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Original

A relatively higher concentration of zinc and strontium within normal levels in breast milk is associated with higher growth rates among breastfed neonate

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Abstract

In this study, we compared differences in the concentration of trace elements in breast milk in women after vaginal and caesarean section deliveries and evaluated the association between the concentration of trace elements in breast milk and infant growth over the first post-natal month. The concentration of the following trace elements was quantified using inductively-coupled plasma mass spectrometry: manganese, cobalt, nickel, copper, zinc, selenium, rubidium, strontium, and molybdenum. The concentration of trace elements was compared between transitional milk and mature milk in 52 women, 26 each after vaginal and caesarean section delivery. Only the concentration of manganese was higher in the vaginal delivery than caesarean section delivery group. The concentration of all trace elements was higher in transitional than mature milk. The concentration of zinc and strontium in mature milk was positively associated with greater neonate height and weight growth rates, per day, over the first post-natal month. Therefore, when concentrations of trace elements in breast milk are within normal limits, a relatively higher concentration of zinc and strontium in mature milk may enhance infant growth during the neonatal period.

Key words : trace element, breast milk, neonates, growth, delivery mode

I. Introduction

Trace elements have been identified in varying concentration in breast milk, with their levels being influenced by several factors that include maternal dietary habits, nutritional status, age, and parity[1-13]. Scott et al. also reported that women who underwent a caesarean section were more likely to experience delayed onset of lactation, compared to women who delivered their foetus vaginally[14]. In general, secretion of breast milk after vaginal delivery is promoted by frequent feeding of infant, while breast

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feeding after emergency caesarean section is likely to be delayed due to maternal stress from protracted labour pain[14]. These differences in infant feeding in the early neonatal period may lead to differences in the concentration of trace elements in breast milk after vaginal and caesarean section deliveries. However, this plausible difference has not been evaluated in previous research.

Breast milk is suitable for infants and includes the nutrients necessary for normal infant growth and development[4]. Trace elements, such as zinc and copper, are nutrients that are as important as protein and lipid for infant development. Generally, infants receive adequate amounts of trace elements from breast milk to meet their needs for normal growth, while deficiency in trace elements can lead to adverse health effects in infants15, 16). However, although the normal concentration of trace elements in human breast milk has been defined, the contribution of trace elements to infant growth is yet to be understood. Therefore, our aim was to compare the concentration of trace elements in breast milk between women who have undergone vaginal and caesarean section deliveries, and to evaluate the association between the concentration of trace elements in breast milk and infant growth rates per day from birth to one month.

II. Materials and Methods

1. Study group

Pairs of mothers and infants were recruited from the maternity service of Iwate Medical University Hospital, Morioka Red Cross Hospital, and the Kurokawa Ladies Clinic, between December 2017 and June 2018. Over the period of observation for this study, 208 deliveries were recorded in these three centres. Among these 208 deliveries, 53 were excluded based on the following criteria: preterm birth (<37 weeks gestation), major anomalies, chromosomal abnormality, and difficulty establishing breastfeeding due to maternal past history or treatment. Among the 155 eligible deliveries, informed consent was obtained from 56 mothers. Of these, 4 mothers failed to collect their breast milk. Ultimately, 52 mother-infant pairs were included in our analysis, 26 in the vaginal delivery group and 26 in the caesarean section delivery group.

The following maternal factors were included in the analysis: age, height and weight (used to calculate the body mass index, BMI), parity, smoking history, and blood pressure at the final health check-up. The following infant factors were included in the analysis: weight of the placenta, gestational age, sex, and birth height and weight, measured immediately after delivery. Infant height and weight were measured again at 1 month (28-43 days) post-delivery, with the height and weight growth rates per day, from birth to 1 month post-delivery, calculated from medical records. The type of infant feeding over the first month after delivery was classified as breast feeding (infant being fed only breast milk) and mixed feeding (infant being fed a combination of breast milk and formula milk).

This study was approved by the Ethics Committee of Iwate Medical University School of Medicine (MH2018-006) and Morioka Red Cross Hospital (30-3). Informed consent was obtained from all individual participants included in the study.

2. Collection of breast milk samples

Breast milk was collected twice after delivery, the first sample at a mean of 4.7 days (transitional milk) and the second at a mean of 32.6 days (mature milk). All breast milk samples of 1.5 ml were collected in plastic containers prior to breakfast and cryopreserved at − 20℃ until measurement.

3. Measurement of the concentrations of trace elements in breast milk

Breast milk samples (0.5 ml) were pipetted into a polypropylene tube (15 ml), and 2.0 ml of ultrapure nitric acid (Kanto Chemical Co., Tokyo, Japan) and 0.1 ml of an internal standard solution (500 mg/L of Beryllium and Rhodium) were added to the sample. The mixed breast milk sample solution was allowed to stand in the laboratory at room temperature for 24 h. Samples were then heated to 60℃ for 24 h. After cooling, 3.0 ml of hydrogen peroxide was added to the sample solution, and the sample solution was heated to 60℃ for 48 h. After cooling, 4.5 ml of ultrapure water (Milli-Q, Merck Darmstad, Germany) was added to the sample solution, and the solution was stored at 4℃ until measurement. The concentrations of the following trace elements were quantified in breast milk using inductively-coupled plasma mass spectrometry (ICP-MS, ELAN DRC-e, PerkinElmer JAPAN, Yokohama): manganese (Mn), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), selenium (Se), rubidium (Rb), strontium (Sr), and molybdenum (Mo). The optical parameters for ICP-MS were as follows: power, 1.1 kw; carrier argon gas flow rate, 0.9 L/min; plasma argon flow rate, 18 L/min; and ion voltage, 6.5 V. For the determination of Se, methane gas was used to suppress the effects of polyatomic ions. As an internal standard, 0.5 mg/L of rhodium solution were used.

The within-run and total imprecision were determined according to the NCCLS Approved Guideline[17]. Synthetic milk on the market was used and five replicates of measurements per day were carried out. It took three days to measure all samples and one run of measurement per day were carried out. This method produced within-run standard deviation of 2.9 ng/mL at 47.7 ng/mL (Mn), 0.04 ng/mL at 1.43 ng/mL (Co), 1.2 ng/mL at 26.2 ng/mL (Ni), 21 ng/mL at 431 ng/mL (Cu), 232 ng/mL at 3285 ng/mL (Zn), 12.5 ng/mL at 53.0 ng/mL (Se), 23 ng/mL at 388 ng/mL (Rb), 13 ng/mL at 134 ng/mL (Sr), 1.8 ng/mL at 21.4 ng/mL (Mo), respectively. Total precision gave a standard deviation of 9.7 ng/mL at 47.7 ng/mL (Mn), 0.14 ng/mL at 1.43 ng/mL (Co), 1.9 ng/mL at 26.2 ng/mL (Ni), 21 ng/mL at 431 ng/mL (Cu), 158 ng/mL at 3285 ng/mL (Zn), 14.8 ng/mL at 53.0 ng/mL (Se), 10 ng/mL at 388 ng/mL (Rb), 10 ng/mL at 134 ng/mL (Sr), 0.5 ng/mL at 21.4 ng/mL (Mo), respectively.

4. Statistical analysis

We calculated mean values of maternal and infant characteristics and compared them between the vaginal and caesarean section delivery groups using a t-test or chi-squared test, as appropriate for the data type. The concentration of trace elements was compared between the vaginal and caesarean section delivery groups and between transitional and mature milk of each group using a t-test. The height and weight growth rates per day were calculated from the change in height and weight from birth to 1 month post-delivery. The association between the concentrations

Table 1. Characteristics of the study group

	Vaginal delivery	Caesarian section	p-value
Maternal factors			
Age (years)	30.8 (5.4)	35.0 (4.9)	0.004
Height (cm)	159.7 (5.0)	159.3 (6.1)	0.804
Weight (kg)	52.8 (5.5)	55.1 (11.4)	0.351
BMI (kg/m^2)	22.4 (8.5)	21.7 (3.8)	0.691
Parity			
Primiparous	13 (50.0)	12 (46.2)	0.695
Multiparous	13 (50.0)	14 (53.8)	
Placental weight (g)	632.2 (143.1)	609.0 (123.6)	0.535
Smoking history	3 (11.5)	3 (11.5)	-
Systolic blood pressure (mmHg)	111.5 (10.3)	116.1 (11.4)	0.132
Diastolic blood pressure (mmHg)	69.9 (9.9)	72.2 (11.5)	0.457
Infant factors			
Gestational age (weeks)	39.6 (1.1)	38.5 (1.2)	0.001
Sex			
Male	10 (38.5)	11 (42.3)	0.777
Female	16 (61.5)	15 (57.7)	
Birth Height (cm)	48.7 (1.9)	48.7 (2.0)	0.907
Birth weight (g)	3160.5 (435.0)	2966.4 (451.7)	0.117
Height at a month old (cm)	53.7 (2.3)	54.1 (2.0)	0.524
Weight at a month old (g)	4222.9 (477.0)	4245.8 (552.0)	0.874
Height growth rates (cm/day)	0.18 (0.04)	0.14 (0.05)	0.009
Weight growth rates (g/day)	34.3 (8.6)	36.8 (7.5)	0.282
Feeding category			
Breast feeding	16 (61.5)	9 (34.6)	
Mixed breast feeding	9 (34.6)	17 (65.4)	0.036
Formula feeding	1 (3.9)	0 (0)	

Data are expressed as means (standard deviations) or n (percentages).
p values were by t-test or χ square test between vaginal delivery group and caesarean section group.

of trace elements and height and growth rates was evaluated using multiple regression analysis. In these regression models, the height and weight growth rates per day were separately used as the dependent variable. Maternal factors (age, height, and weight, which correlated with birth height and weight of infants), infant factors (placental weight and gestational weeks) and the concentration of each trace element in breast milk were used as independent variables. Standardized partial regression coefficients and 95% confidence intervals of these variables were computed after standardizing each variable. P-values <0.05 were considered to be significant. All analyses were performed using SPSS Statistics Desktop version 21 (IBM Japan Inc., Tokyo, Japan).

III. Results

Table 1 summarizes the characteristics of participants in the vaginal and caesarean section delivery groups. With regard to

Table 2. Trace element concentrations in breast milk based on groups: vaginal and caesarean section delivery

	A) Transitional milk			B) Mature milk		
	Concentrations (ng/mL)		p-value	Concentrations (ng/mL)		p-value
	Vaginal delivery	Caesarian section		Vaginal delivery	Caesarian section	
Manganese	16.4 (6.4)	11.9 (3.8)	0.004	12.4 (3.8)	10.4 (5.3)	0.119
Cobalt	0.90 (0.20)	0.86 (0.76)	0.505	0.80 (0.17)	0.76 (0.19)	0.429
Nickel	25.8 (5.7)	24.1 (5.3)	0.261	22.6 (4.3)	21.5 (6.0)	0.485
Copper	610 (188)	536 (167)	0.138	444 (152)	411 (114)	0.381
Zinc	5089 (1529)	5026 (1899)	0.896	2310 (789)	2119 (890)	0.417
Selenium	95.4 (32.5)	102 (25.3)	0.419	60.4 (24.5)	76.6 (42.3)	0.098
Rubidium	824 (171)	780 (191)	0.379	566 (119)	536 (126)	0.379
Strontium	63.9 (18.1)	61.8 (25.4)	0.728	55.1 (16.9)	56.1 (22.0)	0.855
Molybdenum	9.3 (3.2)	10 (3.3)	0.344	4.5 (6.4)	5.1 (5.4)	0.725

Data are expressed as means (standard deviations) .
p values evaluated using a between-group t-test.

maternal factors, women in the caesarean section delivery group were older at the time of delivery than those in the vaginal delivery group (p=0.004), with the groups being comparable on other measured factors. Of note, both groups included 3 mothers with a smoking history. The history of parity was also comparable between the two groups.

With regard to infant factors, the placental weight was comparable between the two groups. However, the mean gestational age was significantly lower in the caesarean section delivery group than in the vaginal delivery group (p = 0.001). Of note, the sex distribution among infants was comparable between the two groups, 10 boys and 16 girls in the vaginal delivery group and 11 boys and 15 girls in the caesarean section delivery group. Similarly, the average height and weight of infants at birth were comparable between the two groups. With regard to the growth rate, although mean weight gain per day was comparable between the two groups, the height growth rate per day was significantly higher in the vaginal delivery group than in the caesarean section delivery group (p=0.009). With regard to the distribution of the type of feeding in the post-natal period, mixed feeding was more frequent in the caesarean section delivery group than in the vaginal delivery group (p = 0.036).

Comparison of the concentration of trace elements is summarized in Table 2A for transitional milk and in Table 2B for mature milk. In the transitional milk, the concentration of Mn was higher in the vaginal delivery group than in the caesarean section delivery group (p = 0.004), with no other between-group differences in the concentration of trace elements. Moreover, there were no between-group differences in the concentration of trace elements in mature milk.

The concentration of trace elements for transitional and mature milk is reported in Table 3. The concentration of all trace elements was higher in transitional milk than in mature milk. In particular, there was a decrease of about 60% in the concentration of Zn (from

Table 3. Trace element concentrations in transitional and mature breast milk

	Concentrations (ng/mL)		p-value
	Transitional milk	Mature milk	
Manganese	14.1 (5.7)	11.4 (4.7)	< 0.001
Cobalt	0.88 (0.20)	0.78 (0.16)	0.001
Nickel	24.9 (5.5)	22.1 (5.2)	0.003
Copper	573 (180)	428 (134)	< 0.001
Zinc	5058 (1708)	2215 (838)	< 0.001
Selenium	98.7 (29.1)	68.5 (35.2)	< 0.001
Rubidium	802 (180)	551 (122)	< 0.001
Strontium	62.9 (21.9)	55.6 (19.4)	0.048
Molybdenum	9.7 (3.2)	4.8 (5.9)	< 0.001

Data are expressed as means (standard deviations).
p values were evaluated using a paired t-test between transitional and mature milk.

Table 4. The association between the concentration of trace elements in mature milk in the breastfed group and infant height and weight growth rates per day

Elements	Height gains (cm/day)			Weight gains (g/day)		
	β	95%CI	p	β	95%CI	p
Manganese	− 0.214	− 0.571 to 0.143	0.225	− 0.143	− 0.586 to 0.300	0.508
Cobalt	− 0.030	− 0.379 to 0.318	0.858	0.257	− 0.142 to 0.655	0.195
Nickel	− 0.026	− 0.373 to 0.321	0.876	0.281	− 0.106 to 0.669	0.146
Copper	0.055	− 0.314 to 0.424	0.759	− 0.127	− 0.544 to 0.289	0.532
Zinc	0.373	0.072 to 0.673	0.017	0.044	− 0.426 to 0.513	0.847
Selenium	− 0.115	− 0.467 to 0.237	0.504	0.038	− 0.462 to 0.539	0.874
Rubidium	0.068	− 0.331 to 0.467	0.726	− 0.192	− 0.628 to 0.245	0.371
Strontium	0.044	− 0.365 to 0.453	0.823	0.411	0.017 to 0.804	0.041
Molybdenum	− 0.159	− 0.503 to 0.185	0.346	0.262	− 0.125 to 0.649	0.175

The association between the concentrations of trace elements and height and growth rates was evaluated using multiple regression analysis. In these regression models, the height and weight growth rates per day were separately used as the dependent variable. Maternal factors (age, height, and weight, which correlated with birth height and weight of infants), infant factors (placental weight and gestational weeks) and the concentration of each trace element in breast milk were used as independent variables. Standardized partial regression coefficients and 95% confidence intervals of these variables were computed after standardizing each variable.
β, Standardized partial regression coefficient; 95%CI, 95% confidence interval

5058 ng/mL to 2115 ng/mL) and of about 50% for Mo (from 9.7 ng/mL to 4.8 ng/mL).

The association between the concentration of trace elements in mature milk and height and growth rates per day is reported in Table 4. A significant positive association was observed between the height growth rate per day and the concentration of Zn (p = 0.017) and between the weight growth rate per day and the concentration of Sr (p = 0.041).

IV. Discussion

The concentrations of trace elements in breast milk were compared between 26 women who delivered vaginally and 26 who delivered via caesarean section. We observed a significantly higher concentration of Mn in transitional milk in the vaginal delivery group, with no differences between the vaginal and caesarean section delivery groups. Overall, the concentration of trace elements was significantly higher in transitional milk than in mature milk. Furthermore, in infants who were only breastfed, there was a significant association between the height growth rate and Zn concentration in mature milk and between the weight growth rate and Sr concentration in mature milk. Our finding of a higher concentration of Mn in transitional milk after vaginal delivery than in that after caesarean section has not been previously reported. Therefore, the underlying mechanism behind this high concentration is not clear. However, we did note that the concentration of Mn in transitional milk tended to be higher with greater gestational age, although this association was only marginally significant. This association might be explained by the longer gestation duration in the vaginal delivery group than in the caesarean section delivery group (p=0.004). There were no significant differences between the two groups in the concentrations of other trace elements in breast milk. Although we did not measure lactation in the present study, delayed onset of lactation in the caesarean delivery has been identified as a risk factor for the early cessation of breastfeeding, and they are expected to receive postpartum breastfeeding support[14].

The concentration of trace elements in breast milk was significantly different between lactation periods (Table 3), with the concentration of all trace elements being significantly lower in mature milk than in transitional milk. Previous studies have similarly reported a decrease in the concentration of trace elements from transitional to mature milk, including a decrease of about 20% in Mn[7], of 30- p-value 40% in Cu[7, 9], 50% in Zn[7, 9], and 50% in Mo[8], these changes being comparable to those in our study.

Hirose et al. reported that the mean daily intake of breast milk was 424 mL at 4 days and 745 mL at a month after delivery[18]. Based on these values of breast milk intake and our measured concentration of trace elements in transitional and mature milk, we calculated the intake of trace elements per day, showing that the increase in milk intake at 1 month compensated for decreases in the concentration of trace elements, such that infants ingested similar quantities of trace elements with transitional and mature milk. Kaneko et al. suggested that Zn concentration might be high in transitional milk (colostrum) to provide a sufficient intake of Zn in neonates, despite a low volume of intake of breast milk[19]. A similar mechanism might exist for all other trace elements.

Several studies have reported on the concentration of trace elements in breast milk[1-13]. These concentrations do vary by country and lactation periods[4-10, 12, 13]. For the same lactation period, the concentration of trace elements was similar to those reported in a previous study in Japan [11], but different from those in other countries [9, 10]. Identified differences between countries might reflect the differences in dietary habit, as well as differences in trace elements in soil [1-3, 5]. Of note, the concentration of Zn and Cu in our study was comparable to previously reported concentrations [6, 7]. Moreover, if maternal dietary intakes of Zn and Cu are sufficient, supplementation does not increase the concentration of Zn and Cu in breast milk [11]. Therefore, the concentration of these two elements appears to be constant, so long as the intake of these elements is sufficient, and the concentration is not further increased by dietary supplementation.

Among infants who were breastfed, we confirmed that Zn and Sr concentrations in mature milk were significantly associated with height and weight growth rates, respectively. Zn deficiency interferes with the metabolism of thyroid hormones, androgens, and growth hormones [20]. Specifically, insulin-like growth factors-1 (IGF-1) exert a growth-promoting action, with Zn playing a specific role in the regulation of circulating IGF-1 concentration [21]. As such, the relatively higher concentration of Zn in mature milk might promote height growth during the neonatal period. As the concentration of Zn in mature milk was within the normal range, the effects of Zn deficiency on growth could not be evaluated.

Studies have confirmed the transfer of Sr from breast milk to infants [22]. In humans, 99% of Sr is distributed in the bone tissue [23]. Therefore, we can assume that the majority of Sr ingested from breast milk would accumulate in the bone tissue of neonates. Although the mechanism is not clear, we speculate that relatively high concentrations of Sr in mature milk may contribute to favourable growth during the neonatal period, increasing the rate of weight gain.

Three main limitations of our study should be considered in the interpretation of our results. First, the number of infants who were breastfed over the 1 month after birth was small, i.e., 36.0% in the caesarean section delivery group and 61.5% in the vaginal delivery group. Furthermore, our follow-up period was short, i.e., a month post-delivery. As breastfeeding influences growth up to six months post-delivery, the association between the concentration of elements in breast milk and infant growth rates could be better clarified using a longer observation period. Second, we did not confirm the accuracy of our method for measurement using a certificated standard material, though we confirmed the within-run and total imprecision. In contrast, our results demonstrate comparable levels with those from previous reports. Therefore, our method is expected to be valid for the construction of this study. Third, the growth of infants is related to intake such as carbohydrates, proteins and lipids, and amount of breast milk sucked by breast feeding infants, however, we had difficulty measuring and analyzing these. Despite these limitations, we did demonstrate an association between a relatively higher concentration of Zn and Sr in mature milk and a greater rate of height and weight gain. We consider this finding to be of clinical value.

In conclusion, our comparison of the concentration of trace elements in transitional and mature milk after vaginal and caesarean section deliveries revealed a significantly higher concentration of Mn in transitional milk in the vaginal delivery group, with no difference between groups for mature milk. We do confirm a higher concentration of all trace elements in transitional milk than in mature milk. We identified a significant association between higher concentrations of Zn and Sr in mature milk and a higher height and weight growth rate among infants who were only breastfed over the first post-natal month.

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母乳中の亜鉛とストロンチウム濃度は新生児期の成長に関与する

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要旨

経腟分娩及び帝王切開で出産した母親の母乳中微量元素濃度の違いが児の成長に及ぼす影響は明らかではない．本研究の目的は，経腟分娩及び帝王切開で出産した母親の母乳中微量元素濃度の違いを明らかにし，母乳中微量元素濃度と新生児期における児の成長との関連を評価することである．母親から移行乳（産後 4-6 日）と成乳（産後 28-43 日）を採取し，誘導結合プラズマ質量分析法を用いて，母乳中のマンガン（Mn），コバルト（Co），ニッケル（Ni），銅（Cu），亜鉛（Zn），セレン（Se），ルビジウム（Rb），ストロンチウム（Sr），モリブデン（Mo）の濃度を測定した．移行乳中の Mn 濃度のみが帝王切開群よりも経腟分娩群で有意に高値だった．全ての微量元素濃度は成乳よりも移行乳で高値を示した．成乳中の Zn 濃度は児の１日あたりの身長増加量と，成乳中の Sr 濃度は児の１日あたりの体重増加量と正の関連を認めた．正常範囲内における高濃度の Zn と Sr は児の成長を促進する可能性がある．