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Minerals and Trace Elements in Human Breast Milk Are Associated with Guatemalan Infant Anthropometric Outcomes within the First 6 Months[1–3]

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Abstract

Background: Breast milk is the recommended source of nutrients for infant growth, but its adequacy to meet infants' mineral and trace element needs is unknown.

Objectives: We used breast-milk mineral and trace element concentrations of Guatemalan mothers at 3 lactation stages to estimate total daily intakes and to determine whether intakes were associated with early infant growth.

Methods: In this cross-sectional study, breast-milk samples were collected from *Mam*-Mayan mothers during transitional (5–17 d, $n = 56$), early (18–46 d, $n = 75$), and established (4–6 mo, $n = 103$) lactation; *z* scores for weight (WAZ), length (LAZ), and head circumference (HCAZ) were measured. Concentrations of 11 minerals (calcium, potassium, magnesium, sodium, copper, iron, manganese, rubidium, selenium, strontium, and zinc) were analyzed by inductively coupled plasma-mass spectrometry (ICP-MS). WHO equations were used to calculate the estimated energy requirement, which was divided by the energy density of breast milk to estimate daily milk volume, and this number was multiplied by breast-milk mineral concentrations to estimate intakes. Principal component analyses identified clusters of minerals; principal components (PCs) were used in regression analyses for anthropometric outcomes.

Results: Estimated breast-milk intakes during established lactation were insufficient to compensate for the lower milk sodium, copper, manganese, and zinc concentrations in male infants and the lower sodium, iron and manganese concentrations in female infants. Estimated intakes of calcium, magnesium, potassium, sodium, and selenium were below the Institute of Medicine Adequate Intake for both sexes at all 3 stages of lactation. In early lactation, multiple linear regressions showed that PC1 (calcium, magnesium, potassium, rubidium, and strontium intakes) was positively associated with WAZ, LAZ, and HCAZ. In established lactation, the same PC with sodium added was positively associated with all 3 anthropometric outcomes; a second PC (PC2: zinc, copper, and selenium intakes) was associated with WAZ and LAZ but not HCAZ.

Conclusions: Breast milk may be inadequate in selected minerals and trace elements where higher estimated intakes were associated with greater infant growth. *J Nutr* 2016;146:2067–74.

Keywords: adequate intakes, human breast milk, infant anthropometry, minerals and trace element concentrations, stage of lactation

Introduction

Human breast milk is regarded as the best source of nutrition for newborn infants (1). Both quality and quantity remain highly conserved because maternal nutritional status has a minimal impact on the macronutrient composition and the total energy content (2). Similarly, there is considerable evidence that breast-milk concentrations of minerals such as calcium and magnesium and trace elements such as copper, iron, and zinc are not affected by maternal diet or nutritional status. They are tightly controlled by homeostatic mechanisms at the level of the mammary epithelial cells (3–6). The exception is selenium; milk selenium concentration is closely related to maternal selenium status (5).

Several studies have examined mineral and trace element concentrations in human breast milk using inductively coupled

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plasma-atomic emission spectrometry (ICP-AES)[8] (7) and, more recently, ICP-MS (8, 9). There is a decline in the concentrations of many elements across the stages of lactation. Reported declines include copper and zinc in Guatemala (10), calcium, magnesium, copper, iron, and zinc by 45 d postpartum in the United States (11, 12), copper, manganese, selenium, and zinc in Austria (13), and sodium, potassium, selenium, and zinc in Japan (7). It has been suggested that this decline in mineral concentrations might reflect lower infant needs for growth (14, 15).

Few studies, to our knowledge, have determined whether breastfeeding infants consume adequate intakes of minerals and trace elements to meet their needs for growth. One study showed that exclusive breastfeeding protected infants from iron deficiency and iron deficiency anemia during the first 4 mo (16), and a review on milk iron and copper stated that there was no need for extra iron or copper in addition to quantities provided by milk for full-term breastfed infants in the first 6 mo (17); the adequacy of other elements in breast milk is not known.

The purpose of the present study was to examine the breast-milk mineral and trace element concentrations of *Mam*-Mayan Guatemalan mothers at 3 stages of lactation (transitional, early, and established) in exclusively and predominantly breastfed infants and to assess the adequacy of breastfed infants' daily intake of minerals and trace elements when breast milk was the sole or predominant source of nutrition. We used breast-milk minerals and trace element concentrations to estimate intakes in infants during transitional, early, and established lactation to determine whether estimated intakes were associated with infant anthropometry at each stage of lactation.

Methods

Study design. In this cross-sectional study, collected milk samples were categorized into 3 lactation stages (18): transitional (5–17 d postpartum, n = 56), early (18–46 d postpartum, n = 75), and established (4–6 mo postpartum, n = 103). Breast-milk mineral and trace element concentrations and their percent of estimated intake divided by Adequate Intake (AI) were compared across the 3 lactation stages. Using principal component analysis (PCA) to control for multicollinearity, we entered individual PCs as independent variables into multiple linear regression models at each stage of lactation to explore associations between estimated breast-milk mineral and trace element intakes and infant anthropometric measurements.

Study site and participants. This study was part of a collaboration between McGill University (Montreal, Canada) and the Center for Studies of Sensory Impairment, Aging, and Metabolism (CeSSIAM; Guatemala City, Guatemala). Field studies were conducted from June 2012 through January 2013 in 8 rural *Mam*-Mayan communities in the San Juan Ostuncalco region of Guatemala (19). Ethical approval was obtained from the McGill University Institutional Review Board and the CeSSIAM Human Subjects Committee.

Lactating mothers of infants aged 5–46 d postpartum and 4–6 mo postpartum were identified and invited by community health workers to participate. All of the mothers provided written informed consent for participation. Mothers whose milk sodium:potassium ratio was >0.6 were excluded because an elevated sodium:potassium ratio indicative of subclinical mastitis (20). Only infants who were exclusively or predominantly breastfed, as defined by WHO, were included (21). Feeding category was defined as exclusive if mothers reported not having fed their infant anything other than breast milk since birth or predominant if mothers had fed their infants ritual fluids (*agüitas)* (22). The types of *agüitas* were boiled water, chamomile tea, sugar water, and corn-dough water.

Breast-milk sample collection. Before milk sample collection, the nipples and areolas of the breasts were cleaned with 70% ethyl alcohol. Breast milk was collected in the morning from the breast not recently used for breastfeeding via full manual expression by a trained midwife. Milk was collected into 60-mL plastic vials and immediately stored on ice. Samples were partitioned into 15-mL tubes and stored at −30°C at the field laboratory before transfer on dry ice to McGill University in 2 separate shipments that were stored at −80°C until analysis 6–24 mo later.

Anthropometric measurements. Maternal weight and height and infant recumbent length, weight, and head circumference were measured as described previously (22). Infant weight-for-age z score (WAZ), length-for-age z score (LAZ), and head circumference-for-age z score (HCAZ) were calculated according to WHO Growth Reference Standards (23) through use of WHO Anthro software version 3.1.

Biochemical analyses. All of the elements were quantified on a Varian ICP-820MS (Analytik Jena) equipped with a collision reaction interface by use of PlasmaCAL Calibration Standards (SCP Science), commercial milk (2% partly skimmed, Dairyland) as the internal quality control, and biological reference QM-S-Q131, QM-S-Q1104, and QM-U-Q1306 (Institut National de Santé Publique du Québec) as external quality controls.

The breast-milk samples arrived in Montreal in 2 shipments. For the first shipment, homogenized milk samples were digested in acid-rinsed glass Pyrex tubes (Corning) using trace metal–grade concentrated nitric acid (70%, Instra-Analyzed Reagent, JT Baker) for 5 h at 125°C in triplicate. Given our concern that the high background concentrations of lead and nickel may interfere with the mineral analysis, the procedure was adjusted for the second shipment. Overnight digestion was completed in plastic DigiTUBEs (SCP Science) through use of trace metal–grade concentrated nitric acid followed by a 3-h reaction with trace metal–grade hydrogen peroxide (30%, Ultrex II, JT Baker), followed by heating at 90°C for 3 h. A comparison of these 2 methods with the use of commercial milk showed no difference in mineral concentrations between the 2 digestion processes (data not shown). Nevertheless, the "shipment" was controlled for in the statistical analyses.

The limits of detection (LOD) for each of the 11 elements, measured on 8 replicates of the lowest calibration standard, were calcium, 1.505 μg/L; copper, 0.396 μg/L; iron, 1.34 μg/L; magnesium, 0.232 μg/L; manganese, 0.005 μg/L; potassium, 4.887 μg/L; rubidium, 0.032 μg/L; selenium, 0.007 μg/L; sodium, 1.816 μg/L; strontium, 0.026 μg/L; and zinc 0.116 μg/L. In the 7 samples in which the manganese concentration was less than the LOD, the manganese concentration was replaced by half of the LOD values of the instrument for statistical analysis (24).

Estimation of daily intake and adequacy of minerals and trace elements. We used a 4-step process that assumed that milk production was driven by infants' energy demands and that the infants consumed the amount of milk needed to meet their daily estimated energy requirement (EER) for age and sex (25). First, infant estimated total energy expenditure (TEE) was calculated using the following equation: TEE = 152 × 92.8 [infant weight (kg)]. Second, EER was calculated as the sum of TEE plus the Food and Agriculture Organization/United National University/World Health Organization energy deposition values for breastfed infants by sex and age in months (26). Third, EER was divided by the assumed energy density of milk (0.67 kcal/mL) to estimate the daily volume of milk consumed (25). Finally, the measured breast-milk mineral concentrations were multiplied by the milk volume to obtain the estimated total daily mineral and trace element intakes. These were compared with the Institute of Medicine AI values (27).

[8] Abbreviations used: AI, adequate intake; EER, estimated energy requirement; HCAZ, head circumference-for-age z score; ICP-MS, inductively coupled plasma MS; LAZ, length-for-age z score; PC, principal component; PCA, principal component analysis; WAZ, weight-for-age z score.

Statistical analysis. Data analyses were performed using SPSS version 22.0 (IBM SPSS Statistics). Significance was set at $P < 0.05$, unless a Bonferroni correction for multiple testing was specified. Before perfoming the analyses, we identified and excluded the outliers using the outlier-labeling rule. Data were checked for normality of residuals using the Shapiro-Wilk test and for homogeneity of variances using Levene's test and [ln(y)] transformed, when necessary. Nontransformed means and SDs are reported in the tables.

One-factor ANCOVAs were performed to compare milk mineral concentrations and infants' estimated daily milk and mineral intakes among lactation stages followed by post-hoc tests; $P < 0.0045$ (Bonferroni corrected) was considered statistically significant. "Shipment" and "feeding category" were entered as covariates. A 2-factor ANOVA (3 × 2) was used to compare the percentage of AIs (ratio of estimated intake divided by AI) by the main effects of the stage of lactation and sex and their interaction. A Bonferroni-corrected $P < 0.0056$ was considered statistically significant.

The multicollinearity of mineral concentrations and intakes was determined by Spearman correlation where $\rho > 0.4$, and $P < 0.00091$ (Bonferroni corrected) were considered statistically significant. To correct for multicollinearity, PCA was applied to transform the highly correlated minerals and trace elements into new sets of linearly uncorrelated variables, PCs. The PCs were then entered as independent variables into multiple linear regression models to assess the association of these PCs (clusters of minerals) with each infant's anthropometric *z* score (dependent variables WAZ, LAZ, and HCAZ) at each of the 3 stages of lactation. Each model controlled for infant sex, maternal height, and shipment.

Results

Population characteristics. There were no differences by stage of lactation for population characteristics, proportion of exclusive and predominant breastfeeding, and infant anthropometry (**Table 1**).

Concentrations and estimated intakes by stage of lactation. **Table 2** shows that concentrations of 9 of 11 minerals and trace elements differed by stage of lactation. Potassium, sodium, copper, and zinc were progressively lower from transitional to early to established lactation. Iron, rubidium, and selenium concentrations were higher during transitional and early lactation, whereas magnesium was higher during established lactation. Manganese was higher in transitional than in established lactation. Calcium and strontium concentrations did not differ by stage of lactation. Several minerals were positively correlated: calcium-magnesium and iron-manganese in all stages of lactation; calcium-strontium in transitional and established lactation; sodium-copper-potassium-selenium in early lactation; sodium-potassium, sodium-selenium, and copper-selenium in established lactation ($\rho > 0.40$, $P < 0.00091$; **Supplemental Table 1**).

Estimated daily milk volume based on estimated energy intakes as well as estimated mineral and trace element intakes for male and female infants are shown in **Table 3**. In male and female infants, estimated milk intake was higher during established than during transitional lactation, but there were sex differences in intakes across lactation stages. In both sexes, estimated calcium intakes were higher during established lactation than during transitional lactation, zinc was lower in established lactation than in either transitional or early lactation, and there were no differences in potassium, manganese, rubidium, and selenium by stage of lactation; however, sex differences were noted for sodium, copper, iron, and strontium. Sodium intakes were lower in established lactation in male infants, whereas sodium intake did not differ by stage of lactation in female infants. A similar pattern was evident for iron intakes. Finally, for magnesium, copper, and strontium, sex differences were found in the pattern of decline or increase across the stages of lactation.

TABLE 1 Characteristics of Guatemalan mother-infant dyads participating in the study[1]

	Stage of lactation		
	Transitional, 5–17 d	Early, 18–46 d	Established, 4–6 mo
Maternal factors			
Age, y	24.4 ± 6.2	24.4 ± 7.6	23.7 ± 6.1
Height, cm	146 ± 6	147 ± 5	147 ± 6
Weight, kg	51.0 ± 8.4	51.1 ± 7.7	50.9 ± 8.9
Parity	3.1 ± 2.1	2.8 ± 2.3	2.7 ± 2.1
Feeding category[2]			
Exclusively breastfed, %	71.4 (58.5, 81.6)	56.0 (44.8, 66.7)	66.0 (56.4, 74.4)
Predominantly breastfed, %	28.6 (18.4, 41.5)	44.0 (33.3, 55.3)	34.0 (25.6, 43.6)
Infant factors			
Male sex, %	55.4 (42.4, 67.6)	65.3 (54.1, 75.1)	51.5 (41.9, 60.9)
WAZ	−0.65 ± 0.93	−0.73 ± 1.10	−0.96 ± 1.16
Underweight,[3] %	7.1 (2.8, 17.0)	12.0 (6.4, 21.3)	15.5 (9.8, 23.8)
LAZ	−1.53 ± 1.06	−1.69 ± 1.09	−1.91 ± 1.40
Stunting,[3] %	30.4 (19.9, 43.3)	37.3 (27.3, 48.6)	44.7 (35.4, 54.3)
WLZ	0.72 ± 1.10	0.96 ± 1.20	0.62 ± 1.06
HCAZ	−0.57 ± 1.41	−0.45 ± 1.19	−0.92 ± 1.53
Delayed cranial growth,[3] %	16.7 (9.0, 28.7)	10.7 (5.5, 19.7)	20.6 (13.8, 29.7)

[1] Values are means ± SDs or 95% CIs for percentages. *n* = 56, 75, and 103 for transitional, early, and established stages of lactation, respectively. ANOVA showed no significant differences in feeding category, maternal factors, and infant factors among stages of lactation. Mother-infant dyads were excluded from this study if the mother's milk had a sodium:potassium ratio >0.6 and/or the infant consumed a mixed diet. HCAZ, head circumference–for-age *z* score; LAZ, length-for-age *z* score; WAZ, weight-for-age *z* score; WLZ, weight-for-length *z* score.

[2] Predominantly breastfed infants in Guatemala consumed only *agüitas* in addition to milk. The types of *agüitas* used included boiled water, chamomile tea, sugar water, and corn-dough water.

[3] Underweight was defined as WAZ <−2 SD, stunting was defined as LAZ <−2 SD, and delayed cranial growth was defined as HCAZ <−2 SD.

TABLE 2 Guatemalan mothers' breast milk mineral and trace element concentrations at 3 stages of lactation[1]

	Stage of lactation			
	Transitional, 5–17 d	Early, 18–46 d	Established, 4–6 mo	P
Minerals,[2] mmol/L				
Calcium	6.56 ± 1.22	6.64 ± 0.90	6.37 ± 0.84	0.15
Magnesium	0.93 ± 0.18^{b}	0.93 ± 0.16^{b}	1.45 ± 0.22^{a}	<0.0001*
Potassium	15.1 ± 2.1^{a}	13.4 ± 1.8^{b}	11.0 ± 1.4^{c}	<0.0001*
Sodium	6.35 ± 1.44^{a}	5.41 ± 1.17^{b}	4.17 ± 0.96^{c}	<0.0001*
Trace elements,[2] μmol/L				
Copper	9.24 ± 1.81^{a}	7.24 ± 1.51^{b}	4.13 ± 1.25^{c}	<0.0001*
Iron	10.0 ± 7.9^{a}	10.4 ± 8.0^{a}	5.73 ± 4.48^{b}	<0.0001*
Manganese	0.21 ± 0.15^{a}	$0.20 \pm 0.17^{a,b}$	0.14 ± 0.13^{b}	0.002*
Rubidium	13.1 ± 2.9^{a}	12.3 ± 2.7^{a}	9.48 ± 2.22^{b}	<0.0001*
Selenium	0.21 ± 0.06^{a}	0.21 ± 0.08^{a}	0.17 ± 0.07^{b}	<0.0001*
Strontium	0.50 ± 0.16	0.53 ± 0.18	0.52 ± 0.16	0.59
Zinc	66.7 ± 14.4^{a}	53.0 ± 17.7^{b}	20.0 ± 11.9^{c}	<0.0001*

[1] Values are arithmetic means ± SDs. n = 55, 73, and 100 for transitional, early, and established stages of lactation, respectively. Labeled means in a row without a common superscript letter differ, $P < 0.05$. Shipment was entered as a covariate into the model. *Significance at Bonferroni corrected probability = 0.05 ÷ 11 = 0.0045.

[2] All minerals except calcium were log transformed [ln(y)] to achieve normality during ANCOVA.

At all 3 stages of lactation correlations between mineral and trace element intakes also were positive: calcium-magnesium, iron-manganese, potassium-sodium, potassium-copper, calcium-strontium, and potassium-rubidium (ρ > 0.4, $P < 0.00091$; **Supplemental Table 2**).

Prevalences and percent of AIs (estimated intake/AI). The prevalence of inadequacy was common (**Supplemental Table 3**). For minerals, <15% of infants reached adequate intakes for calcium, magnesium, potassium, and sodium, with the exception of male infants for calcium and magnesium in established lactation. For trace elements, <10% met AI for selenium at any stage of lactation. The proportion of infants with adequate copper and zinc intakes during established lactation was much lower than during transitional and early lactation. The prevalence of iron adequacy ranged from 21.6% to 45.7% across the 3 stages of lactation, and the prevalence of manganese adequacy ranged from 54.0% to 82% (Supplemental Table 3).

The comparison of the means for percent of AIs for each mineral or trace element by infant sex at each stage of lactation is presented in **Table 4**. The percent of recommended AI differed by stage of lactation for calcium, magnesium, copper, and zinc and by infant sex for calcium, magnesium, potassium, sodium, and copper. The interaction between the 2 main effects was not significant. For calcium, magnesium, potassium, and sodium, mean estimated intakes were below the AI for both sexes at all stages of lactation. For trace elements, mean estimated intakes for copper and zinc exceeded AI during transitional lactation but were lower (90% and 44–46%, respectively) during established lactation. Manganese intakes exceeded the AI at all stages of lactation for both sexes and did not differ between the groups. In contrast, selenium ranged from 48% to 60% of the AI but did not differ by stage of lactation or by sex.

Associations of mineral and trace element concentrations with anthropometry. During transitional lactation (**Supplemental Table 4**), 4 PCs were identified from the PCA. When these PCs were used in multiple linear regressions, PC2 (concentrations of copper, magnesium, and zinc) was negatively associated with the HCAZ, but PC1 (potassium, rubidium, and sodium), PC3 (iron and manganese), and PC4 (strontium, calcium, and selenium) were not significant. The model captured 20.5% of the total variation in infant HCAZ. The models for WAZ and LAZ were not significant.

During early lactation (Supplemental Table 4), 3 PCs entered the multiple regression models, but only PC1 (copper, potassium, sodium, selenium, and zinc) was negatively associated with WAZ (R^2_{adj} = 0.138) and LAZ (R^2_{adj} = 0.107). PC2 (calcium, rubidium, magnesium, and strontium) and PC3 (manganese and iron) were not significant in either model. The model for HCAZ was not significant. During the established lactation, only PC3 (copper, sodium, selenium, and zinc) was positively and associated with HCAZ (R^2_{adj} = 0.146). Models for WAZ and LAZ were not significant.

Associations of estimated mineral and trace element intakes with anthropometry. During transitional lactation, none of the 3 PCs were significant in any of the regression models. In contrast, during early lactation PC1 (intakes of calcium, potassium, magnesium, rubidium, and strontium) was positively associated with WAZ, LAZ, and HCAZ. PC2 (intakes of copper, sodium, selenium, and zinc) was positively associated with WAZ. These models captured 41.7%, 19.7%, and 27.9% of the variance in WAZ, LAZ, and HCAZ, respectively (**Table 5**).

During established lactation (Table 5), PC1 (copper, potassium, magnesium, sodium, rubidium, and strontium) and PC3 (copper, selenium, and zinc) were positively associated with infant WAZ and LAZ and captured 67.4% and 46.1% of the total variation in WAZ and LAZ, respectively. Moreover, PC1 (copper, potassium, magnesium, sodium, rubidium, and strontium) was positively associated with HCAZ in a model that explained 23.5% of its variability.

Discussion

Several studies have measured concentrations of minerals and trace elements in breast milk and reported concentrations

TABLE 3 Estimated daily milk, mineral, and trace element intakes by sex at 3 stages of lactation in breastfed Guatemalan infants[1]

	Stage of lactation			
	Transitional, 5–17 d	Early, 18–46 d	Established, 4–6 mo	*P*
Male infants				
Milk consumed, mL/d	539 ± 56[c]	607 ± 80[b]	712 ± 120[a]	<0.0001*
Mineral intake,[2] mg/d				
Calcium	148 ± 35[b]	164 ± 33[b]	190 ± 39[a]	<0.0001*
Magnesium	12.6 ± 2.0[b]	13.6 ± 3.3[b]	25.9 ± 6.0[a]	<0.0001*
Potassium	314 ± 37	311 ± 48	307 ± 58	0.78
Sodium	81.1 ± 18.3[a]	74.2 ± 15.3[a,b]	67.6 ± 20.2[b]	0.003*
Trace element intake,[2] μg/d				
Copper	321 ± 65[a]	270 ± 63[b]	182 ± 63[c]	<0.0001*
Iron	342 ± 270[a]	327 ± 229[a]	215 ± 180[b]	<0.0001*
Manganese	6.05 ± 4.24[a]	7.35 ± 6.13[a]	4.62 ± 5.22[b]	0.01
Rubidium	607 ± 116[a,b]	636 ± 159[a]	564 ± 126[b]	0.04
Selenium	9.54 ± 3.44	9.39 ± 3.32	9.53 ± 4.72	0.65
Strontium	23.3 ± 10.4[b]	29.3 ± 11.1[a]	33.8 ± 12.6[a]	<0.0001*
Zinc	2370 ± 453[a]	2000 ± 607[a]	931 ± 643[b]	<0.0001*
Female infants				
Milk consumed, mL/d	483 ± 61[b]	520 ± 58[b]	686 ± 105[a]	<0.0001*
Mineral intake,[2] mg/d				
Calcium	125 ± 29[b]	140 ± 21[b]	173 ± 34[a]	<0.0001*
Magnesium	10.4 ± 2.0[c]	12.2 ± 2.4[b]	23.5 ± 4.7[a]	<0.0001*
Potassium	286 ± 56	279 ± 49	294 ± 57	0.53
Sodium	66.5 ± 16.5	64.5 ± 15.9	66.2 ± 16.2	0.88
Trace element intake,[2] μg/d				
Copper	274 ± 61[a]	249 ± 51[a]	184 ± 59[b]	<0.0001*
Iron	203 ± 99	316 ± 255	224 ± 159	0.07
Manganese	5.81 ± 4.39	4.77 ± 4.06	5.87 ± 4.62	0.07
Rubidium	524 ± 118	546 ± 141	572 ± 204	0.82
Selenium	7.21 ± 2.12	8.47 ± 3.27	9.01 ± 3.45	0.11
Strontium	21.2 ± 4.5[b]	22.6 ± 7.0[b]	30.4 ± 9[a]	<0.0001*
Zinc	2040 ± 484[a]	1960 ± 691[a]	878 ± 432[b]	<0.0001*

[1] Values are arithmetic means ± SDs. n = 31, 47, and 51 for male infants and n = 24, 25, and 49 for female infants during transitional, early, and established lactation, respectively. Labeled means in a row without a common superscript letter differ, $P < 0.05$. Shipment and feeding category were entered as covariates into the model. *Significance at Bonferroni corrected probability = 0.05 ÷ 11 = 0.0045.

[2] Magnesium, chromium, iron, manganese, rubidium, selenium, strontium, and zinc were log transformed [ln(y)] to achieve normality during ANCOVA.

similar to ours (8, 9), but ours is the first study to our knowledge to attempt to associate these concentrations with infant growth during the first 6 mo of life. Although one study had examined minerals in breast milk from northern Guatemala (10), our study has several advantages over this and previous studies. First, we examined a wider range of minerals and trace elements. Second, we controlled for the multicollinearity among elements by using PC in the multiple regression analyses. Third, we determined whether estimated intakes were associated with infant anthropometry at 3 stages of lactation. With this approach, several notable observations emerged. First, calcium, potassium, sodium, copper, iron, manganese, selenium, and zinc concentrations were lower during established than during transitional and early lactation; only the magnesium concentration was higher. Second, higher milk intakes in established lactation were insufficient to compensate for the lower milk concentrations of sodium, copper, iron, manganese, and zinc for male infants and the lower milk concentrations of sodium, iron, and manganese for female infants. Estimated intakes of calcium, magnesium, sodium, potassium, and selenium were below AIs for both sexes at all stages of lactation. Finally, during early lactation, a PC composed of calcium, magnesium, potassium, rubidium, and strontium intakes was associated with higher WAZ, LAZ, and HCAZ. The same PC plus sodium also was associated with better growth for all 3 anthropometric parameters in established lactation. Moreover, in established lactation, a second PC that included copper, selenium, and zinc intakes also was associated with WAZ and LAZ but not with HCAZ. Taken together, these results suggest that inadequate intakes of selected minerals and trace elements in exclusively or predominantly breastfed infants may be associated with compromised infant growth during their first 6 mo in the western highlands of Guatemala.

Despite differences in methodological approaches and differences in population characteristics among studies, our milk concentrations compared favorably with previous ICP-MS studies on milk (8, 9), with the exception of iron and rubidium concentrations, which were higher in our study. Furthermore, the decline in concentrations as lactation progressed was consistent with previous observations in developing (10) and developed countries (7, 13). It is accepted widely that concentrations of several minerals (calcium and magnesium) and trace elements (copper, iron, and zinc) are tightly regulated in milk and largely unaffected by maternal diet (5, 6); the known exception is milk selenium (5). Additionally, several other factors have been associated

TABLE 4 Comparison of percentages of AI among Guatemalan infants by stage of lactation and sex during the first 6 mo[1]

	Transitional lactation		Early lactation		Established lactation		P^2	
	Male	Female	Male	Female	Male	Female	Lactation stage	Infant sex
Minerals,[3] % of AI								
Calcium	74.1 ± 17.5[c,d]	62.3 ± 14.4[d]	82.0 ± 16.6[b,c]	70.0 ± 10.4[c,d]	95.0 ± 19.4[a]	86.4 ± 16.9[a,b]	<0.0001*	<0.0001*
Magnesium	41.9 ± 6.8[b,c]	34.5 ± 6.8[c]	45.2 ± 10.9[b]	40.7 ± 7.9[b,c]	86.2 ± 19.9[a]	78.4 ± 15.7[a]	<0.0001*	0.001*
Potassium	78.4 ± 9.3	71.5 ± 14.1	77.8 ± 12.0	69.8 ± 12.2	76.8 ± 14.4	73.5 ± 14.4	0.80	0.001*
Sodium	67.6 ± 15.2[a]	55.4 ± 13.8[b]	61.8 ± 12.8[a,b]	53.7 ± 13.2[b]	56.3 ± 16.9[b]	55.2 ± 13.5[b]	0.06	0.001*
Trace elements,[3] % of AI								
Copper	160 ± 32[a]	137 ± 30[a, b]	135 ± 32[b]	124 ± 25[b]	90.9 ± 32[c]	92.2 ± 30[c]	<0.0001*	0.011*
Iron	127 ± 100	75.1 ± 36.6	121 ± 85	117 ± 94	79.8 ± 66.6	83.0 ± 58.7	0.01	0.11
Manganese	202 ± 141	194 ± 146	245 ± 204	159 ± 136	154 ± 174	196 ± 154	0.54	0.46
Selenium	63.6 ± 22.9	48.0 ± 14.1	62.6 ± 22.2	56.5 ± 21.8	63.6 ± 31.5	60.1 ± 23.0	0.34	0.01
Zinc	118 ± 23[a]	102 ± 24[a]	99.8 ± 30.3[a]	97.8 ± 34.5[a]	46.6 ± 32.1[b]	43.9 ± 21.6[b]	<0.0001*	0.08

[1] Percentages (means ± SDs) were calculated as infant's mineral intake divided by recommended AI values (27). n = 31, 47, and 51 for male infants and n = 24, 25, and 49 for female infants during transitional, early, and established lactation, respectively. Labeled means in a row without a common superscript letter differ, $P < 0.05$. *Significance at Bonferroni corrected probability = 0.05 ÷ 9 = 0.0056. AI, Adequate Intake.
[2] Two-factor ANOVA showed no significant interaction for stage of lactation × infant sex on milk minerals.
[3] Recommended AI for infants aged 0–6 mo: 200 mg Ca/d, 30 mg Mg/d, 400 mg K/d, 120 mg Na/d, 200 μg Cu/d, 270 μg Fe/d, 3 μg Mn/d, 15 μg Se/d, and 2000 μg Zn/d. There was no established AI for rubidium and strontium.

with variability in milk mineral concentrations. Diurnal variations have been noted for sodium and iron (11, 28). Other factors include maternal age, parity, length of gestation (29), and infection (5). We assessed whether maternal age and parity differed by stage of lactation, and they did not. We also noted a negative association between milk mineral and trace element concentrations and anthropometric outcomes until we used estimated intakes, which included milk volume in their calculation; then our associations using PCs were positively associated with our anthropometric outcomes, suggesting to us that changes in milk volume could underscore the mineral intakes in addition to concentrations. Milk volume is known to be regulated by lactose, sodium, and calcium (30, 31). Interestingly, the estimated intakes of the latter 2 minerals were below the AIs in our study population.

Several PCs were significant in our multiple linear regressions with anthropometric outcomes. Explanations for these sets of minerals emerging as PC clusters could relate to their multicollinearity and common transporters (6). The first PC, which included calcium, magnesium, and potassium, as well as strontium and rubidium for which recommended AIs are unavailable, was associated with WAZ, LAZ, and HCAZ during all 3 stages of lactation. In our study, estimated intakes for calcium, magnesium, and potassium as percent of AI were below the recommended AI. Explanations for lower breast-milk calcium may be related to repeated pregnancies (32) and lactating teenage mothers, although there were no adverse effects on infant growth (33). In contrast, magnesium is not affected by adolescent motherhood, maternal undernutrition or

TABLE 5 Multiple linear regressions associating estimated daily milk mineral and trace element intakes with WAZ, LAZ, and HCAZ of breastfed Guatemalan infants at 2 stages of lactation[1]

	WAZ[2]		LAZ[2]		HCAZ[2]	
	Coefficient (B)	P	Coefficient (B)	P	Coefficient (B)	P
Early lactation, 18–46 d						
PC1 (calcium, magnesium, strontium, rubidium, potassium)	0.694 ± 0.116	<0.0001*	0.457 ± 0.124	<0.0001*	0.705 ± 0.137	<0.0001*
PC2 (sodium, selenium, copper, zinc)	0.277 ± 0.123	0.028*	0.007 ± 0.129	0.96	0.277 ± 0.143	0.06
PC3 (iron, manganese)	−0.130 ± 0.141	0.36	−0.152 ± 0.153	0.32	−0.080 ± 0.170	0.64
Infant sex	0.516 ± 0.244	0.038*	−0.018 ± 0.269	0.95	0.118 ± 0.457	0.80
Maternal height	0.015 ± 0.015	0.31	0.041 ± 0.024	0.10	0.075 ± 0.034	0.034*
Shipment	−0.216 ± 0.318	0.50	0.129 ± 0.348	0.71	−1.30 ± 0.654	0.05
R^2_{adj}, overall P	0.417	<0.0001*	0.197	0.003*	0.279	<0.0001*
Established lactation (4–6 mo)						
PC1 (calcium, magnesium, potassium, rubidium, sodium, strontium)	0.903 ± 0.069	<0.0001*	0.917 ± 0.110	<0.0001*	0.559 ± 0.147	<0.0001*
PC2 (iron, manganese)	0.060 ± 0.072	0.41	−0.104 ± 0.110	0.35	−0.159 ± 0.145	0.27
PC3 (zinc, copper, selenium)	0.306 ± 0.071	<0.0001*	0.322 ± 0.111	0.005*	−0.032 ± 0.149	0.83
Infant sex	0.583 ± 0.136	<0.0001*	0.537 ± 0.211	0.013*	0.719 ± 0.281	0.12
Maternal height	0.012 ± 0.008	0.13	0.011 ± 0.019	0.55	0.051 ± 0.025	0.047*
Shipment	−0.497 ± 0.151	0.001*	−0.364 ± 0.235	0.13	−0.609 ± 0.311	0.05
R^2_{adj}, overall P	0.674	<0.0001*	0.461	<0.0001*	0.235	<0.0001*

[1] Values are coefficients ± SEs. n = 73 for early lactation and n = 100 for established lactation. PCs were obtained from PCA of milk minerals. *Significance at $P < 0.05$. HCAZ, head circumference-for-age z score; LAZ, length-for-age z score; PC, principal component; PCA, principal component analysis; WAZ, weight-for-age z score.
[2] Infant sex, maternal height, and shipment were controlled as confounding factors during multiple linear regression analyses.

dietary magnesium, race, and stage of lactation (34). Our study does report differences in magnesium by stage of lactation and with infant growth, which to our knowledge had not been reported previously. Lastly, rubidium emerged in our PC1 along with potassium, both of which were highly correlated. Rubidium is considered a potential potassium antagonist (35) and has been found in breast milk (36), but there is controversy as to its toxicity (35, 36). Moreover, one study found no correlation between milk rubidium and newborn weight (36). There appears to be little scientific literature that describes the contribution of this cluster of minerals to early infant growth and no one has explored their interrelations. Further studies are warranted.

The second PC clustered copper, selenium, and zinc, and it was associated with WAZ and LAZ but not HCAZ and only in established lactation. The association of copper, selenium, and zinc intakes into a single PC may relate to their common antioxidant properties (37). Mean copper intakes approached or exceeded the recommended AI, and zinc also approached or exceeded its AI during transitional and early lactation but not during established lactation. Interestingly, selenium, which, unlike the other 2 trace elements, reflects dietary intake, averaged ≤63% of the AI for both sexes across all 3 lactation stages. More research is needed to determine whether selenium deficiency in the diet is a problem in these Guatemalan communities.

In the third PC, both iron and manganese intakes consistently grouped together into a single PC but were not associated with any of the anthropometric outcomes at any stage of lactation. Despite their sharing a common transporter (6, 38), we suggest that the absence of an association of this mineral pair with growth may exist because the mean intake of manganese exceeded adequacy in both male and female infants at all stages of lactation, and because the mean intake of iron generally exceeded the AI, with the exception of male infants during established lactation and female infants during early lactation. Furthermore, it is well known that infants may not require iron supplements during the first 6 mo (39), given adequate storage from birth and supply through milk. Our finding would support this knowledge.

It is interesting to note that sodium chloride intakes (5.2 ± 1.7 g/d) among Guatemalan women have been found to be low in comparison with European and Beninese women (40) and that none of the breastfed infants in our population had adequate intakes of sodium from breast milk. A Gambian study using standardized sodium concentrations but variable estimates of breast-milk volume concluded that sodium concentrations differed considerably and did not stabilize; the authors suggested that differences in breast-milk sodium concentrations were the result of lower dietary intakes (41). Low sodium concentrations may impair infant growth (42). It also has been suggested that the secretion of water by the mammary gland could be driven partly by the secretion of lactose and partly by the secretion of ions via sodium, potassium, and chlorine cotransport across the basolateral membrane (30, 31). We can infer, however, only that low maternal dietary intakes may underscore low breast-milk sodium concentrations, reduced breast-milk volumes, and poor growth because we observed that sodium entered our PC1 and was associated with WAZ, LAZ, and HCAZ during established lactation. This inference requires further investigation.

Despite its strengths, our study has several limitations. Interpretation of our findings is constrained because we did not directly measure milk volumes. Milk volume was estimated and based on the assumption that milk production is driven by infant energy demands, the maternal ability to produce milk was not compromised, and the volume of milk consumed by the infants was adequate to maintain their EER (25). This could have led to an underestimate of intake if the mother had compromised milk production or to an overestimate of intake if the infant had consumed milk in excess of energy demands. Although the bioavailability of minerals and trace elements from milk is high, other milk constituents that could have modulated intestinal absorption were not considered. Finally, although we adjusted for type of feeding as a covariate for intakes, we may have underestimated the dietary contribution of minerals in the various *agüitas* consumed by infants who were predominantly breastfed.

In conclusion, there has been a long-held belief that breast milk satisfies all of the nutritional needs of all infants (27), but the results from our study provided evidence that breast milk may not be a sufficient source of minerals and trace elements for infants, especially in developing countries such as Guatemala. The prevalence of inadequate intakes was high. Our findings also show that mean estimated intake as a percentage of the AI for calcium, magnesium, sodium, potassium, selenium, and zinc fell below the AI for both sexes at all stages of lactation. We conclude that both low concentrations in breast milk and/or reduced milk volumes leading to inadequate intakes could impair infant WAZ, LAZ, and HCAZ during early and established lactation in breastfeeding infants in the western highlands of Guatemala.

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