MINERAL CONTENT OF THE MILK OF FULANI WOMEN AND THE SERA OF THEIR BREAST-FED INFANTS

By

Dorothy J. VanderjaGT, *Ph.D[1],* ***Jaimie T. Shores,*** *B.Sc., B.A.[1],* ***Selina N. Okolo,*** *M.D.[2],* ***Mark Millson,*** *M.A.[3],* ***Ada F. Ezeogu,*** *M.S.[4],* ***Wadinga Wadinga,*** *M.D.[5], and* ***Robert H. Glew,*** *Ph.D[1].*

[1]Department of Biochemistry & Molecular Biology, University of New Mexico School of Medicine Albuquerque, New Mexico, U.S.A.
[2]Department of Paediatrics Jos University Teaching Hospital Jos, Nigeria
[3]National Institute of Occupational Safety and Health Cincinnati, Ohio, U.S.A.
[4]Department of Chemical Pathology Jos University Teaching Hospital Jos, Nigeria
[5]Department of Obstetrics & Gynaecology Jos University Teaching Hospital Jos, Nigeria

To whom all correspondence and reprint requests should be addressed:
Robert H. Glew, Ph.D., Department of Biochemistry and Molecular Biology, Room 249 BMSB, University of New Mexico School of Medicine, Albuquerque, NM 87131. Telephone (505) 272-2362; FAX: (505)272-6587;
E-mail rglew@salud.unm.edu

ABSTRACT

Objectives: To evaluate the mineral and trace element content of breast milk of the Fulani of Northern Nigeria with respect to levels obtained with sera from Fulani mother-baby pairs.

Methods: We obtained milk and serum from 34 Fulani mother-infant pairs. The samples were collected through bimanual expression midway through feeding. The age, height, weight, skin-fold thickness, parity, and information regarding the general state of health of each subject were recorded. Following collection, milk and serum specimens were aliquoted into 1.5ml cryovials and stored at 20°C until which time they were analysed.

Results: The levels of calcium, chromium, copper, iron, magnesium, manganese, and zinc in the milk were within or exceeded the range of values reported for other populations worldwide and were comparable to those of the United States. Copper, zinc, iron, and calcium decreased in concentration by 50-75% over the first six months post-gestation. Copper and iron displayed a positive correlation between their maternal sera and milk concentrations. No significant positive correlations were observed between minerals in milk and the sera of nursing infants; in fact, a negative correlation was observed with copper levels of those fluids. Furthermore, high milk concentration of zinc appeared to antagonize the absorption of copper in the breast-fed infants.

Conclusion: These findings indicate that the milk of Fulani women provides sufficient amounts of critical minerals and trace elements to satisfy the needs of their exclusively breast-fed infant during the first six months of life. In addition, the concentration of each of these minerals in infant' serum is not related to their respective levels in milk.

Keywords: Human milk, trace elements, minerals, Fulani, nutrition.

INTRODUCTION

In many underdeveloped areas of the world, breast milk is often the exclusive dietary item during the first six months of life[1-5]. Even when non-breast milk sources of infant nutrition are available, problems often arise from excessive dilution and use of contaminated water sources, while other nutritional and immunologic concerns are present[6,7], prompting government and health care agencies the world over to emphasize the importance of breastfeeding[6].

Whereas even in the most economically deprived populations the concentrations of protein, lactose and fat of human milk are usually sufficient to satisfy the caloric and protein requirements of the growing infant, minerals and trace elements represent a class of nutrients for which there is well-founded concern in the field of pediatric nutrition[8]. The challenge of providing the newborn infant with adequate amount of minerals such as calcium and iron, and trace elements such as copper, zinc and manganese is nowhere greater than it is in the western Sahel where the infant mortality rates for particular ethnic groups who inhabit this region of Africa, including the Fulani, are exceptionally high.

The Fulani (or Fulbe as they call themselves) inhabit the Western Sahel of Africa and extend from Mauritania in the west of Cameroon in the east. They are nomadic semi-pastoralists whose livelihood and culture depend largely upon herding cattle. The Fulani represent one of the major ethnic African groups who inhabit the border region between Nigeria and the Republic of Niger. Surprisingly little is known about the general health and nutritional-deficiency based health problems of the Fulani. Even though the Fulani reside in close proximity to Hausa populations, they continue to speak the Fulbe language and adhere to Fulani customs. In recent years, we have analyzed the milk of Fulani women for its content of vitamins A and E and B carotene[5], n-3 and n-6 fatty acids[9], and for its total antioxidant capacity[4].

The purpose of this study were to measure the concentrations of seven minerals and trace elements in the blood and milk of nursing Fulani women, and to correlate these data with the concentrations of these same micronutrients in the blood of the infants they were nursing. To this end, we collected blood and milk from 34 Fulani women in a hamlet near the town of Vom in Northern Nigeria and, simultaneously, blood from their exclusively breast-fed infants and analysed these fluids for their content of calcium, chromium, copper, iron, magnesium, manganese and zinc.

METHODS

Subjects

The Fulani of the Jos Plateau, which is located in central Nigeria, are semi-pastoralists. while they inhabit permanent settlements in the grasslands of the Plateau, the males spend 4-6 months/year trekking as far as 200 miles (particularly during the cold-dry season) in search of pasture for their animals.

The Hamlet of Kaduna-Vom is located 30km south of the city of Jos. The women residing in this hamlet provided a convenient population to study because of their proximity to the teaching hospital in Jos where the samples were processed and stored. We obtained milk and serum from 34 Fulani mother-infant pairs living in Kaduna-Vom. The samples were collected through bimanual expression midway through feeding. The age, height, weight, skin-fold thickness, parity, and information regarding the general state of health of each subject were recorded.

All specimens and anthropometric data were gathered during the rainy season, between June and August of 1998. following collection, milk and serum specimens were aliquoted into 1.5ml cryovials and stored at 20°C until which time they were transported in the frozen state to Albuquerque, NM for analysis.

Analysis of minerals and trace elements

The milk and serum specimens were thawed and vortexed vigorously and aliquots were quantitatively transferred to 50ml Griffin beakers using 5 %(v/v) nitric acid. The samples were transferred with successive rinses of first the caps, then the threads of the vials, and finally the sample vials. All of the samples, including the supplied blanks and a reagent blank, were digested with 4ml concentrated HNO_3 and 1ml of 70%$HClO_4$. the acids that were used throughout were Fisher Brand's Optima (Pittsburgh.PA).

The beakers were covered with watch glasses and refluxed at 150°C for 4 hrs. The watch glass covers were removed and the undersides rinsed into the samples using a 5% HNO_3 solution. The samples were taken to near dryness at 150°C. One-fourth ml of 4:1 HNO_3-$HClO_4$ was used to redissolve each sample which was then quantitatively transferred to a graduated centrifuge tube and brought to a final volume of 5.0ml with deionized water.

The resulting solutions were analyzed for their trace metal content by inductively coupled plasma-optical emission spectrometry (ICP-OES) using a Spectre Analytical end on plasma-axial view spectrometer (EOP) which was equipped with a fixed cross-flow nebulizer and dual pass spray chamber.

Quality control samples, which were supplied by the National Institution of Occupational Safety and Health, consisted of the analyst's serum and breast milk from a co-worker. Three replicate aliquots (0.5ml) of each were taken to establish baselines. Three additional aliquots were 'spiked' with multi-element ICP standards and analyzed concurrently with test samples.

Statistical Analysis

Group comparisons and correlations were performed using NCSS 97 statistical system for Windows (Kaysville, UT). A p value <0.05 was considered statistically significant.

RESULTS

Comments on the study population

As shown in Table 1, the ages of the 34 mothers ranged from 15 to 45 years (mean, 26.0 years; standard deviation (SD), 7.5 years). The parity of the women ranged from 1 to 10 (mean, 4; SD, 3), and their body mass index (BM) ranged from 14.5 to 24.0kg/m^2 (mean, 19.4kg/m^2; SD, 2.4kg/m^2). Given the wide range of the mothers' BMI, values, we initially separated the study population into two groups (BMI<19kg/m^2 and BMI>19kg/m^2) and subjected our mineral and trace element data to statistical analysis to determine if there were any BMI-based differences. Since no statistically significant differences were observed, the mineral and trace element data for all 34 women were pooled.

The average age of the infants, and hence the time post-gestation when the milk specimens were collected, was 11 weeks (range, 2-24 weeks). Their average weight was 4.7kg (range, 1.8-8.0kg) and their average height was 56.6cm (range, 46-68 cm).

Concentrations of minerals and trace elements in the milk of Fulani women.

We compared the minerals in the milk of the Fulani with values reported for the milk of populations elsewhere in the World (Table 2). The median concentrations in the Fulani mothers' milk were within the range of values reported for calcium (244mg/L), chromium (18ug/ml), magnesium (31mg/L), and manganese (11ug/L). elements whose concentration in the milk of the Fulani women exceeded the levels that have been reported for other populations included copper (377ug/L), iron (755ug/L), and zinc (2.75mg/L). Noteworthy was the strong positive correlation we observed between the copper and zinc concentrations of the milk of the Fulani women in our study (Fig. 1 r=0.56, p<0.001).

These results for possible correlations between the levels of the various minerals and trace elements in the milk specimens versus the age, parity, post-gestational time, and BMI of the women, several noteworthy findings emerged. First, there was a statistically significant (p=0.029) positive correlation (r=0.38) between the copper concentration of Fulani milk and the BMI of the mothers. In addition, there were negative correlations between the calcium (r=0.47, p=0.005), iron (r=0.38, p=0.031), copper (r=0.5, p=0.003), and zinc (r=0.63, p<0.001) concentrations in the milk with the time post-gestation (Fig. 2).

Correlations between the concentrations of copper in material serum and milk

Regression analysis of the data for maternal serum and milk revealed that, except for copper, there was no correlation between the concentration of any mineral in the mothers' serum and that of their milk. However, with regard to copper, a correlation between the two fluids was observed: as shown in Figure 3, the correlation between maternal serum copper and breast milk copper was positive (r=0.57) and highly significant (p<0.001).

Comparison of the concentrations of minerals and trace elements in the milk of Fulani women and the serum of their exclusively breast-fed infants

A major goal of our study was to assess the overall mineral and trace element status of exclusively breast-fed Fulani infants. The mean serum concentrations of calcium, iron, manganese and zinc in the nursing infants were within the reference range of values for infants at the University Hospital of the University of New Mexico in Albuquerque: calcium, 92.0ug/ml, (reference range of 88-108ug/L); iron, 1.58ug/mL, (reference range of 0.60-2.23ug/mL); magnesium exceeded the upper limits of the pediatric reference ranges for these three elements (Table 3).

A moderately strong (r=0.42) and statistically significant (p=0.02) negative correlation was seen between the copper concentration of the milk of Fulani women and the serum copper concentration of the infants they were nursing (Fig. 4). Noteworthy, however, was our findings of a statistically significant (p=0.001) negative correlation between the concentration of zinc in the mothers' milk and the copper levels in the blood of the Fulani infants (Fig. 5): the higher the zinc concentration in the milk, the lower was the copper concentration of the infant's serum. These data indicate the possibility that an antagonism exists between zinc and copper when it comes to absorbing these elements in the intestines of the exclusively breast-fed Fulani infants.

Table 1: Characteristics of Fulani Mother-Baby Pairs

	Mother				Infacts				
Mother-baby pairs	Age (yrs)	Weight (kg)	Height (cm)	BMI (m/kg2)	Parity	Age (months)	Weight (kg)	Length (cm)	Gender (M/F)
1	18	43	161	16.5	1	5	3.7	54.5	M
2	17	47	164	17.6	1	12	5.4	58.0	M
3	30	59	164	21.9	5	2	3.5	52.5	M
4	32	56	163	21.1	6	2	2.0	50.0	F
5	17	47	155	19.6	1	8	1.8	46.0	M
6	25	54	150	24.0	2	20	6.0	62.0	M
7	30	68	171	23.2	7	20	5.0	60.0	M
8	28	50	171	17.1	6	20	7.0	65.0	M
9	20	45	162	17.2	3	8	7.0	54.5	M
10	25	55	166	20.0	5	20	7.0	61.5	M
11	18	44	162	16.8	1	12	5.0	59.0	M
12	30	52	155	21.6	8	5	4.0	54.0	M
13	18	34	149	15.3	1	16	6.0	60.0	F
14	18	43	147	19.6	2	21	5.0	54.5	M
15	35	52	158	20.8	4	2	4.0	51.0	M
16	25	42	161	18.9	2	6	4.0	52.0	F
17	15	46	163	18.4	1	16	8.0	63.0	M
18	28	49.6	170	19.1	7	16	6.5	67.0	M
19	45	56	170	21.1	10	7	3.0	56.0	M
20	30	52	157	18.0	8	5	3.0	55.0	M
21	25	61	168	21.1	2	3	2.0	57.5	M
22	18	53	157	21.5	4	20	5.0	60.5	M
23	15	57	168	20.2	1	20	7.0	68.0	F
24	25	52	157	21.1	5	8	4.0	57.5	F
25	32	43	172	14.5	4	7	4.0	59.0	M
26	30	39	157	15.8	7	16	3.0	54.4	F
27	25	42	149	19.0	6	2	4.0	49.4	F
28	20	50	150	22.4	1	3	4.0	48.5	F
29	35	40	164	14.9	8	8	4.0	54.0	F
30	38	51	156	21.0	8	20	6.0	64.5	M
31	18	53	167	19.0	1	6	4.0	57.5	M
32	36	43	153	18.4	8	8	5.0	54.0	M
33	27	49	152	21.2	2	24	6.0	64.0	M
34	35	49.5	157	20.1	7	5	3.5	50.0	M

Table 2: Macro-and trace element concentrations in the milk of Fulani women and women of selected populations worldwide

Mineral	Fulani (This Study)	Nigeria	Guatemala	Zaire	Ethiopia	Reference Range
Ca (mg/L)	244(182-389)	266	303	274	462+133	267+20
Cr (ug/L)	18(11-44)	4.36	1.32	1.07	NR	22.9+13
Cu (ug/L)	377(93 891)	278	246	201	370+200	280+26
Fe (ug/L)	755(208 3400)	556	385	523	470+190	450+71
Mg (mg/L)	31 (18 49)	29.0	34.1	37.8	25.62+2.87	30.2+15
Mn (ug/L)	11 (4 90)	15.8	3.79	11.2	NR	20.0+1.4
Zn (mg/L)	2.75(0.75 6.99)	1.68	2.61	1.92	6.59+2.1	1.60+0.30

[a] Median (range), [b] Median, [c] Mean + Stand. Dev.
NR, Not reported due to insufficient numbers of specimens containing measurable levels of mineral [(1)], Fransson GB, Gebre-Medhin M, Hambraeus L. The human milk contents of iron, copper, zinc, calcium, and magnesium in a population with habitually high intake of iron. Acta Paediatr Scand. 73: 471-476, 1984
[(2)], Minor and Trace Elements in Breast Milk, Report of a Joint WHO/IAEA Collaborative Study; pp. 11-97. World Health Organization Geneva, 1989; England.

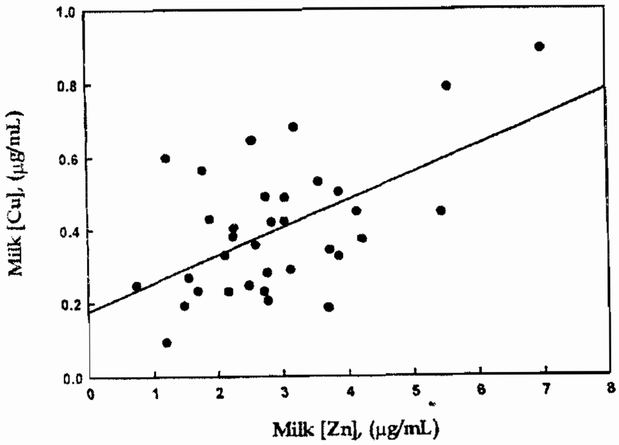
Table 3: Summary of the concentrations of trace elements in maternal and baby sera (ug/mL)

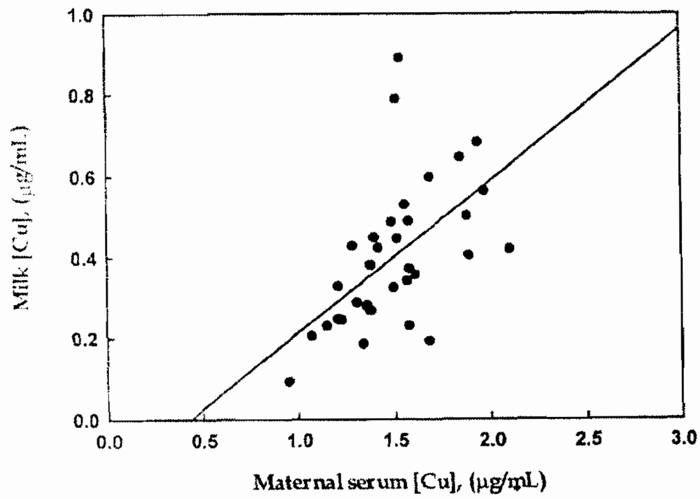
Mineral	Mother's serum Median (range)	Baby's serum	P-value	Adult	Reference Ranges Infant
Cu	85 (74 96)	93. (76.06)	0.35	86 104	88 - 108
Cr	0.025 (0.013 0.081)	0.015 (0.011 0.025)	0.74	0.000 0.002	0.000 0.002
	1.51 (0.95 2.10)	1.73 (0.70 3.56)	0.34	0.8 1.55	0.59 0.70 (1-5mn) 0.27 1.53 (6mn-5yr)
Cu					
Fe	0.68 (0.30 4.64)	1.56 (0.627 3.61)	0.08	0.3 1.5	0.60 2.25
Mg	19.2 (11.4 21.9)	20.1 (16.0 24.9)	0.15	12 24	1.6 2.2
Mn	0.017 (0.008 0.020)	0.005 (0.003 0.012)	0.92	0.000 0.008	0.000 0.08
Zn	0.53 (0.12 1.22)	1.31 (0.81 1.96)	0.20	0.65 2.56	0.66 - 1.44 (male 0-16 yr) 0.66 2.44 (female 0-16yr)

Reference values of University Hospital, University of New Mexico.

Table 4: Summary of correlations between the concentrations of minerals and trace elements in the milk of Fulani women and the sera of their exclusively breast-fed babies

Mineral	*Maternal serum* r	*Vs. Milk* p	*Milk Vs.* r	*Baby serum* p
Ca	0.08	NS	0.29	NS
Cr	0.43	NS	0.29	NS
Cu	0.57	< 0.001	0.43	0.02
Fe	0.37	0.05	0.11	NS
Mg	0.28	NS	0.03	NS
Mn	0.69	NS	0.21	NS
Zn	0.24	NS	0.05	NS





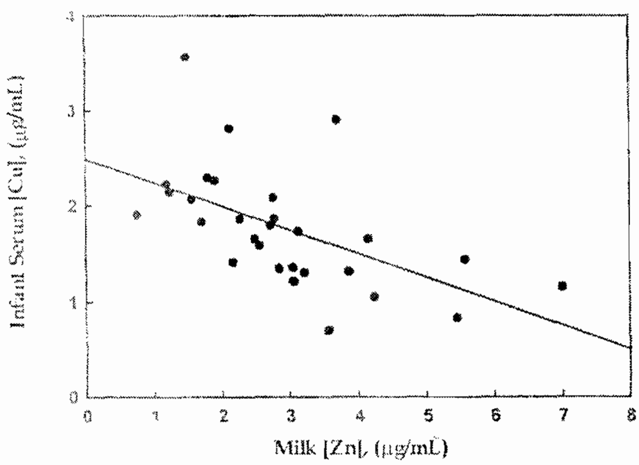


Figure Legends

Figure1, Scatter plot milk zinc concentrations vs. milk copper concentrations. $Y = 0.08x + 0.18$; $r = 0.56$; $p < 0.001$

Figure 2, Panel A: Scatter plot of concentrations of Ca in breast milk vs. time post-gestation. $Y = -3.75x + 303.9$; $r = 0.47$; $p = 0.005$.

Panel B: Scatter plot of concentrations of Cu in breast milk vs. time post-gestation. $Y = -1.26x + 0.54$; $r = 0.50$; $p = 0.003$.

Panel C: Scatter plot of concentrations of Fe in breast milk vs. time post-gestation. $Y = -3.74x + 1.35$; $r = 0.37$; $p = 0.03$.

Panel D: Scatter plot of concentrations of Zn in breast milk vs. time post-gestation. $Y = -0.11x + 4.21$; $r = 0.62$; $p < 0.001$.

Figure 3: Scatter plot of maternal serum copper concentrations vs. milk copper concentrations. $Y = 0.37x \; 0.16$; $r = 0.57$' $p < 0.001$.

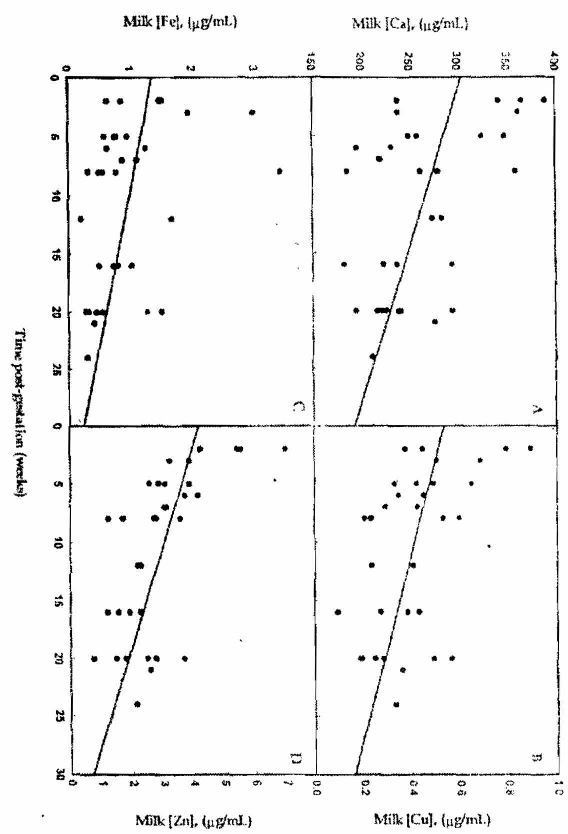
Figure 4: Scatter plot of milk copper concentrations vs. infant serum copper concentrations $Y = -1.45x + 2.33$; $r = 0.42$; $p = 0.020$.

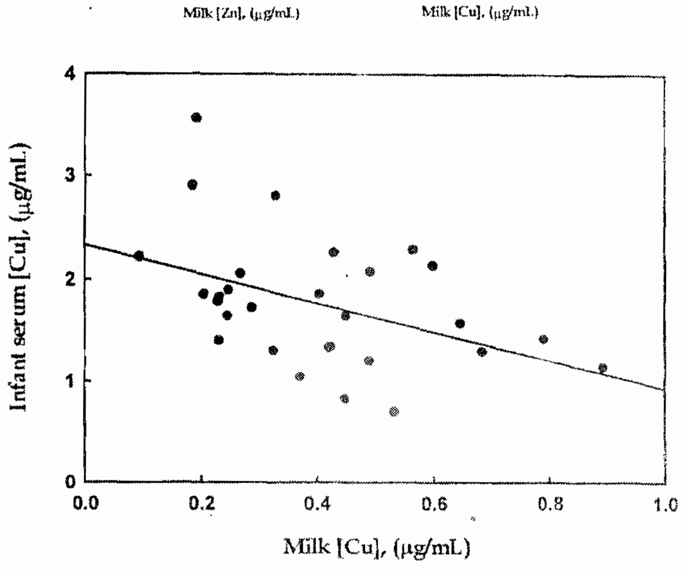
Figure 5: Scatter plot of milk zinc concentrations vs. infant serum copper concentrations. $Y = -0.25x + 2.48$; $r = 0.56$; $p = 0.00$

DISCUSSION

The main purpose of this study was to assess the quality of the milk of Fulani women with regard to its content of minerals and trace elements. Although detailed dietary analyses of the women who participated in this study are not available, it is generally recognized that the Fulani of the Jos Plateau consumes substantial quantities of dairy products including milk, cheese, and butter oil. In addition, millet provides the major source of carbohydrate calories. Green vegetables and mangos are obtained from farms surrounding the hamlets.

It is noteworthy that the concentrations of the minerals calcium and magnesium as well as the trace elements copper, zinc, iron, chromium and manganese in the milk of the Fulani women were within the range of values reported for the milk of other human populations worldwide (Table 2).





In fact, the levels of copper, iron and zinc in the milk of the Fulani women we studied were actually higher than those of most other populations that have been reported. We conclude that the levels of minerals and trace elements in the milk of Fulani women are comparable to those of women elsewhere in the world and potentially capable of satisfying the nutritional needs of their exclusively breast-fed infants.

With regard to the issue of how closely the mineral and trace element levels in the milk of the Fulani women paralleled those of the blood serum of these same lactating women, except for copper and iron (Table 4), no statistically significant correlations were noted. The copper concentration of the milk of the Fulani women correlated positively ($p<0.001$) and strongly ($r=0.57$) with that of their sera. Although the level of zinc in maternal serum did not correlate with the zinc concentration in the milk specimens ($p>0.05$), we did find a strong positive correlation ($r=0.56$, $p<0.001$) between the zinc and copper levels of the milk of the Fulani women (Fig. 1), meaning that when one of these metals was present in high concentration in milk, the other was high as well. The relatively high zinc concentrations in the milk of the Fulani women may be a result of the redistribution of high amounts of maternal tissue zinc that has been built up during pregnancy(10). The return to pre-pregnant zinc levels in the lactating woman may occur through loss during lactation. However, this may lead to a need for relatively high copper concentrations in milk to ensure adequate copper absorption due to possible antagonism that exists between copper and zinc absorption (discussed below). We hypothesize that this may include use by copper and zinc of a shared divalent cation transport system or a common component involved in the induction of separate secretory/transport mechanisms in mammary tissue involved in the secretion of trace minerals into milk.

Another reason we conducted our study was to evaluate the mineral and trace element status of the infants who were being exclusively breast-fed by Fulani women. The concentrations of calcium, iron and zinc we determined in the infants' sera were within the range of values reported for healthy infants elsewhere (Table 4). As for copper, its concentration in the serum of the Fulani infants was relatively high, exceeding the upper limits of the range of values for infants reported by other investigators(11,12). Our overall conclusion regarding this particular aspect of this present study is that the mineral and trace element requirements of the exclusively breast-fed Fulani infants appear to have been satisfied by the milk provided by their mothers.

Apart from answering these two questions that are of a practical nutritional nature, our data revealed an interesting biological phenomenon, that is, when we plotted either the copper (Fig. 4) or zinc (Fig. 5) concentration of the mothers' milk versus the copper concentration in the serum of the infants who were being nursed by these women, in each case a strong negative correlation was found. This observation points to a possible interaction between zinc and copper and one that may occur at the level of the infant's small intestine when these elements are absorbed. The relationship shown in Figure 5 is consistent with the hypothesis that zinc antagonizes the absorption of copper from the intestine of the Fulani infants. Indeed, this phenomenon has been well documented, although its mechanism remains elusive(13-

15). Previous studies have implicated metallothionine (MT), a cysteine-rich metal binding protein whose expression can be induced by zinc (16,17), in the mechanism of this antagonism. The hypothesis that high zinc levels induce MT expression and that MT sequesters copper in the enterocyte(16, 17) may account, in part at least, for the apparent antagonism that exists between co er and zinc in the intestine. However, more recent studies involving MT gene-null mice have shown similar antagonism of copper transport by zinc in the absence of MT, thus implicating an MT independent mechanism to account for the apparent antagonism between copper and zinc(18,19). One possibility is the gene product identified in Menkes disease, a disorder affecting copper absorption from the intestine and which is caused by a recessive mutation on the X chromosome in a region encoding a membrane-bound P-type ATPase that transports copper(9,20-22). Although copper uptake by enterocytes in Menkes disease is relatively normal, copper transport across the basolateral membrane is impaired, resulting in a frank copper deficiency. How zinc may be interacting with this protein, if at all, remains to be seen.

CONCLUSION

In conclusion, our main findings are that, despite the remarkable low BMI's of many of the women who participated in our study (some as low as 14.5kg/m^2), the milk of the Fulani women and the blood of their exclusively breast-fed infants contain levels of seven important minerals and trace elements essential to the proper development of infants during the first six months of life that compare favourably to those of populations living in more developed parts of the world. Finally, our data raise questions about possible interactions between zinc and copper that might be occurring in the intestine of the breast-fed infant.

REFERENCES

1. Boerma JT, Rutstein SO, Sommerfelt AE, Bicego GT. Bottle use for infant feeding in developing countries: data from the Demographic and Health Surveys. Has the bottle battle been lost? J. Trop. Pediatr. 1991; 37: 116-123.
2. Calvani S. Health crisis in African Countries. Lancet 1992; 339-126.
3. Okolo SN, VanderJagt TJ, Vu T, VanderJagt TA, VanderJgt DJ, Okonji M, Huang Y-S, Chuang L-T, Onwuanaku C, Glew RH. The fatty acid composition of human milk in northern Nigeria. J. Hum. Lact 2000 (in press).
4. Reeves LM, Onwuanaku BS, Okolo SN, Glew RH, Blackwell W, Magnussen MA, Bhatt SK, Okonji M, VanderJgt DJ. Total antioxidant capacity of human milk from Nigeria and Nepal. Life Sci. 2000 (submitted).
5. Schmeits B, Okolo SN, VanderJgt DJ, Huang VS, Chuang L-t, Mata JR, Tsin A, Glew RH. Content of lipid mutrients in the milk of Fulani women. Nut. Res. 1999, 15: 113-120.
6. Jason J. Breast-feeding in 1991. N. Engl. J. Med. 1991: 325-1036.
7. Wilmoth TA, Elder JP. An assessment of research on breast-feeding promotion strategies in developing countries. Soc. Sci. Med. 1995; 41: 579-594.
8. Lonnerdal B. Effects of milk and milk components on calcium, magnesium, and trace element absorption during infancy. Phys. Rev. 1997; 77:643-669.
9. Mercer JFB, Livingston J, Hall B, Paynter JA, Begy C, Chandrasekharappa S, Lockhart P, Grimes A, Bhave M, Siemieniak D, Grover TW. Isolation of a partial candidate gene for Menkes disease by positional cloning. Nat. Genet. 1993; 3: 20-23.
10. Trace Elements in Human Nutrition and Health. Geneva, Switzerland: 1996. World Health Organization (WHO) 84.
11. Fransson GB, Gebre-Medhin M, Hambraeus L. The human milk contents of iron, copper, zinc, calcium and magnesium in a population with habitually high intake of iron. Acta Paediatr. Scand 1984; 73: 471-476.
12. Minor and Trace Elements in Breast Milk, Report of a Joint WHO/IAEA Collaborative Study. Geneva, Switzerland: 1989. World Health Organization (WHO) 11-97.
13. Bremner I, Beattie HJ. Copper and zinc metabolism in health and disease: Speciation and interactions. Proc. Nutr. Soc. 1995: 54: 489-499.
14. Reeves PG, Briske-Anderson M, Johnson LA. Physiologic concentrations of zinc affect the kinetics of copper uptake and transport in the human intestinal cell model. Caco-2. J. Nutr. 1998; 128: 1794-1801.
15. Reeves PG, Briske-Anderson M, Newman SM, Jr. High zinc concentrations in culture media affect copper uptake and transport in differentiated human colon adenocarcinoma cells. J. Nutr. 1996: 126: 1701-1712.
16. Fischer PWF, Giroux A, L'Abbe MR. The effect of dietary zinc on intestinal copper absorption. An. J.Clin. Nutr. 1981; 34: 1670-1675.
17. Fischer PWF, Ginoux A, L'Abbe MR. Effects of zinc on mucosal copper binding and on the kinetics of copper absorption. J. Nutr. 1983; 113: 462-468.
18. Masters BA, Kelly EJ, Quaife CJ, Brinster RL, Palmiter RD. Targeted disruption of metallothionine I and II genes increases sensitivity to cadmium. Proc. Natl. Acad. Sci. 1994; 91: 584-588.
19. Reeves PG. Copper metabolism in metallothionein-null mice fed a high zinc diet. J. Nutr. Biochem. 1998; 9: 598-601.
20. Harris ED, Yongchang Q, Reddy MCM. Genes regulating copper metabolism. Molecular and Cellular Biochemistry 1998; 188: 57-62.
21. Tumer Z, Horn N. Menkes disease: recent advances and new aspects. J. Med. Gen. 1997; 34: 265-274.
22. Vulpe C, Levinson B, Whitney S, Packman S, Gitschier J,. Isolation of a candidate gene for Menkes disease and evidence that it encodes a copper-transporting ATPase. Nat. Genet. 1993; 3: 7-13.