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Background

Exclusively breastfed infants require that adequate amounts of carbohydrates, fatty acids, proteins, and mineral nutrients are available to foster normal skeletal and neuromuscular growth and development. Minerals such as calcium, sodium, and potassium, which are crucial for normal physiological function, are found in variable concentrations in human milk. As well, antibodies and other rarefied minerals - selenium, zinc, manganese, and copper – are critical for protection against infection and proper function of the immune system and are supplied to the neonate by the mother's milk. Although many studies have investigated the trace mineral concentrations in breast milk (1,2) few have examined the correlation between maternal dietary intake of these minerals and their concentration in breast milk. Furthermore, few studies have explored the mineral content of human milk in New Mexican populations, specifically non-Hispanic whites and southwestern Hispanics.

In a study of 25 lactating women in Libya whose daily intake of copper, selenium, and zinc were within or above the recommended daily allowance (RDA), the average milk content of copper, selenium, and zinc were 0.84, 0.104, and 16.1 mg/L. (1) Another study, more closely related geographically to our own study population, analyzed the serum calcium levels in a rural Mexican population to assess the effects of lactation on these levels throughout the first year postpartum (1, 3, 6, and 12 months) when compared to a group of non-lactating women of similar age, socioeconomic status, and geography. Quantitative data for breast milk mineral concentration was not reported;

however, because the lactating subset of subjects had a significant decrease in serum calcium levels ($P < 0.05$), it was suggested that increased dietary intake of calcium during lactation is necessary to mitigate decreases in serum calcium concentrations and is needed to provide an adequate mineral concentration in breast milk. (2)

Exposure to increased mineral dietary intake influences corresponding breast milk levels (3). In agriculture, sodium selenate is supplemented in fertilizers since the early 1980s. With this soil nutrient enrichment, dietary exposure of individuals ingesting foods grown with this product is significantly increased. A study of Finnish women reported dietary exposure to selenium and copper correlate with increased amounts of both minerals in human milk at one month of lactation. These examiners investigated the changes in breast milk concentration with respect to selenium, cadmium, zinc, and copper over a period of 8 years (1987–1995) in two Finnish populations – urban (Helsinki) and rural (Kuopio – a rural area designated due increased in bedrock copper concentrations). The Finnish research group collected 257 samples at one month of lactation in these two distinct areas. As was initially hypothesized, selenium breast milk concentrations increased in both the Helsinki and Kuopio populations from 16.4 μg/l to 18.9 μg/l ($P < 0.001$) over this 8-year span. Increased selenium levels also correlated with decreased cadmium, zinc, and copper concentrations in these samples. (3) Furthermore, mothers living in the rural area – Kuopio – had increased breast milk copper concentrations when compared to their urban counterparts.

Prior to this investigation of two southwestern US populations, Glew and VanderJagt accumulated data on breast milk mineral concentrations from various groups in sub-Saharan Africa, specifically the inhabitants of Jos, Nigeria. In a study comparing Jos population breast milk mineral concentrations to values obtained by the WHO/IAEA study, Glew and collaborators (5) found the relative breast milk concentrations of eight minerals in the breast milk of Jos women – copper, iron, sodium, potassium, magnesium, manganese, zinc, and phosphorus – fell within concentration intervals established in other areas worldwide (4), with only the iron mean concentration falling below the established interval. (5) Furthermore, their study also demonstrated, with two exceptions – sodium and copper – that certain minerals effectively concentrate in breast milk, regardless of low serum values.

In a similar study of the Fulani population of the Western Sahel of Africa, the breast milk and serum were obtained from 34 Fulani mother–infant pairs. This study determined that concentrations of crucial minerals again fell within or exceeded the intervals of other populations worldwide. Furthermore, these data demonstrated a positive correlation between maternal serum concentrations of copper and iron and concentration of these minerals in breast milk. (6)

With the evidence suggesting the serum concentrations of particular minerals correlates with their milk concentration, I investigated the correlation between dietary intake of eight nutrient minerals – calcium, selenium, magnesium, zinc, copper, iron, sodium, and potassium - and their respective breast milk concentrations. My primary hypothesis was that breast milk mineral concentrations will directly correlate with dietary intake. Along with this, I investigated the daily mineral dietary intake variation between non-Hispanic white and Hispanic lactating mothers in Albuquerque and the surrounding area.

Methods

Subjects

The 29 study participants – 16 Hispanic and 13 non-Hispanic White - were healthy lactating women residing in the Albuquerque metropolitan area in central New Mexico. Milk samples were collected at the General Clinical Research Center from subjects who were designated into one of two dominant subpopulations via ethnic self-reporting - either white non-Hispanic or Hispanic. Anthropometric data including age, weight, height, parity, and general state of health information were recorded for each individual.

Mothers included in the study were required to have been lactating for greater than one month but less than six months. Furthermore, smokers, alcohol users, and subjects with a present medical history of chronic or acute illness were excluded.

This study was approved by the Institutional Research Review Committee at the University of New Mexico Health Sciences Center, and informed written consent was obtained from each subject.

Samples

Specimens were collected at the General Clinical Research Center through bimanual expression midway through feeding cycle. Milk specimens were aliquoted into 1.5 ml cryovials and stored at -20°C for frozen transport to the National Institute of Occupational Safety and Health for mineral analysis that were carried out using Inductively Coupled Plasma Optical Emission Spectroscopy.

Diet record

Collection of reliable quantitative information about an individual's food consumption was obtained via the validated and widely used three-day dietary record (7). Participants completed a written diet record of all food and drink ingested during the three days prior to giving blood and milk samples. Along with written and verbal instructions on maintain a dietary record, participants were provided a standardized form to record daily intake. Subjects met with a university dietitian at the General Clinical Research Center who reviewed and confirmed each entry on the dietary record in order to obtain an accurate estimate of the kinds and amounts of food consumed. These amounts of food and beverage are checked, when necessary, using three-dimensional food models (8). The three-day diet records were coded and analyzed using Food Intake Analysis System, versions 3.99 and Millenium (1999, 2005 The University of Texas, School of Public Health, Houston, TX, USA) (9) which uses the same databases as the United States Department of Agriculture, Agricultural Research Service and the Continuing Survey of Food Intake by Individuals (1994-1996).

Milk analysis

Samples were thawed for approximately 3 hours prior to preparation, then vortexed vigorously. Two milliliter aliquots of each sample, with the exception of sample S1003AL (1.5 mL), were weighed into 150 mL Phillips beakers and treated with nitric and perchloric acids. Samples were covered and refluxed overnight at 150°C on a hot plate. The following morning, the covers were removed and the samples were allowed to come to near dryness at the same temperature. Samples were then treated with 0.50 mL of 4:1 nitric-perchloric acid (v/v) and a minimal amount of water. Samples were cooled to room temperature and then transferred to a graduated centrifuge tubes and brought to a final volume of 10.0 mL. Any samples that did not come to near dryness were recovered and left on the hot plate for an additional night at 110°C. The following day, these remaining samples came to dryness and were treated as above. These sample solutions were then analyzed by ICP-OES for their content of metals using a Spectro Analytical EOP (end on plasma-axial view) spectrometer using a fixed cross-flow nebulizer and a dual pass spray chamber. Samples were over ranged in calcium and therefore were diluted 20:1 and reanalyzed.

Statistical analyses

For group statistics, the subjects reported in Table 1 were stratified by ethnicity – Hispanic and Non-Hispanic White – and included population description by mean and standard deviation for the variables age, weight, height, and length of lactation. Mean breast milk concentration and standard deviation for each mineral are reported in Table 2. A third table, in identical format to those discussed above, will include similar statistical analysis of values reported for dietary intake.

Regression analysis and descriptive statistics were obtained using NCSS2006 statistical system for Windows. The metric of statistical significance was considered at a p value < 0.05. A power of 80% to detect a difference is achieved by analysis of the two groups' mean values for each mineral. A difference of ±1.1x standard deviation between group mean values suggests only a 20% probability of accepting a false null hypothesis. Regression analysis of data provided in Tables 2 and 3 were used to test for correlations between mineral dietary intake and breast milk mineral concentration.

Results

Comments on the study population. The average age of the 29 study participants was 28.0 ± 4.3 years, with the 13 non-Hispanic white subjects having a mean age approximately 6 years greater than the 16 Hispanic participants (31.6 versus 25.3 years, $p = 0.01$) (Table 1). As for body composition parameters, the Hispanic subjects had a higher mean body mass index (BMI), mid-arm circumference, and percent body fat; however, differences were not significant ($p > 0.05$). Of the body composition parameters evaluated, only height showed a modest difference ($p = 0.049$) favoring the NHW subjects. At time of breast milk sampling, each subject had been lactating for one to six months.

Table 1. Comparison of body composition parameters between Hispanic and non-Hispanic White (NHW) women in New Mexico

Parameter	Hispanic (n = 16)	NHW (n = 13)	Total Population (n = 29)
		Mean (± 1 SD)	
Age (yrs)	25.3 (3.9)	31.6 (4.8)	28.1 (5.3)
Height (cm)	162 (5)	166(5) *	164 (6)
Weight (kg)	73.1 (15.8)	68.0 (10.7)	70.8 (13.8)
BMI (kg/m^2)	28.0 (6.4)	24.8 (3.5)	26.6 (5.4)
MAC (cm)	30.1 (4.9)	29.8 (4.8)	30.0 (4.8)
TSF (mm)	25.9 (8.3)	23.5 (8.2)	24.9 (8.2)
FFM (kg)	45.5 (5.2)	45.8 (4.5)	45.7 (4.8)
Body fat (kg)	27.4 (11.6)	22.2 (7.1)	25.1 (10.0)
Body fat (%)	35.9 (8.5)	32.0 (5.8)	34.2 (7.5)

* p = 0.049; other differences were not significant; BMI, body mass index; MAC, mid-arm circumference; TSF, triceps skin-fold; FFM, fat-free mass

Mineral content of breast milk. With the exception to iron, the breast milk mineral concentrations were within the range of values reported by investigators elsewhere (4,5,6,9). The mean iron concentration in the milk of 13 non-Hispanic white subjects was 247 mg/L, whereas the mean breast milk iron concentration for the Hispanic group was 356 μg/L. The mean iron breast milk concentration was 307 μg/L for the total southwestern population (n = 29).

Table 2. Concentration of selected mineral in breast milk of women in New Mexico

Mineral (mg/L)	Non-Hispanic white (n = 13)	Hispanic (n = 16)	Total Population (n = 29)
		Mean (±1 SD*)	
Calcium	341 (37)	311 (49)	324 (46)
Copper	0.300 (0.139)	0.344 (0.105)	0.325 (0.043)
Iron	0.247 (0.106)	0.356 (0.139)	0.307 (0.135)
Magnesium	34.0 (4.8)	32.7 (4.4)	33.3 (4.6)
Potassium	558 (64)	571 (69)	565 (66)
Selenium	0.090 (0.041)	0.128 (0.046)	0.111 (0.047)
Sodium	127 (41)	172 (88)	152 (74)
Zinc	1.32 (0.60)	2.23 (1.03)	1.81 (0.96)

* SD = Standard deviation

Correlation of dietary intake with breast milk mineral concentration. Evaluation of the two southwestern subpopulations showed no significant difference in the dietary intake of any of the eight analyzed minerals. Interestingly, selenium was the only one of the eight minerals analyzed that exhibited a positive correlation between dietary intake and breast milk mineral concentration. By regression analysis, selenium dietary intake demonstrated a weak correlation ($r^2 = 0.20$) with selenium breast milk concentration. This model is describe by the function MSe = DSe * 3.399 X 10^{-4} + 6.699 X 10^{-2}, where DSe is dietary intake of selenium and MSe is selenium breast milk concentration in μg/g.

Table 3. Dietary intake of selected minerals by women in New Mexico

Mineral (mg/day)	Non-Hispanic white (n = 13)	Hispanic (n = 16)	Total Population (n = 29)
		Mean (±1 SD*)	
Calcium	1216 (497)	804 (451)	989 (508)
Copper	1.55 (0.60)	1.39 (0.53)	1.46 (0.56)
Iron	17.9 (5.1)	16.1 (4.7)	16.9 (4.9)
Magnesium	354 (116)	298 (93)	323 (106)
Potassium	3077 (1083)	2734 (992)	2888 (1029)
Selenium	102 (25)	131 (75)	118 (59)
Sodium	3457 (735)	3912 (1161)	3708 (1003)
Zinc	13.9 (5.5)	12.1 (4.1)	12.9 (4.8)

* SD = Standard Deviation

Discussion

The goal of the present study was, first, to assess the correlation between mineral dietary intake and breast milk mineral concentration, and second, to assess dietary intake and breast milk mineral concentration differences between the two designated southwestern subpopulations. No significant difference in dietary intake of any of the eight minerals was found between the two groups.

Interestingly, however, both ethnic populations had mean breast milk iron concentrations significantly lower than values reported in Table 4 (446 – 1510 μg/L) by investigators elsewhere in the world (4, 5, 6, 9). As stated here, a slight correlation between dietary intake of selenium and its milk concentration was evident.

What is the significance of low iron concentrations in human milk? In pulmonary physiology, iron plays an integral part in heme complex synthesis, which is found in both hemoglobin and myoglobin and is therefore important in oxygen tissue delivery and prolonged muscular oxygenation. Divalent iron forms four coordinate bonds with porphyrin rings of heme. This heme complex is then incorporate into the macromolecule hemoglobin of red blood cells and used to transport oxygen from the pulmonary vasculature to the body's periphery (10). Alternatively, the heme complex may be associated with the protein myoglobin, found in increase concentration in slow twitch muscle fibers, and used to store oxygen for use when rate of pulmonary oxygenation is insufficient to provide muscle tissue with adequate oxygen for a required task.

Iron is also a crucial component of proteins involved in cell respiration. The electron transfer chain, found in the inner mitochondrial membrane of aerobic, eukaryotic cells, is composed of four cytochrome proteins (Complexes I – IV) responsible for the oxidation of the reduced substrates NADH and $FADH_2$ (11). The reducible moiety of these cytochrome proteins is a ferrosulfide compound. Iron sulfide allows for the loss of electron from NADH and $FADH_2$. The reduction of these proteins causes a change in protein conformation and induces the pumping of protons into the intramembranous space of the mitochondria. Accumulation of protons in this space creates the chemiosmotic gradient that is the source of potential energy responsible for activating ATP synthetase F1F0 complex. The low pH and high electropositivity of intramembranous space acts as the repulsive force to accelerate protons through the ATP synthetase complex, which provides energy for the phosphorylation of ADP to ATP, the primary source of energy for eukaryotic cells.

Although regression analysis of the data collected in this study did not show a positive correlation between dietary iron intake and breast milk iron concentration. Iron supplementation may be advisable to ensure that breastfed infants in these populations receive adequate iron. Arguably, the suggestion of supplementation should be regarded with caution, due to the well-recognized low bioavailability of supplemental iron. This decreased bioavailability of supplemental iron can lead to increased intestinal levels of iron that increase the risk of serious gastrointestinal infections.

The results and implications of this study are limited by three keys factors – small sample size, failure to analyze for phytates and dietary fiber intake for each subject, and lack of information regarding the duration of lactation. First, the two groups described in the present study were comprised of 16 Hispanic and 13 Non-Hispanic white females. This relatively small sample size, along with a wide distribution of values for both dietary intake and breast milk mineral concentration, limited the power of the study and made detection of true differences between groups problematic. As well, though dietary intake data was collected with a reliable collection instrument, cationic mineral absorption in the duodenum is hindered by increased intraluminal concentrations of fiber and other chelating molecules, such as phytates. This study did not take into consideration the variably in mineral absorption caused such molecules. Finally, though one criterion for participation required each mother to be lactating for greater than but less than six months, a specific value for duration of lactation for each individual was not collected. Prior investigators suggest that duration of lactation can significantly effect breast milk mineral concentrations (1).

With regard to future projects, because of our small sample size it is incumbent upon us to expand this study to include a much larger number of subjects. It would also be useful to sample other region of the state including both rural and urban communities. The results of the present study raise questions about the need for iron supplementation both for the lactating mother and their nursing infants. We would also like to know if the low levels of iron in the mother's milk of the subjects in our study had any consequences in their babies. Specifically, it would be interesting to assess the iron status of these infants using standard clinical laboratory methods, i.e. ferritin, transferring, transferring saturation, red cell morphology and indices.

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Table 4. Comparison of the mineral content of human milk from various populations worldwide

	Nigeria/Hausa	Nigeria/Yoruba	Japan	Phillipines	Zaire	Sweden	Southwest US
Calcium (mg/L)	-	-	250	-	-	-	324
Copper (µg/L)	170	279	-	339	199	186	325
Iron (µg/L)	1510	487	1190	642	851	446	307
Magnesium (mg/L)	24.9	32.2	27.0	28.0	38.9	34.2	27.0
Potassium (mg/L)	424	509	470	534	548	-	470
Selenium (µg/L)	-	-	17.0	-	-	-	111.0
Sodium (mg/L)	178	87	135	118	120	88	152
Zinc (µg/L)	1070	1860	1450	1870	1680	700	1810

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