Mineral and Trace Element Content of Human Transitory Milk Identified with Inductively Coupled Plasma Atomic Emission Spectrometry

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Summary Mineral and trace element content of the human transitory milk collected on days 6 to 9 postpartum in Tokyo was measured by inductively coupled plasma atomic emission spectrometry (ICP-AES). Ten elements (Na, Mg, P, S, K, Ca, Fe, Cu, Zn, and Sr) were detected in ten-fold diluted milk sample with this method. With the stepwise multiple regression analysis, maternal and infants' biological attributes, such as weight, stature, sex, or age, were noted as factors contributing to the interindividual variation in Na, Mg, P, S, and Sr. Biological mechanism responsible for this selection cannot be found. Days postpartum for the milk collection was selected as a highly significant (p<0.001) variable with negative coefficient to explain milk S variation.

Key Words transitory milk, minerals, trace element, ICP-AES, variation, multiple regression analysis, biological attributes of mother and infant, days postpartum

Mineral and trace element contents of human milk have been a matter of concern among nutritionists in relation to the nutritional requirement of essential elements for newborns. Extensive work has been done on the levels of essential elements, such as Ca, Mg, P, Mn, Cu, Zn, and Se, in human mature milk, along with the sources of variation in the elemental concentrations (1-8). Longitudinal variation is well established for Zn (1,4,5,7) and Se (2,3). In general, elemental concentrations are higher in the early period of lactation, i.e., in colostrum and...
transitory milk. Composition of the milk in this period is in the phase of dramatic change; however, few data on elemental concentration in the milk of this period are available (9–14) when compared with those in mature milk. One of the purposes of the present study is to increase our knowledge on the elemental composition of human transitory milk with inductively coupled plasma atomic emission spectrometry (ICP-AES), which is routinely used for multi-element analysis of various biological materials but seldom for that of human milk to date.

In our preliminary study on Zn and Se content of transitory milk of Japanese (measured by atomic absorption spectrometry and fluorometry, respectively), the following variables were extracted as contributory factors to the interindividual variation in the concentrations of these elements: maternal age and infant’s sex for Zn; and the days postpartum for milk collection, the admitted hospital for delivery, and infant’s sex for Se (15). Another purpose of the present study is to further examine whether these factors explain interindividual variation in the concentrations of other elements than Zn and Se, which can be determined with ICP-AES.

SUBJECTS AND METHODS

Subjects. Subjects and milk samples are the same as those in our previous study (15). Twenty-seven healthy Japanese females, who gave their informed consent, participated in this study. On days 6 to 9 after their full-term delivery, milk samples were obtained by manual expression into acid-washed Pyrex® tube at the hospitals in Tokyo (Hosp. A and Hosp. B) during May to September 1988. Sampling was done by themselves in the morning at their health examination for dehospitalization. The characteristics of the present subjects (mothers and infants) are summarized in Table 1. After delivering to our laboratory, samples were stored at −20°C in the dark until analysis.

Analytical methods. Milk sample (1 ml) was digested with 1 ml of nitric acid (Ultrapure grade, Kanto Chemical) in PTFE vial set in the Teflon-lined stainless-steel bomb (San-ai Kagaku) at 120°C for 3 h. After cooling, PTFE vial was taken out and placed on the hot plate in class 100 clean bench to evaporate the digestant at 140°C to a volume of approximately 0.1 ml. The residual solution was made up to 10 ml with Millipore® distilled water. Elemental concentrations were measured by ICP-AES (JY48P, Daini Seiko-sha). Standard solution contained the following elements at the concentration of 10 ppm in 1% nitric acid; Li, B, Na, Mg, Al, Si, P, S, K, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Zn, As, Se, Mo, Cd, Hg, and Pb. It also contained Cu and Sr (both with higher sensitivity) at 1 ppm. Detection limits for these elements, expressed in µg of element per 1 ml of milk, are presented in Table 2. Human serum (NIES No. 4), Bovine Liver (NBS, 1577a), and Wheat Flour (NBS, 1568) were analyzed concomitantly for the analytical quality assurance and obtained results were in good agreement with the certified values.

Statistical methods. Skewness of the distribution of the elemental concentration was calculated; log-transformation was applied to the concentration.
Table 1. The characteristics of the present subjects.

|                  | Hosp. A     | Hosp. B     | Total       |
|------------------|-------------|-------------|-------------|
| Mothers          |             |             |             |
| Age (yrs)        | 29.7 (4.4)  | 28.0 (4.8)  | 28.9 (4.6)  |
| Stature (cm)     | 156.7 (6.8) | 158.5 (5.8) | 157.4 (6.3) |
| Weight (kg) a    | 53.9 (4.4)  | 53.9 (5.9)  | 53.9 (5.0)  |
| BMI (kg/m²) b    | 22.0 (1.7)  | 21.4 (1.6)  | 21.7 (1.6)  |
| d-p-p (days) c   | 7.2 (0.4)   | 6.9 (0.9)   | 7.1 (0.7)   |
| Infants          |             |             |             |
| Birth weight (g) | 3145 (426)  | 3088 (259)  | 3120 (356)  |
| Birth order      | 1.8 (0.8)   | 2.0 (0.9)   | 1.9 (0.8)   |
| Sex (male)       | 7           | 5           | 12          |
| (female)         | 8           | 7           | 15          |

Recapitulated from Li et al. (15). Figures in the table are arithmetic mean with its standard deviation in parenthesis except for infants’ sex where number of subjects are given. Statistically significant difference did not exist in any variables between the subjects from the two hospitals (t-test, Chi-square test for infants’ sex.). a Estimated from body weight before delivery by subtracting weight of infant and placenta, amount of blood loss and amniotic fluid. b Body mass index. c Days postpartum for the milk collection.

Table 2. Detection limit of the present analysis (µg/ml). a

| Element | Detection limit | Element | Detection limit |
|---------|-----------------|---------|-----------------|
| Li      | 0.1             | Mn      | 0.05            |
| B       | 0.05            | Fe      | 0.1             |
| Na      | 0.2             | Co      | 0.1             |
| Mg      | 0.05            | Ni      | 0.1             |
| Al      | 0.2             | Cu      | 0.05            |
| Si      | 0.3             | Zn      | 0.02            |
| P       | 1               | As      | 1               |
| S       | 0.2             | Se      | 0.5             |
| K       | 1               | Sr      | 0.05            |
| Ca      | 0.05            | Mo      | 0.2             |
| Ti      | 0.1             | Cd      | 0.05            |
| V       | 0.1             | Hg      | 0.2             |
| Cr      | 0.2             | Pb      | 2               |

a Microgram per unit volume of milk (ml).

when the skewness exceeds 1. Student t-test, the calculation of Pearson correlation coefficient between elemental concentrations, and multiple regression analysis were conducted using SPSS-X program (16). In the multiple regression analysis, each
elemental concentration detected was used for the dependent variable. The independent variables used were as follows: days postpartum for the milk sampling, maternal age, maternal stature, maternal weight, infant’s sex (male: 0; female: 1), infant’s birth weight, birth order, and the hospital for delivery (Hosp. A: 0; Hosp. B, 1). Variables were selected with the stepwise method, using the probability of F-value as criteria for entrance ($P_{in}=0.05$) into, and elimination ($P_{out}=0.1$) from, the multiple regression equation to select variable with strict criteria taking the sample size into consideration. Zinc and Se concentrations, which were recapitulated from our previous study (15), were also used as dependent variables for re-analysis with the present statistical method.

RESULTS

Table 3 presents the arithmetic and geometric mean concentration of the elements in the present milk samples, which was detected in the present ICP-AES analysis. Since there were no statistically significant differences between the two hospitals data, both data were combined and shown in the table. The concentrations of Na, K, Mg, Ca, P, S, Cu, Zn, and Sr were above detection limit in all the samples. Iron was detected; however, duplicate analysis revealed discrepancy between the two determinations, probably because of low concentrations in the present samples which were close to the detection limit for Fe (0.1 µg/ml in milk):

| Element (unit) | Arithmetic mean (SD) | Geometric mean (SD) |
|----------------|----------------------|---------------------|
| Na (µg/ml)     | 310 (138)            | 288 (1.4)           |
| Mg (µg/ml)     | 32.5 (5.2)           | 32.1 (1.2)          |
| P (µg/ml)      | 194 (34)             | 191 (1.2)           |
| S (µg/ml)      | 209 (27)             | 208 (1.1)           |
| K (µg/ml)      | 882 (105)            | 877 (1.1)           |
| Ca (µg/ml)     | 309 (48)             | 306 (1.2)           |
| Cu (µg/ml)     | 0.67 (0.14)          | 0.66 (1.2)          |
| Zn (µg/ml)     | 5.53 (2.18)          | 5.12 (1.5)          |
| Zn (µg/ml)     | 5.74 (1.95)          | 5.40 (1.4)          |
| Se (ng/ml)     | 29.2 (6.5)           | 28.5 (1.3)          |
| Sr (µg/ml)     | 0.06 (0.02)          | 0.06 (1.4)          |
| Na/K (mol/mol) | 0.59 (0.24)          |                     |
| P/Ca (mol/mol) | 0.83 (0.17)          |                     |
| Mg/Ca (mol/mol)| 0.18 (0.04)          |                     |

*Number of samples = 27. Recapitulated from Li et al. (15). Zn was determined by AAS and Se by fluorometry. Only the arithmetic mean was given for molar ratio.
Table 4. Correlation coefficient between elemental concentrations.

| Mg  | P     | S     | K     | Ca    | Cu    | Zn    | Se* | Sr |
|-----|-------|-------|-------|-------|-------|-------|-----|----|
|     |       |       |       |       |       |       |     |    |
|     |       |       |       |       |       |       |     |    |
|     |       |       |       |       |       |       |     |    |

Figures in the table are Pearson correlation coefficients which are significant (*, p<0.05; **, p<0.01; ***, p<0.001). *Recapitulated from Li et al. (15).

hence analytical value for Fe was not given in the table. None of the samples were above detection limit for other elements (see Table 2). The skewness of the distribution for Na, Cu, and Sr exceeded 1; thus the log-transformed value for Na, Cu, and Sr, and the crude value for other elements detected (K, Mg, Ca, P, S, and Zn) were used in the following statistical analysis. In Table 3, molar ratios between selected elements (Mg/Ca, P/Ca, Na/K) are also presented.

Table 4 presents the interrelationship between the concentrations of elements. This correlation matrix includes Se which is recapitulated from our previous study (15). Significantly positive correlation was observed between Na and S, Mg and S, Ca and Sr, Ca and Cu, Cu and P, Mg and K, and Se and S. Negative correlation between Na and P was significant.

The result of multiple regression analysis is summarized in Table 5. The following variable(s) was selected as significant (p<0.05) to explain the interindividual variation in each elemental concentration: Na, maternal stature (with negative coefficient); Mg, infant’s sex; P, maternal stature; S, days postpartum for milk collection and infant’s birth weight; Sr, maternal age. No independent variables were selected as significant for K, Ca, Cu, and Zn variation. Using Zn and Se data recapitulated from our previous study and the present $P_{in}=0.05$ and $P_{out}=0.1$ criteria, maternal age ($F$-value: 5.56, $p<0.05$) for Zn and days postpartum (17.04, $p<0.01$) and admitted hospital for delivery (5.73, $p<0.05$) for Se (Table 5) were selected.

DISCUSSION

With ICP-AES, ten elements (Na, Mg, P, S, K, Ca, Fe, Cu, Zn, and Sr) were detected simultaneously in tenfold diluted milk samples. As in the cases with other biological materials, ICP-AES provides information on the elemental composition
of human milk, especially for the elements with relatively high concentration. When compared with literature data on the elemental concentrations in human transitory milk (9-13) andcolostrum and transitory milk (14), the present results for Na, Mg, Ca, P, S, Cu, and Zn were in fairly good agreement. The present mean K level (882 µg/ml) was higher than those reported (628 µg/ml for the milk collected on the 6 to 10th day postpartum (10), 650 µg/ml for colostrum and transitory milk (14), 640-755 µg/ml for transitory milk (9)). Iron concentration in human colostrum and transitory milk was reported to be 0.28 µg/ml (14), and the present result (around 0.1 µg/ml) may be consistent with this level. Failure to detect other elements is reasonable when we take the present detection limit (Table 2) and literature data (9, 12, 13, 17) into consideration, although these reports do not cover all of the present analyte.

Molar ratios of Na to K, P to Ca, and Mg to Ca in the present milk samples were 0.59, 0.83, and 0.18, respectively (Table 3). When calculating from the recent data on mature milk (3 months after delivery) from 6 countries (Guatemala, Hungary, Nigeria, Philippines, Sweden, and Zaire) (8), these ratios were between the range of 0.27 to 0.46, 0.62 to 0.83, and 0.18 to 0.24, respectively. Longitudinal variations in these ratios calculating from the data presented by Yamamoto et al. (10) (3 days to 13 months or more after delivery) fell in the range of 0.56 to 0.85 for Na/K, 0.65 to 0.85 for P/Ca, and 0.15 to 0.20 for Mg/Ca. These indicate that the balance of these elements in human milk does not substantially vary with lactation, and/or geographic locations. On the other hand, the balance of the dietary intake of these elements differs from that of human milk. From the reported mineral-to-

Table 5. Results of multiple regression analyses.

| Dependent variable | Independent variables selected | Beta | F-value^a |
|--------------------|-------------------------------|------|-----------|
| Na"               | Maternal stature              | -0.557 | 11.26** |
| Mg                 | Infant’s sex                  | 0.474  | 7.25*    |
| P                  | Maternal stature              | 0.453  | 6.47*    |
| S                  | Days postpartum               | -0.673  | 24.23*** |
|                    | Infant’s birth weight         | -0.357  | 6.28*    |
| K                  | None                          |       |          |
| Ca                 | None                          |       |          |
| Cu"               | None                          |       |          |
| Zn                 | None                          |       |          |
| Zn^c               | Maternal age                  | -0.427  | 5.56*    |
| Se^c               | Days postpartum               | -0.641  | 17.04**  |
|                    | Hospital                      | 0.372  | 5.73*    |
| Sr^b               | Maternal age                  | 0.457  | 6.59*    |

^aSignificance level is represented with asterisk: *, **, and *** denote p < 0.05, 0.01, and 0.001, respectively. ^bLog-transformed value was used. ^cRecapitulated from Li et al. (15).
mineral ratios, or calculated from the reported data on dietary intake of minerals,
the following values were obtained: Na/K, 3.2 (18) and 4.6 (19) for Japanese; P/Ca,
2.25 to 2.87 for Japanese infants (20) and 1.7 for Finns (21); Mg/Ca, 0.6 for
Japanese (19) and 0.47 for Finns (21). Hongo et al. (22) reported similar values for
P/Ca (2.1 to 2.5) and Mg/Ca (0.6 to 0.9), while lower (thus close to that of human
milk) Na/K (<0.5) for Papuans, whose diet consisted of mostly “local food,” such
as hunted game, wild plants, or tuberous crops. Thus alterations of mineral balance
in human diet occurs at the weaning. If we accept the idea that human milk is an
ideal food for humans in terms of its nutritional value, this alteration of mineral
balance at weaning is worthy to note. Implication for this alteration will be a subject
of further investigation in relation to human health.

Few studies have dealt with interrelationship between the elemental con
centrations in human milk. Finley et al. (23) examined relationship between the
concentrations of Fe, Cu, Zn, Ca, Mg, Na, K, and organic constituents (lactose,
lipid, and protein) in human milk. They revealed several interrelationships between
elemental concentrations; however, their results were not consistent with the present
ones except for significant Ca-Cu pair. The most likely reason for this inconsistency
is that they used mature milk (1–20 months) in contrast to our transitory milk.
Significant correlation between Mg-K, Ca-Sr, and S-Se, found in the present study,
might be attributed to the similar chemical property between the elements in each
pair. However, the reason for other significant correlations between elements is not
known. Speciation of the chemical form of each element in human milk will provide
further insight into the relationship between elements.

As shown in Table 5, multiple regression analysis extracted maternal or infant’s
biological attributes as possible contributory factors to vary elemental concentrations, such as Na, Mg, P, S, and Sr. Biological mechanisms responsible for
these selections cannot be readily found. Meanwhile, absence of significant variables
to explain milk Zn variation provokes attention in the interpretation of the present
results of multiple regression analysis because our previous study showed different
results, i.e., maternal age and infant’s sex were selected as contributory factors to
milk Zn variation (15). In that study, we employed atomic absorption spectrometry
(AAS) for Zn determination. The analytical results of Zn by ICP-AES and AAS fairly agreed: Zn (AAS) = 0.81 × Zn (ICP) + 1.3, r = 0.908 (p < 0.001). The statistical
method used in the present and previous study was essentially the same with minor
change which did not substantially affect the result. However, variable with F-value
larger than 2 but probability of the F-value larger than 0.05 did not enter the present
equation, e.g., variable “infant’s sex” for Zn (F-value: 2.95, p = 0.099) (Table
5, (15)). Variation due to different analytical chemical methods, which is within the
range of analytical error, led to discrepancy in the results. This indicates that the
magnitude of contribution of the maternal age (F-value: 6.29, p = 0.019 (19)) and
infant’s sex to milk Zn variation, if any, was very weak. This inconsistency is
probably due to rather small sample size for this kind of statistical analysis. Therefore
the above-mentioned relations between maternal and infant’s biological attributes
and elemental concentration, particularly those with marginal significance, must be carefully evaluated taking the case with Zn into consideration.

Multiple regression analysis selected the variable “days postpartum” for the variation in S concentration with negative coefficient and highly significant F-value (24, 23, $p < 0.001$, Fig. 1). This variable was also selected for Se with negative coefficient and large F-value (17.04, $p < 0.01$). These strong relations indicate that the 6 to 9th day after delivery is just the period when S and Se concurrently decrease to the levels of mature milk.

From the present results, it can be concluded that 1) with ICP-AES, ten elements (Na, K, Mg, Ca, P, S, Fe, Cu, Zn, and Sr), which were relatively abundant and of nutritional importance, could be detected in tenfold diluted human transitory milk; 2) contribution of maternal and infant's biological attributes to interindividual variation in Na, Mg, P, S, and Sr concentrations in milk was statistically significant, but we should be careful to draw any firm conclusion that these represent some biological mechanism; 3) S content in human milk might vary within a narrow range of days postpartum, i.e., 6 to 9 days after delivery.

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