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CLINICAL NUTRITION RESEARCH

Association of Maternal Diet With Zinc, Copper, and Iron Concentrations in Transitional Human Milk Produced by Korean Mothers

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The aims of this study were to evaluate zinc, copper, and iron concentrations in the transitory milk of Korean lactating mothers and to investigate the relationship between these concentrations and maternal diet. Human milk samples were collected between 5 and 15 days postpartum from 96 healthy, lactating mothers in postpartum care centers in Seoul, Korea. Dietary intake during lactation was determined based on a 3-day dietary record. The mean zinc, copper, and iron concentrations in the human milk samples collected were 3.88 ± 1.74 mg/L, 0.69 ± 0.25 mg/L, and 5.85 ± 8.53 mg/L, respectively. The mothers who consumed alcoholic beverages during pregnancy had tended to have lower concentrations of zinc and copper, as well as significantly lower concentrations of iron, in their milk ($p < 0.047$). In contrast, the mothers who took daily supplements had much higher iron concentrations in their milk ($p = 0.002$). Dietary intakes of zinc, copper, and iron during lactation did not affect the concentrations of zinc, copper, and iron in the milk samples analyzed. Intakes of vitamin C, selenium, and iodine were associated with the concentration of copper in the milk samples analyzed, and consumption of food categorized as 'meat and meat products' was positively associated with the concentration of zinc. Consumption of rice was the top contributor to the concentrations of all three minerals. In conclusion, associations between maternal diet and nutrient concentrations in transitory human milk can provide useful information, particularly in regard to infant growth.

Keywords: Human transitional milk, Zinc, Copper, Iron, Dietary intake

Introduction

Human milk is considered to provide an infant with all the essential nutrients needed in the early stages of life following birth [1]. Correspondingly, the World Health Organization recommends that breastfeeding should constitute the exclusive source of nutrition for infants up to at least 6 months of age [2]. Therefore, investigations of human milk content and the factors affecting human milk composition are important for improving the growth and development of breastfeeding infants and for establishing nutritional requirements for lactating mothers.

Minerals are essential nutrients for the growth and development of healthy tissues for both adults and infants [3]. The concentrations of most minerals in human milk remain fairly constant during lactation, except for zinc, copper, and iron. The latter three minerals have their highest concentrations immediately after delivery, and then these concentrations decrease over several months thereafter [4-6]. For infants with deficiencies in these trace elements, adverse effects on growth, motor development, cell-mediated immunity, and skeletal development have been observed [7].

Zinc is essential for cellular metabolic processes, immunity, neurological function and sexual development. Zinc also plays a fundamental role in growth and development [8]. Copper has an important role in the growth and formation of bone and in the nervous system [9], while iron is a component of hemoglobin in the blood and has an essential role in biological oxidation [10]. Copper and iron affect the absorption of zinc by competing with each other, thereby demonstrating how the excessive intake of one essential element may compromise the intake and status of another.

Studies of maternal diet in relation to concentrations of zinc, copper, and iron in human milk have produced mixed results. For example, in one study [4], no relationship between the iron and zinc content of the human milk and maternal nutritional status was observed, while another study [11] also reported the absence of relationship between maternal intake of zinc and zinc concentration in human milk. However, Arnaud and Favier [12] reported that the concentration of copper in human milk is related to a mother's body mass index (BMI). A study by Leotsinidis et al. [13] further revealed that dietary habits play a role in determining mineral levels in human milk, while smoking affected copper levels. Similarly, both diet and environment have been shown to strongly influence zinc distribution [14].

Transitory milk is secreted between 5 and 15 days after delivery [15]. During this period, the nutritional components and immune substances vary in human milk. Several researchers have reported that changes in trace elements concentrations, particularly zinc, copper, and iron in human milk can vary according to a mother's diet and its characteristics [12,14]. However, many studies have focused on examining the components of colostrum and mature milk, while data regarding the concentrations of zinc, copper, and iron in transitory human milk and in relation to maternal diet and environmental factors are limited. Therefore, the aim of the present study was to investigate whether maternal diet and other environmental factors during lactation affect the concentrations of zinc, copper, and iron in transitory human milk, thereby providing useful information for promoting healthy infant growth and development.

Materials and Methods

Study subjects

Ninety-six women who gave birth to healthy full-term babies between 10 June 2013 and 27 January 2014 agreed to participate in this study. All of the women were staying at postpartum care centers in Seoul and Gyeonggi province in South Korea and were in good health, they did not have any known diseases (e.g., hypertension, gestational diabetes, etc), and they provided informed written consent. Convenience sampling was conducted between 5 and 15 days postpartum. This was approved by the Institutional Review Board (IRB) of Ewha Womans University (IRB no: 2013-51-18).

Each participant in this study was interviewed by a trained dietician. Information on general characteristics, including age, height, present weight, BMI, and weight gain during pregnancy, supplementation, and smoking and drinking status, was collected for each mother. For the newborn infants, age, gender, and birth weight were recorded.

Dietary intake assessment

All subjects submitted a 3-day dietary record for dietary assessment that was performed within two weeks of their interview. Each participant was instructed on how to complete the dietary record and was asked to record details regarding the quantity and types of foods they consumed. Food models were used to represent the amount of foods consumed. Dietary intake data were assessed with the Computer Aided Nutritional Analysis Program software (CANPro, version 4.0, The Korean Nutrition Society, Seoul, Korea 2011). Following the assessment of each mother's dietary intake, 28-nutrient and

16-food group classifications during lactation were identified. Each daily nutrient intake profile was compared with the estimated average requirements recommended by the 2010 Korean Dietary Reference Intakes 2010 (KDRIs 2010, the Korean Nutrition Society, Seoul, Korea).

Breast milk collection

Human milk samples (20-50 mL) were collected from 96 healthy lactating mothers between 5 and 15 days after they gave birth. The samples were obtained with an electronic breast pump and were collected into polyethylene bottles. The samples were sealed, placed on ice, then transported to our laboratory to be stored at -20°C until use.

Sample assessment

The contents of each milk sample were analyzed by spectrophotometry. Briefly, nitric acid (HNO_3) was added to 1 mL of each milk sample. After each sample underwent digestion under pressure, the samples were quantitatively transferred to clean polyethylene tubes and were diluted in 10 mL of high purity water. Copper and zinc content were analyzed by using an inductively coupled plasma mass spectrometer (NEXION 350D, Perkin Elmer, Waltham, MA, USA). To analyze the iron concentration of each sample, samples were centrifuged at 690 x g for 20 min and then the aqueous phase was collected. Iron concentrations were determined with an automated biochemical analyzer (BS-2000M, Mindary, Shenzhen, China). The detection limits, defined as three standard deviations for the blank, were 0.15 μg/L for copper, 39 μg/L for iron, and 11 μg/L for zinc. The accuracy of these analyses were checked by various methods and a reference material (BCR, Geel, Belgium) was included for each test.

Statistical analysis

Statistical analyses were performed using the SAS statistic software package, version 9.3 (SAS Institute, Cary, NC, USA). For continuous data, mean ± standard deviation was calculated. Categorical data were reported as the number and percentage being occupied. Normal probability plots and the Kolmogorov–Smirnov test were used to determine whether the variable followed a normal distribution. Differences between two groups were compared with an independent t-test. When the variables did not exhibit a normal distribution, the variables were subjected to the Wilcoxon's-rank sum test to compare two groups. To analyze the association between continuous variables, Pearson's correlation test was used for data

with a normal distribution, while Spearman's rank correlation method was used for data that were not normally distributed. The correlation analysis for dietary intake was adjusted for age, weight, alcohol drinking history, and supplementation during pregnancy. Association between the food items consumed and zinc, copper, and iron concentrations in the human milk samples analyzed were identified with multiple regression models. A stepwise forward selection procedure was used where independent variables and confounders were added to the model according to their significance. For all of the analyses performed, a p-value less than 0.05 was considered statistically significant.

Results

General characteristics of the subjects

The general characteristics of the mothers and their babies that were examined are presented in Table 1. The mean age of the mothers was 31.8 ± 3.9 years, their mean height was 162.0 ± 4.8 cm, and their mean weight was 60.6 ± 8.2 kg. The mean BMI for the mothers was 23.1 ± 2.8 kg/m^2. The mothers gained an average of 13.4 ± 5.3 kg during pregnancy and 82.3% took nutritional supplements during pregnancy. None of the mothers smoked during their pregnancy, although 11.5% of the lactating mothers reported a history of smoking before their pregnancy and 88.5% had no history of smoking. Only 14.6% of the lactating mothers consumed alcoholic beverages during pregnancy. Of the babies born, 52.1% were male and the average birth weight was 3.2 ± 0.4 kg. The average age of babies was 11.2 ± 2.8 days.

Zinc, copper, and iron concentrations in the human transitional milk and their concentrations according to alcohol drinking and mineral supplement intake

The concentrations of zinc, copper, and iron that were detected in the human milk samples analyzed are listed in Tables 2. The mean concentrations were 3.88 ± 1.74 mg/L (range: 0.80 – 10.00 mg/L), 0.69 ± 0.25 mg/L (range: 0.08 – 1.50 mg/L), and 5.85 ± 8.53 mg/L (range: 0.05 –34.00 mg/L), respectively.

The concentrations of these three minerals in the human milk samples examined according to alcohol and supplement intake of each mother are presented in Tables 3. The mothers who consumed alcoholic beverages during pregnancy tended to have lower concentrations of zinc and copper in their milk, as well as a significantly lower concentration of iron (p = 0.047). In contrast, the iron concentration in the milk samples

Table 1. General characteristics of the subjects and their baby (n = 96)

	Mean $\pm$ SD or N (%)
Mothers	
Age, years	31.8 $\pm$ 3.9[*]
Height, cm	162.0 $\pm$ 4.8
Present weight, kg	60.6 $\pm$ 8.2
BMI, kg/m^2	23.1 $\pm$ 2.8
Weight gain in pregnancy, kg	13.4 $\pm$ 5.3
Supplement in pregnancy	
Yes	79 (82.3)[†]
No	17 (17.7)
Supplement type	
Mineral supplement	53 (55.2)
Mineral supplement and vitamins	26 (27.1)
None	17 (17.7)
Supplement intake frequency	
Daily	64 (66.7)
Non-Daily	15 (15.6)
None	17 (17.7)
Smoking before pregnancy	
Yes	11 (11.5)
No	85 (88.5)
Drinking in pregnancy	
Yes	14 (14.6)
No	82 (85.4)
Newborn infants	
Age, days	11.2 $\pm$ 2.8
Sex	
Male	50 (52.1)
Female	46 (47.9)
Birth weight, kg	3.2 $\pm$ 0.4

BMI: body mass index (kg/m^2), Mineral supplement: calcium supplement, iron supplement, or multi-mineral supplement, Non-Daily: Under once or twice per week. [*]Values are Mean $\pm$ SD; [†]Values are N (%).

obtained from the mothers who took daily supplements was significantly higher than in the milk samples from the mothers who did not take daily supplements (p = 0.002).

Daily nutrient intakes during lactation and concentration of zinc, copper, and iron in human milk

Table 4 presents the daily nutrient intake data during lacta-tion and the correlation of these data with zinc, copper, and iron concentrations that were detected in the milk samples. The average energy intake for the mothers was 2092.0 $\pm$ 314.7 kcal, and their average daily intake of iron, zinc, and copper were 23.6 $\pm$ 15.5 mg, 12.5 $\pm$ 2.2 mg, and 1.8 $\pm$ 1.9 mg, respec-tively. These amounts of mineral intakes were sufficient to meet KDRIs 2010. While zinc concentrations in the milk sam-ples had no relationship with any particular nutrient intakes during lactation, copper concentrations in the milk samples were positively associated with the consumption of vitamin C (R = 0.236, p = 0.022) and selenium (R = 0.232, p = 0.025), and were inversely associated with iodine intake (R = -0.267, p = 0.013). In addition, iron concentrations in the milk samples significantly correlated with the maternal daily intake of fat (R = 0.215, p = 0.038).

Food intakes by groups and zinc, copper, and iron concentrations in human milk

Table 5 lists the daily food intake profiles during lactation for the present study, as well as their correlation with zinc, copper, and iron concentrations in the milk samples analyzed. The intake data were divided into 15 categories, and were also classified into an animal-based group and a plant-based group. Among the former group, 'meat and meat products' was found to positively correlate with zinc concentrations in the milk samples. The other food groups, except 'beverage' was shown no significant correlation with the concentrations of zinc, copper, and iron.

The cumulative percent contribution and cumulative R^2 of the top 20 food items that affected zinc, copper, and iron concentrations are presented in Table 6. For this analysis, the dietary intake of the mothers was used to determine the pri-ority and cumulative R^2 of each related food item. Rice was the top contributor to the intake of all three minerals. For zinc, beef, followed by pork and dried seaweed were key food items. However, after considering between-person variability, soy bean sauce, wheat flour, and raw seaweed were the top three food items that contributed to zinc concentrations. In con-trast, higher levels of both copper and iron were associated with the intake of lemon, bread with jam, and fly fish caviar.

Discussion

Transitory milk shares some of the characteristics of colos-trum, and then milk production is rapidly increased to support the nutritional and developmental requirements of a growing

Table 2. Analysis of zinc, copper, and iron levels in transitional human milk and comparison with reported studies

Reference	Country	Stage of lactation	Zinc, mg/L	Copper, mg/L	Iron, mg/L	Ref. No.
Present study	Korea	5–15 day	3.88 ± 1.74[*]	0.69 ± 0.25	0.21[§]	
			(0.80–10.00)[†]	(0.08–1.50)	(0.05–34.00)	
Similar study about transitory milk						
Trugo et al., 1988	Brazil	6–36 day			0.90	[50]
Friel et al., 1999	Canada	7 day		0.85		[51]
		14 day		0.82		[51]
Mendelson et al., 1982	Canada	8–10 day		0.73		[21]
Arnaud and Fabier et al., 1995	France	Transitory	5.03	0.95	0.31	[12]
Sann et al., 1981	France	7–14 day		0.63		[20]
Dorner et al., 1989	Germany		2.06	0.83	0.43	[17]
Leotsinidis et al., 2005	Greece	14 day	2.99	0.39	0.46	[13]
Tripathi et al., 1999	India		1.77	1.95		[52]
Aquilio et al.,1996	Italy	2–6 day		0.27	0.26	[53]
		12–16 day				[53]
Perrone et al., 1994	Italy	1 week		0.49	0.62	[54]
		1–2 week		0.36	0.46	[54]
Kwon et al., 2004	Korea	Transitory	2.92[†]		0.87	[19]
Kim et al., 2004	Korea	7–15 day	3.7	0.43	0.48	[55]
Lee et al., 2000	Korea	0.5 month	3.94	0.61		[18]
Yang et al., 1995	Korea	7 day	4.2			[56]
Choi et al., 1991	Korea	7 day	3.5	0.34	0.32	[57]
		14 day	3.4	0.32	0.28	[57]
Atinmo and Omololu et al., 1982	Nigeria	1 week		0.34	0.59	[58]
		1–2 week		0.31	0.46	[58]
Wasowicz et al., 2001	Poland	Transitory	0.37	0.39		[59]
Jirapinyo et al., 1985	Thailand	7–28 day		0.42		[60]
Turan et al., 2001	Turkey	Transitory	0.60–8.70	0.10–3.60	0.20–4.00	[61]
Parr et al., 1996	Turkey	Transitory	0.70–2.61	0.19–0.31	0.35–0.72	[23]
Lemons et al., 1982	UK	7–56 day			0.04	[62]
Casey et al., 1989	US	Transitory		0.59–1.33	0.12–28.28	[22]
Moran et al., 1983	US	6–10 day		1.04		[63]

All units of values are converted to mg/L.
[*]Values are Mean ± SD; [†]Values are Minimum–Maximum; [‡]Values are Mean; [§]Values are median only for iron in the present study.

infant [15]. Zinc, copper, and iron concentrations are higher in colostrum and transitory milk compared with mature milk, and this corresponds with the greater need of newborn infants for larger amounts of these minerals and the low volume of milk intake early in their life [16].

In Table 2, zinc, copper, and iron concentrations in tran-sitional human milk samples that were analyzed in previously reported studies are listed. Zinc (3.88 mg/L) and copper concentrations (0.69 mg/L) in the lactating participants of the present study are within the range previously reported. Regarding zinc, the mean concentrations from the present study were similar or slightly lower than that reported in other

Table 3. Zinc, copper, and iron concentrations in human transitional milk according to alcohol drinking and mineral supplement intake

	Zinc, mg/L		Copper, mg/L		Iron, mg/L	
Alcohol drinking in pregnancy						
Yes (n = 14)	0.38 ± 0.18	p = 0.136	0.67 ± 0.24	p = 0.108	5.12 ± 7.96	p = 0.047
No (n = 82)	0.45 ± 0.16		0.79 ± 0.29		10.10 ± 10.67	
Mineral supplement intake frequency during pregnancy						
Daily (n = 64)	0.36 ± 0.18	p = 0.280	0.69 ± 0.27	p = 0.717	7.36 ± 9.10	p = 0.002
Non daily (n = 15)	0.40 ± 0.17		0.70 ± 0.22		2.83 ± 6.36	

Values are concentration of zinc, copper and iron in breast milk according to lifestyle of mothers (Mean ± SD). Comparison between two groups by t-test for zinc and copper, but Wilcoxon rank sum for Iron.
Mineral supplement: calcium supplement or iron supplement or multi-mineral supplement, Non daily: Under once or twice per a weeks.

studies conducted in Germany [17] (2.06 mg/L), Korea [18,19] (2.92 mg/L, 3.94 mg/L), and Greece [13] (2.99 mg/L). Conversely, zinc concentrations in the transitory milk of lactating mothers in France were 5.03 mg/L, and this is higher than the concentration determined in the present study [12]. Regarding mean copper concentrations in human milk, various values have been reported worldwide for similar stages of lactation. However, the mean concentration of copper determined in the present study did not markedly differ from those reported in other studies, particularly the values reported by studies conducted in France (0.61 mg/L) [20], Korea (0.63 mg/L) [18], and Canada (0.73 mg/L) [21]. However, the copper concentrations reported by studies conducted in the United States (0.59–1.33 mg/L) [22] and Turkey (0.10–3.60 mg/L) [23] are higher than that of the present study. These differences may be due to geographical and nutritional influences associated with these populations [22]. In contrast with the zinc and copper concentrations, the mean iron concentrations reported in the present study tended to be higher than the values previously reported in other studies. However, iron concentrations in the milk samples of the present study did not exhibit a normal distribution, and this could account for the difference in results. Iron supplementation up to comparatively high levels is common in many affluent countries during pregnancy, and often continues into the lactation period [13]. Thus, a wide deviation in milk iron concentrations is possibly due to iron supplementation. Several studies have also mentioned that these differences may be explained by geographical and nutritional influences [22-24], as well as differences in sampling procedures [25].

Among the trace elements present in human milk, only iron was significantly associated with some of the general characteristics of the participants examined. For example, a higher concentration of iron was detected in the milk sample that was obtained from mothers who gained less weight during pregnancy, who did not consume alcoholic beverages during pregnancy, and who took daily supplements. Numerous studies have studied the effects of mineral supplements on the concentrations of trace elements in human milk, and most have reported that maternal supplements do not affect the concentrations of trace minerals in human milk [26,27]. For example, Mello-Neto et al. [27] found no difference in iron concentrations in the human milk produced by women who took daily supplements during pregnancy and the lactating period versus those that did not. In another study, the effect of maternal zinc supplements was very weak and was only apparent in woman who had taken zinc supplements for long periods of time [28]. In the present study, an association between daily iron supplementation and iron concentration in the milk samples analyzed was observed. In Korea, iron supplementation is recommended to minimize the harmful effects of iron-deficiency-based anemia during pregnancy, based on the assumption that long-term iron supplementation during pregnancy and lactation influences the iron concentrations in human milk. A potential detrimental aspect of iron supplementation is the risk of inhibiting the absorption of other micronutrients, including copper and zinc [29-34]. Iron metabolism involves both zinc and copper, and this mainly mediated via divalent metal transporter 1 [35-38] and human copper transporter [38]. It has been reported that women receiving iron supplements can have zinc malabsorption [32]. However, in the present study, iron supplementation did not appear to influence the levels of copper and zinc in transitional human milk.

In previous studies, a correlation between maternal intakes of zinc, copper, and iron and the concentrations of these minerals, in human milk has not been observed [11,28,39]. Correspondingly, the results of the present study also did not show

Table 4. Correlations between daily nutrient intakes during lactation and zinc, copper and iron concentrations in human milk (n = 96)

	Daily intakes (Mean ± SD)	%RDA	Zinc		Copper		Iron	
			Correlation coefficients	p-value	Correlation coefficients	p-value	Correlation coefficients	p-value
Nutrients								
Energy, kcal	2092.0 ± 314.7	92.0*	−0.003	0.980	0.130	0.214	0.113	0.283
Protein, g	91.5 ± 18.3	130.7	0.077	0.464	−0.054	0.604	0.030	0.772
Fat, g	74.5 ± 33.6	-	0.022	0.835	0.154	0.141	0.215	0.038
Carbohydrate, g	324.8 ± 291.7	-	−0.023	0.827	0.031	0.766	0.077	0.462
Cholesterol, mg	405.2 ± 1115.9	-	−0.029	0.785	−0.087	0.409	0.137	0.191
Dietary fiber, g	31.6 ± 7.0	126.5	0.040	0.705	0.113	0.280	−0.022	0.834
Vitamin A, μg RE	1132.0 ± 355.0	99.3	0.101	0.335	−0.141	0.179	−0.100	0.343
Retinol, μg	149.9 ± 252.6	-	0.015	0.886	0.176	0.091	0.065	0.539
β-carotene, μg	6138.0 ± 1998.0	-	0.113	0.281	−0.136	0.193	−0.102	0.332
Vitamin B_1, mg	4.4 ± 20.6	294.2	0.014	0.891	0.171	0.102	0.083	0.430
Vitamin B_2, mg	1.6 ± 0.6	98.2	−0.047	0.653	−0.046	0.661	−0.052	0.623
Niacin, mg	20.6 ± 4.6	108.2	0.017	0.873	−0.038	0.718	0.039	0.708
Vitamin B_6, mg	2.5 ± 3.2	112.8	0.005	0.956	0.143	0.170	−0.057	0.587
Folate, μg	587.4 ± 144.0	106.8	0.029	0.782	−0.053	0.616	0.127	0.225
Vitamin C, mg	122.4 ± 75.5	90.7	0.036	0.734	0.236	0.022	0.021	0.843
Vitamin E, mg	27.9 ± 6.7	214.5†	0.023	0.825	−0.029	0.781	0.201	0.054
Calcium, mg	659.5 ± 171.6	64.7	0.081	0.438	0.001	0.999	0.025	0.811
Phosphorus, mg	1287.0 ± 266.1	183.9	0.040	0.701	−0.029	0.782	0.037	0.726
Iron, mg	23.2 ± 15.5	165.3	−0.002	0.981	0.129	0.217	−0.195	0.062
Potassium, mg	4084.0 ± 846.1	104.7†	0.071	0.501	−0.045	0.671	0.047	0.653
Sodium, mg	6345.0 ± 1334.0	423.0†	0.048	0.651	−0.080	0.445	0.112	0.287
Zinc, mg	12.5 ± 2.2	96.3	0.037	0.723	0.064	0.542	−0.097	0.354
Copper, mg	1.8 ± 1.9	144.1	−0.004	0.972	0.138	0.187	−0.075	0.478
Magnesium, mg	190.5 ± 668.7	68.1	0.008	0.942	0.166	0.112	0.194	0.062
Selenium, μg	159.2 ± 227.4	244.9	0.041	0.694	0.232	0.025	−0.101	0.337
Manganese, mg	4.3 ± 1.4	121.8†	0.010	0.928	0.133	0.203	−0.170	0.103
Chlorine, mg	533.1 ± 1089.0	23.2†	−0.003	0.979	0.186	0.075	0.175	0.094
Iodine, μg	1699.0 ± 465.0	514.9	0.165	0.115	−0.267	0.013	0.060	0.567

Analyzed by Pearson's correlation coefficient for zinc and copper, but Spearman's rank correlation for Iron. Adjusted for age, weight experience of drinking, and supplement.
RDA: Recommended dietary intake by dietary reference intake of Korean 2010.
*Estimated energy requirements by dietary reference intake of Korean 2010; †Adequate intake by dietary reference intake of Korean 2010.

a clear correlation between dietary zinc intake of the mother and zinc concentrations in human milk. This may be due to active transport mechanisms involving zinc, copper, and iron in the mammary gland [40-43]. However, iron concentrations in human milk were positively associated with a mother's dietary intake of fat, cholesterol, and magnesium, which may be due to the consumption of meat and meat products. Numerous studies have also reported that intake of dietary copper does not affect copper concentrations in human milk [44-48]. However, in the present study, daily intakes of vitamin C, selenium,

Table 5. Correlations between food intakes by groups during lactation and zinc, copper and iron concentrations in human milk (n = 96)

	Daily intakes (Mean ± SD)	Zinc		Copper		Iron	
		Correlation coefficients	p-value	Correlation coefficients	p-value	Correlation coefficients	p-value
Food groups							
Meat and meat products, g	114.1 ± 37.9	0.206	0.048	-0.071	0.500	-0.054	0.607
Fish and fish products, g	99.4 ± 35.6	0.135	0.196	-0.083	0.432	-0.187	0.073
Egg and egg products, g	28.6 ± 7.3	-0.177	0.090	0.041	0.694	0.009	0.934
Milk and milk products, g	20.9 ± 20.8	0.095	0.366	0.045	0.648	-0.079	0.452
Cereals and Cereal products, g	319.7 ± 88.0	0.074	0.479	0.031	0.772	0.121	0.246
Potatoes and starch products, g	55.4 ± 34.0	-0.191	0.066	0.135	0.197	-0.138	0.186
Sugar and sugar products, g	11.6 ± 4.4	-0.023	0.827	0.031	0.766	0.077	0.462
Beans and bean products, g	174.7 ± 100.3	-0.093	0.374	-0.109	0.297	0.058	0.584
Nut, seeds and products, g	12.6 ± 13.4	0.118	0.260	-0.066	0.531	-0.038	0.712
Vegetables, g	423.0 ± 137.0	0.086	0.414	0.098	0.352	-0.090	0.391
Fruits, g	115.8 ± 58.3	-0.035	0.740	0.048	0.649	0.148	0.158
Mushrooms, g	21.3 ± 16.9	-0.086	0.412	-0.149	0.155	0.112	0.285
Seaweeds, g	16.5 ± 4.1	0.124	0.235	0.048	0.650	-0.007	0.948
Fats and oils, g	23.1 ± 6.5	0.201	0.053	-0.002	0.986	-0.195	0.061
Beverage, g	2.7 ± 5.3	0.032	0.760	-0.019	0.860	0.389	<0.001
Animal group total, g	263.1 ± 73.6	0.194	0.063	-0.039	0.712	-0.169	0.106
Plant group total, g	1174.0 ± 238.9	0.010	0.925	0.040	0.707	0.048	0.647
Total, g	1437.0 ± 286.0	0.058	0.581	0.023	0.827	-0.005	0.963

Analyzed by Pearson's correlation method for zinc and copper, but Spearman's rank correlation for Iron. Adjusted for age, weight experience of drinking, and supplement.

and iodine were related to copper concentrations in the milk samples. Maru et al. [49] previously reported that differences in copper concentrations in human milk between rural and urban mothers could be attributed to variations in the dietary intake of the mothers.

A human's requirement for iron and zinc may be more easily met with animal-based, higher-protein meal plans which provide these nutrients in their most bioavailable form. Many studies have investigated the contents of human milk in relation to dietary nutrient intake, although the use of categorized food groups in these studies has been limited. In the present study, zinc concentrations in the milk samples analyzed were found to be positively influenced by the intake of meat and meat products, while fats and oils appeared to be positive factors for zinc. In contrast, dietary intakes appeared to have less effects on copper and iron concentrations in human milk in the present study. Regarding iron concentrations, there

were found to be positively associated with the consumption of certain beverages, including drinks containing fruit nectar, shakes, coffee, and soda. It is difficult to explain these observations, and thus, further studies are needed.

When the major food items that influenced zinc, copper, and iron concentrations in human milk were analyzed by stepwise multiple regression, rice, a staple food of the Korean diet, was the top contributor to all three minerals. For zinc intake, animal-based food items, including beef, pork, eggs, and chicken, were also important contributors. In a similar study [13], zinc levels were found to be associated with the consumption of fruit and rice, while copper levels were appeared to increase following the consumption of fruits and potatoes. Moreover, plant-based food items such as rice, soy milk, cucumber, and red beans were found to have greater effects on copper intake than animal-based food items.

Table 6. Cumulative %contribution and cumulative R² of top 20 for zinc, copper, and iron intakes

Zinc					Copper					Iron				
Food item	No	Cum%	Food item	Cum R²	Food item	No	Cum%	Food item	Cum R²	Food item	No	Cum%	Food item	Cum R²
Rice	1	25.5	Soy bean sauce	0.23	Rice	1	21.4	Lemon	0.57	Rice	1	15.0	Lemon	0.56
Beef	2	35.8	Wheat flour	0.34	Soy milk	2	33.0	Bread with jam	0.88	Beef	2	25.9	Bread with jam	0.84
Pork	3	40.9	Raw seaweed	0.40	Cucumber	3	36.7	Fly fish caviar	0.98	Dried seaweed	3	31.7	Fly fish caviar	0.94
Dried seaweed	4	44.7	Cockle	0.46	Red bean	4	39.6	Salt	0.98	Soy milk	4	37.3	Salt	0.95
Soy milk	5	47.4	Shrimp	0.53	Shrimp	5	42.4	Soy milk	0.98	Pumpkin	5	39.7	Pie	0.96
Red bean	6	50.0	Lemon	0.58	Egg	6	45.2	Webfoot octopus	0.99	Clam	6	41.7	Tofu	0.97
Egg	7	52.5	Tofu	0.62	Beef	7	47.6	Shrimp	0.99	Red bean	7	43.5	Ginger	0.97
Chicken	8	54.5	Gelatin	0.65	Oyster	8	49.7	Perilla	0.99	Egg	8	45.1	Beet root	0.98
Shrimp	9	56.5	Salt	0.67	Sweet potato	9	51.6	Chestnut	0.99	Soy bean sauce	9	46.7	Spaghetti	0.98
Tomato	10	58.1	Bamboo shoot	0.69	Tofu	10	53.5	Eggplant	0.99	Cucumber	10	48.2	Trunk of sweet potato	0.98
Squid	11	59.4	Walnut	0.71	Potato	11	55.4	Mushroom soup	0.99	Red pepper	11	49.6	Soy oil	0.98
Tofu	12	60.7	Radish	0.72	Perilla	12	57.1	Dried seaweed	0.99	Canned tuna	12	51.0	Raw lettuce	0.98
Cucumber	13	61.9	Pepper	0.74	Spaghetti	13	58.7	Bamboo shoot	0.99	Spinach	13	52.3	Oyster mushroom	0.98
Soy bean sauce	14	63.0	Crown daisy	0.75	Sesame	14	60.2	Pear	0.99	Tofu	14	53.5	Button mushroom	0.98
Corn	15	64.1	Dried lettuce	0.77	Squid	15	61.6	Pork	0.99	Shrimp	15	54.8	Peanut oil	0.99
Sandwich	16	65.2	Spaghetti	0.79	Button mushroom	16	62.9	Mustard sauce	0.99	Radish	16	56.0	Laver	0.99
Pumpkin	17	66.2	Salmon	0.80	Mung bean	17	64.2	Yogurt	0.99	Pork	17	57.1	Tomato	0.99
Perilla	18	67.2	Ginger	0.81	Garlic	18	65.4	Raw lettuce	0.99	Mussel	18	58.3	Potato starch	0.99
Yangjangpi	19	68.2	Pear	0.82	Pork	19	66.5	Konjak	1.00	Spaghetti	19	59.5	Peach	0.99
Oyster	20	69.1	Mandarin	0.83	Soy bean paste	20	67.6	Italian dressing	1.00	Perilla	20	60.6	Cucumber	0.99

Analyzed by stepwise multiple regression.
Cum%: cumulative contribution percent, Cum R²: cumulative R² of regression analysis.

Conclusion

In summary, maternal daily intakes of zinc, copper and iron during lactation did not directly affect zinc, copper, and iron concentrations that were detected in the milk samples that were analyzed. However, zinc concentrations were related to the intake of meat and meat products, and copper concentrations correlated with the intake of vitamin C, selenium, and iodine. Furthermore, iron concentrations was found to be more closely associated with the general characteristics of a pregnancy (e.g., drinking during pregnancy and daily supplements during pregnancy) than dietary factors, although food items contributed greatly to iron concentrations in the milk samples than to the zinc and copper concentrations. food items contributed greatly to the concentrations of iron in the milk samples more than to the zinc and copper concentrations. Based on these results, further studies to investigate critical dietary factors that are associated with zinc, copper, and iron concentrations are warranted, with the goal of promoting healthy infant growth and development.

Conflicts of Interest

The authors declare no conflict of interest.

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