygiene and/or lack of access to clean water.

Cranial growth and low head circumference

Interestingly, indicators consistent with SCM and breast inflammation, breastfeeding practices, and fecal-oral contamination emerged in statistical models for cranial growth. To begin, this study showed that indicators consistent with SCM and breast inflammation did not act in the same manner toward cranial growth. On the one hand, a higher milk Na:K ratio was negatively associated with HCAZ attainment in early lactation. This finding is similar to previous research in Guatemala (29) and globally, which has shown a negative trend between SCM and head circumference (12, 13). On the other hand, higher milk IL-8 was positively associated with HCAZ attainment in established lactation. Proinflammatory cytokines are usually considered neurotoxic (46), but there is emerging evidence that some can be neuroprotective (47). It is known

that peripheral cytokines cross the blood–brain barrier (BBB) (48) and that transendothelial migration of neutrophils across the BBB uses IL-8 as a chemoattractant (49). Also, microglial cells in the postnatal brain, when activated by proinflammatory cytokines, play a role in normal brain development (50). These findings support a potential biological basis for the neuroprotective role of specific proinflammatory cytokines, but more studies are warranted.

Importantly, having fed colostrum was both positively associated with HCAZ attainment and associated with decreased odds of low head circumference in early lactation. The direct relation between colostrum and brain development is unknown but could be due to the direct benefit of nutrients and immunoglobulins on growth. It could also be due to the indirect effect of early initiation of breastfeeding to improve overall infant health with maternal transfer of passive immunity associated with critical early life protection from infections (43). In comparison, the present study reported that higher breastfeeding frequency was negatively associated with HCAZ attainment in established lactation and infant head circumference velocity. This aligns with our previous cross-sectional study finding that suggested higher feeding frequency was associated with decreased odds of low head circumference (29). On the one hand, it is not illogical to think that this longitudinal study finding could be in part a natural response of mothers to want to feed their infants more frequently to compensate for their smaller head size. Alternatively, it could be that when our previous cross-sectional study pooled infant head circumference results from both early and established lactation, we were unable to tease out with precision as this study has done.

In this study, maternal stool *Endolimax nana* was negatively associated with HCAZ attainment in established lactation. This aligns with our previous cross-sectional study, which found that maternal *Entamoeba coli* was associated with increased odds of low head circumference (29). Defining a specific mechanistic role in the pathogenesis of nutritional impairment is challenging. Despite the fact that protozoa in this study are ubiquitous environmental organisms and generally considered nonpathogenic, this study did show that *Endolimax nana* and *Entamoeba coli* negatively impacted WAZ attainment by 6 wk and 4–6 mo, respectively, that *Entamoeba coli* was associated with lowered growth velocity and with increased odds of the infant being underweight at 4–6 mo, but that *Iodamoeba bütschlii* was associated with increased cranial growth velocity.

For others, the clinical significance of some so-called "nonpathogenic" intestinal protozoa remains unresolved as prior exposure, parasite load, and genetic variability as factors modifying clinical presentation (51). For example, in Zambian school-age children, there was a positive association between diarrhea and *Endolimax* infections (52). Chronic episodes of diarrhea could underscore growth faltering (53); however, in our study, diarrhea did not emerge as a factor associated with poor growth. Nonetheless, these findings suggest the need to rethink the approach of classifying protozoa as either pathogenic or nonpathogenic.

In contrast to *Endolimax nana*, this study found maternal *Iodamoeba bütschlii* was positively associated with infant head circumference velocity. This latter observation raises the possibility that *Iodamoeba bütschlii* might have unique properties with consequences to the infant brain, but this has not been studied. It is known that gut microbiota can play a regulatory role in neurodevelopment during the first 1000 d (38). Therefore, it is plausible that *Iodamoeba bütschlii* or other nonpathogenic protozoa might influence gut-brain microbial cross-talk. In fact, child cohort studies from LMICs have reported that enteric infection is predictive of cognitive delay later in childhood (54) but inflammatory markers of an environmental enteric disorder have demonstrated positive associations with neurodevelopment (55). Given that reducing intestinal pathogens could influence gut microbiota dynamics, the microbiome-gut-brain axis should be considered further in this context.

Strengths and limitations

Despite the identification of SCM and nonpathogenic protozoa as modifiers of infant growth, we acknowledge the following study limitations. First, anthropometry was measured once during early lactation and once during established lactation; more anthropometric measurements of infant growth patterns would have permitted better modeling of infant growth patterns. Second, breastfeeding practices were self-reported and therefore could have been subject to recall bias. Third, only maternal and not infant stool samples were collected, which limited our assessment of the direct understanding of the impact of fecal-oral contamination on growth. Fourth, we did not use Kato Katz technique to measure nematodes in maternal stool samples and measured protozoan infections using a direct smear, which was the focus of our study. Moreover, our findings do not suggest a causal connection between the presence of these protozoa and growth but suggest they may be possible surrogate markers. Lastly, we cannot rule out that other maternal and/or infant factors not measured might have contributed to differences in early infant growth.

Conclusion

Our investigation in the Western Highlands of Guatemala has highlighted that several maternal nonpathogenic protozoa impair infant weight throughout the first 6 mo postpartum. Likewise, a higher milk Na:K ratio in early lactation contributed to infant underweight and stunting by 6 wk. In contrast, cytokines in milk were unexpectedly positively associated with linear velocity and cranial attainment, confirming that more attention needs to be given to the inflammation-growth paradigm as it relates to milk composition. Similarly, feeding colostrum had a protective effect on infant head circumference and linear attainment and the protective nature of having a toilet at one's home was observed, which could interrupt one pathway by which fecal-oral contamination is spread. Finally, these findings expand the paradigm for early infant growth faltering from a focus on dietary interventions to include the contribution of SCM and breast inflammation, maternal fecal-oral contamination, and breastfeeding practices on infant growth faltering.

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