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Trace Elements in Human Transitory Milk

Variation Caused by Biological Attributes of Mother and Infant

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ABSTRACT

Multielement analysis was performed on human milk collected on 5–9-d postpartum from 51 Japanese females using inductively coupled plasma (ICP) mass spectrometry (MS), ICP atomic emission spectrometry (ICP-AES) and fluorometry. Thirty-one elements were detected by these analytical methods in milk. Twelve elements (Na, Mg, P, S, K, Ca, Cu, Zn, Se, Sr, Rb, and Mo) were detected in all of the samples. Al, Cs, and Ba were the elements detected by ICP—MS in more than half of the samples. Multiple regression analysis extracted biological attributes of mother and infant, such as maternal stature, maternal wt, or infant's birth wt, as statistically significant factors contributing to the variation in elemental concentration in milk. How-

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ever, the rates of contribution were small in all cases. It was concluded that the biological attributes of mother and infant examined in this study were not the major factors that contribute to elemental variation in human milk.

Index Entries: Transitory milk; multielement analysis; ICP-MS; ICP-AES; fluorometry; element variation; biological attributes of mother and infant.

INTRODUCTION

Human milk is the first food for humans and it serves as the sole source of all the nutrients required for the biological functions and growth during the early stages of life. At the same time, it is the first and the sole source of oral exposure to the pollutants for humans. Element content of human milk, including minerals, essential trace elements, and pollutant elements, is, therefore, of importance from both nutritional and environmental points of view, and many works have been done on this subject. We have analyzed elemental composition of the transitory milk of Japanese with inductively coupled plasma atomic emission spectrometry (ICP-AES) and fluorometry and detected 10 elements with the concentration above several tens of ng/mL (Na, Mg, P, S, K, Ca, Fe, Cu, Zn, Sr) (*1*). However, elements at lower level could not be detected and more sensitive method was needed to further clarify elemental composition of human milk. Recently, ICP mass spectrometry (ICP-MS) has developed as a highly sensitive method with the capability of simultaneous multielement analysis (*2*). It is expected that the ICP-MS method will further increase our knowledge on the elemental composition of human milk.

One of the findings in the studies on elemental concentrations in human milk, so far, is the large inter- and intraindividual variation in the concentrations of essential elements, such as zinc and selenium. This seems to contradict the notion that human milk is an ideal source of nutrients for humans, in both quality and quantity and, therefore, that the concentration of essential elements in human milk should have been regulated to a constant level to meet the requirement of the newborn. Sources of this variation have been a subject of concern. Element intake of mother (*3–7*), mother's age (*7–9*), birth order (*7,9*), history of lactation (*8,9*), geographic factor (*10,11*), and lactating period (*12–15* and many others) have been examined as the sources of variation.

In our previous studies (*1,16*), biological attributes of mother and infant, e.g., mother's stature, mother's age, or infant's sex, were notified as the statistically significant factors contributing to the elemental variation in the transitory milk, collected from 27 Japanese women, by the multiple-regression analysis. However, firm conclusions could not be drawn about whether the selection of these factors were causal partly because of the small sample size. It seemed necessary to further examine

whether these factors contribute to the elemental variation using larger number of samples.

The present study is basically the extension of the previous one. Twenty-four subjects were added to the previous 27. Further, ICP-MS method was employed in addition to ICP-AES and fluorometry for the multielement analysis. On the multielement data for 51 subjects in total, statistical analysis was conducted under almost identical design to the previous study.

MATERIALS AND METHODS

Transitory Milk Samples

Human milk samples were collected from 51 Japanese women at three hospitals (A, B, C) in Tokyo. Characteristics of the subjects (mother and infant) were summarized in Table 1. Of 51 samples, 27 from A and B, collected during May to September, 1988, were the same as those analysed in our previous studies. Twenty-four samples from C were collected during August to September, 1989. Sampling was done 5–9-d postpartum by means of manual expression into acid-washed Pyrex or Teflon tube by the subjects, who gave their informed consent, at the hospitals. As shown in Table 1, one-way analysis of variance revealed significant variation owing to the hospitals for the infant's birth order and days postpartum for milk sampling, both being earlier in C than in A and B. Samples were stored at −20°C in the dark until analysis.

Analytical Methods

The procedure of sample preparation was described in detail in the previous study (*1*). Multielement analysis was performed by ICP-AES (JY48P, Daini Seiko-sha) and ICP-MS (PMS-100, Yokogawa Electric). Selenium was determined by fluorometric method (*17*). Elements analyzed by each analytical method was shown in Table 2.

Statistical Methods

Student's *t*-test, one-way analysis of variance (ANOVA), and stepwise multiple-regression anaysis were performed using SPSS-X program (*18*) after appropriate transformation of the crude value. In the stepwise multiple-regression analysis, the dependent variable was the concentration of each element and the independent variables were as follows: days postpartum for the milk sampling, mother's age, mother's stature, mother's wt, infant's sex (male:0; female:1), infant's birth wt and birth order. The variable "hospital for delivery", which was included in the previous studies, was not included in the present one since the samples in the present study were from three hospitals and, therefore, could not cate-

Table 1
Characteristics of the Present Subjects*

	Hosp. A	Hosp. B	Hosp. C	Total	
N	12	15	24	51	ANOVA[a]
Mother's					
age (yr)	28.0 ± 4.5	29.7 ± 4.4	27.3 ± 4.4	28.2 ± 4.5	NS
Stature (cm)	158.5 ± 5.8	156.7 ± 6.8	159.3 ± 4.9	158.3 ± 5.7	NS
weight (kg)	53.9 ± 5.9	53.9 ± 4.4	54.5 ± 7.2	54.2 ± 6.1	NS
BMI (kg/m^2)[b]	21.4 ± 1.6	22.0 ± 1.7	21.5 ± 2.5	21.6 ± 2.1	NS
Infant's birth weight (g)	3088 ± 259	3145 ± 426	3172 ± 366	3144 ± 358	NS
birth order	2.0 ± 0.9	1.8 ± 0.8	1.4 ± 0.6	1.6 ± 0.8	$p < .05$
sex male	5	7	11	23	
female	7	8	13	28	NS[c]
Days postpartum for milk sampling	6.9 ± 0.9	7.2 ± 0.4	5.6 ± 0.5	6.4 ± 0.9	$p < .001$

*Figures in the table are arithmetic mean ± SD except for N and infant's sex, where number of subjects are given.

[a]One-way analysis of variance; NS: not significant.

[b]Body mass index.

[c]Chi-square test (not significant).

Table 2
Elements Analyzed in the Present Study[a]

Analytical method	Element									
ICP-MS	Li	Na	Mg	Al	Sc	Ti	V	Cr	Mn	Fe
	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Rb	Sr
	Y	Zr	Nb	Mo	Pd	Ag	Cd	In	Sn	Sb
	Te	Cs	Ba	La	Ce	Pr	Nd	Sm	Eu	Gd
	Tb	Dy	Ho	Er	Tm	Yb	Lu	Hf	Ta	W
	Pt	Au	Tl	Pb	Bi	Th	U			
ICP-AES	Li	B	Na	Mg	Al	Si	P	S	K	Ca
	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	As
	Se	Sr	Mo	Cd	Hg	Pb				
Fluorometry	Se									

[a]Elements that are contained in the standard solution employed in the present analyses.

Table 3
Elemental Concentrations
in Japanese Transitory Milk Determined
by ICP-AES and Fluorometry

Element[a]	Mean ± SD
Na	301 ± 113
Mg	31.7 ± 4.9
P	196 ± 31
S	203 ± 28
K	872 ± 92
Ca	307 ± 47
Cu	0.67 ± 0.15
Zn	5.12 ± 2.01
Se[b]	0.029 ± 0.006
Sr	0.057 ± 0.018

[a]Concentration is expressed in μg/mL.
[b]Determined by fluorometry; others were by ICP-AES.

gorize into dummy variable 0 and 1 as used in the previous study. The criteria for the selection into, and elimination from, the multiple-regression equation was the significance of the F-value (p_{in} = 0.05 and p_{out} = 0.1).

RESULTS

Table 3 presents arithmetic mean elemental concentrations in 51 Japanese transitory milk, which were determined by fluorometry (Se) and ICP-AES (other elements). Since one-way ANOVA revealed absence of significant variation among the samples from three hospitals, data are combined and are shown in this table. The skewness of the distribution of the elemental concentration exceeded 1 for Na and Sr: log-transformed value was used for these elements in all of the statistical analyses.

Table 4 presents median elemental concentrations detected by ICP-MS. Median value was shown because there were many cases with the concentration below detection limit for some of the elements. In addition, medians for three hospitals were separately shown because ICP-MS analyses were conducted on two separate occasions (one for A and B samples and one for C samples) and this resulted in different detection limits between the two deteminations for some elements as a result of the difference in the acid blank level. Although Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Ga, Ge, and As were detected by ICP-MS, interference from molecular ion was suspected at the mass number used for the determination of each element. Therefore, analytical results for these elements were excluded from the table. Y, Rh, In, Sb, Te, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, W, Re, Tl, and U were not detected in any of the samples (detection limit for the individual element ranged from 0.5 to 11 ng/mL).

Table 4
Median Elemental Concentrations in Japanese Transitory Milk Determined by ICP-MS

Element[a], *m/z*	Hosp. A median,	Hosp. A min–max	Hosp. B median,	Hosp. B min–max	Hosp. C median,	Hosp. C min–max
Li (7)	<0.9	(<0.9–0.95)	<0.9	(<0.9–3.98)	<2.4[b]	(<2.4–7.00)
Al (27)	24.5	(<8.5–223)	78.6	(28.9–528)	<76	(<76–537)
Rb (85)	732	(539–872)	728	(374–940)	857	(708–1220)
Nb (93)	0.23	(<0.4–5.48)	0.43	(<0.4–4.40)	<1.3	(<1.3–2.38)
Zr (90)	<0.7	(<0.7–58.9)	<0.7	(<0.7–8.30)	<1.7	(<1.7–1.69)
Mo (98)	11.0	(6.60–18.8)	10.9	(5.90–36.6)	9.60	(1.80–21.2)
Pd (105)	<1.6	(<1.6–1.93)	<1.6	—[c]	<2.0	(<2.0–2.43)
Ag (107)	<1.5	(<1.5–5.06)	<1.5	(<1.5–7.49)	<2.4	—
Cd (114)	<3.1	(<3.1–3.35)	<3.1	(<3.1–4.92)	<3.0	(<3.0–9.51)
Sn (118)	<2.9	(<2.9–45.1)	<2.9	(<2.9–16.3)	<4.8	(<4.8–14.6)
Cs (133)	1.25	(0.44–3.43)	1.12	(<0.4–2.49)	2.48	(1.39–4.25)
Ba (138)	2.15	(<0.9–38.3)	1.60	(<0.9–6.10)	2.07	(<0.7–26.2)
La (139)	<0.6	—	<0.6	—	<0.8	(<0.8–0.84)
Ce (140)	<0.4	(<0.4–0.68)	<0.4	(<0.4–0.77)	<0.8	(<0.8–2.92)
Hf (180)	<1.5	(<1.5–3.50)	<1.5	—	<2.0	(<2.0–2.42)
Ta (181)	<1.0	(<1.0–8.54)	<1.0	(<1.0–12.8)	<0.6	(<0.6–0.75)
Pt (195)	<2.5	(<2.5–29.6)	<2.5	—	<5.8	—
Au (197)	<1.4	—	<1.4	(<1.4–2.40)	<3.9	—
Pb (208)	<32	(<32–32.7)	<32	(<32–171)	<40	—
Bi (209)	<0.7	—	<0.7	—	<1.7	(<1.7–2.08)
Th (232)	<0.7	—	<0.7	(<0.7–0.82)	<3.4	—

[a]Not all of the elements analyzed are shown. Some elements are not shown for the following reasons: Not detected in any of the samples (Y, Rh, In, Sb, Te, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, W, Re, Tl, U); determined by other analytical methods (Na, Mg, Cu, Zn, Sr by ICP-AES and Se by fluorometry); interference from molecular ion was suspected (Sc(*m/z* = 45), Ti (47), V (51), Cr (52), Mn (55), Fe (57), Co (59), Ni (60), Ga (69), Ge (72), As (75)).

[b]Detection limit for Hosp. C is not identical to that for Hosps. A and B because ICP-MS analysis was performed on separate occasions for Hosp. C and Hosps. A and B.

[c]Not detected in any of the samples from the hospital.

Table 5
Results of the Multiple Regression Analysis

Dependent variable	Independent variable selected[a]	Beta	F-value	R^2
Na[b]	Mother's stature	−.379	8.20**	0.13
Mg	Infant's birth wt	−.392	10.00**	
	Mother's stature	−.300	5.85*	0.25
P	Mother's stature	.320	5.80*	
	Infant's birth wt	−.307	5.35*	0.14
S	Infant's birth wt	−.311	5.23*	0.08
K	None	—	—	—
Ca	Infant's sex	.340	6.42*	0.10
Cu	None	—	—	—
Zn	None	—	—	—
Se	Infant's sex	.296	4.69*	0.07
Rb	Days postpartum	−.391	8.86**	0.14
Sr[b]	Mother's age	.277	4.07*	0.06
Mo[b]	None	—	—	—
Cs	Days postpartum	−.444	12.06**	0.18

[a]Criteria for entrance into, and elmination from, the multiple regression function are the significance of the F-value: $p_{in} = 0.05$; $p_{out} = 0.1$.

[b]Log-transformed value is used for the analysis.

* and ** denotes that the F-value is significant at $p = 0.05$ and $p = 0.01$, respectively.

Table 5 presents the results of the multiple-regression analysis. Dependent variables used were the concentrations of the element listed in Table 2, and Rb, Mo, and Cs (log-transformed value was used for Na, Sr, and Mo). We did not use the data obtained by ICP-MS for Na, Mg, Cu, Zn, Se, and Sr in this statistical analysis. Instead, data for these elements obtained by ICP-AES and fluorometry were used because ICP-AES or fluorometry is widely used and, therefore, considered to be a reliable method for the determination of these elements. Other elements were excluded from the analysis since there were many cases with the concentration below the detection limit. Mother's stature was selected as a significant factor to explain Na, Mg, and P concentration: infant's birth wt for Mg, P, and S: infant's sex for Ca and Se: days postpartum for Rb and Cs: mother's age for Sr. None of the independent variables were selected for K, Cu, Zn, and Mo variation.

DISCUSSION

Elemental Composition of Transitory Milk Identified with ICP-MS

By using the three analytical methods employed in the present study, i.e., ICP-MS, ICP-AES, and fluorometry, 31 elements were detected in the human transitory milk of Japanese (Tables 3 and 4). As

shown in Table 4, ICP-MS is sensitive enough to detect the elements in human milk at ng/mL level or less. Among the elements listed in Table 4, Sn and Mo are essential elements for animals, whereas others are not. Molybdenum concentration in the milk at early lactating period is reported in several studies (*19–21*), in which comparable values to the present ones are obtained. Variation of Mo concentration attributable to the duration of the lactation (*20*) and that attributable to socioeconomical factor (*22*) are recognized. On the other hand, literature data on Sn concentration in human milk is limited. Kosta et al. (*23*) reported that Sn concentrations in transitory milk (*3–8*-d postpartum) of 18 Yugoslavs did not exceed 0.4 ng/mL (converted from reported μg/kg dry wt value to ng/mL value by the factor of 7.5). The range of Sn concentration of the present result is from <2.9 to 45 ng/mL and it is apparently higher than reported. The source of this difference is not clear but geographical variation as well as the possibility of contamination during the sampling and analytical procedure should be considered. Other essential trace elements intended to be determined in this tudy, i.e., V, Cr, Mn, Fe, Co, Ni, and As, were detected by ICP-MS. However, the analytical results were not fully acceptable; interference from molecular ions was suspected from isotopes of the elements. The oxide of matrix element in the sample can be problematic in determining V, Mn, Fe, and Ni at the concentrations normally found in the human milk when the following isotopes were used: ^{51}V ($^{35}Cl^{16}O^+$), ^{55}Mn ($^{39}K^{16}O^+$), ^{57}Fe ($^{41}Ca^{16}O^+$), ^{60}Ni ($^{44}Ca^{16}O^+$). Argon in plasma also forms a molecular ion with matrix elements, such as $^{40}Ar^{12}C^+$, $^{36}Ar^{23}Na^+$ and $^{40}Ar^{35}Cl^+$, interfering at ^{52}Cr, ^{59}Co, and ^{75}As, respectively (*24*). In the present study, a simple sample preparation procedure was employed involving only digestion and dilution. It allowed rapid sample treatment but was not satisfactory for the determination of trace elements of less than *m/z* 80 in human milk by ICP-MS.

Since even a low level exposure to heavy metals is considered to affect neural development, the concentration in human milk should be monitored. In this analysis, toxic heavy metals, such as Cd or Pb, could hardly be detected (Cd: detected in six samples out of 51 cases, Pb: 5/51). Cadmium concentration in transitory milk is reported to be around 0.4 ng/mL (*23*) and reliable data for Pb are not available. Higher values were obtained in WHO/IAEA survey, i.e., the range for Cd was <0.1–18.6 ng/mL and that for Pb was <0.1–219 ng/mL (*11*), although data were on mature milk. The present results (Cd: <3–9.5 ng/mL; Pb: <40–171 ng/mL, Table 4) seem to be within these ranges. We must note that detection limit of Pb (30–40 ng/mL of milk, Table 4) was higher than those of other elements. The higher blank Pb is attributable to Pb analysis at this level being liable to be affected by contamination during the analytical procedure. For the monitoring of Pb exposure level of the newborn, this contamination problem must be overcome.

Aluminum, Rb, Cs, and Ba were the elements that were detected in almost all of the samples. Aluminum at pharmaceutical dose is known to induce neurological damage (*25*), hence frequent detection of Al in human milk attracts caution although the level is not serious in terms of daily Al intake of the adult (*20–40 mg/d/average American (26)*). Contamination during the analytical procedure should also be considered in evaluating the Al data. It might be that Rb, Cs, and Ba are present as "impurities" of the major alkali and alkaline earth elements in milk (Na, K, and Ca). However, it is worth noting that Rb and Cs concentration decrease with days postpartum whereas major alkali elements in milk, i.e., Na and K, do not (Table 5). It thus may be speculated that Rb and Cs are not merely present as "impurities" of major alkali elements in transitory milk but may have some biological role or meaning. Regarding other elements analyzed in this study, there were many samples with concentrations below the detection limits (21 elements were not detected in any of the samples; *see* the footnote of Table 4). We cannot evaluate whether the present concentration levels of individual elements are not harmful to the newborn because valid impact evaluation is not yet established. Human exposure to various trace elements such as Ga, In, lanthanides, is expected increase with the rapid development of high-technology industries, hence we will have to keep monitoring the levels of these elements in human milk because the newborn forms a susceptible population to toxicants.

Variation of Elemental Concentrations

Some of the biological attributes of mother and infant were selected as statistically significant variables to explain the variation in element concentration in human transitory milk by stepwise multiple-regression analysis (Table 5). The data from A and B were statistically analyzed under our almost identical design (the variable "hospital for delivery" was included as an independent variable in the previous study) before (*1*), but the results are not consistent with the present ones: Selection of mother's stature for Na and P variation, mother's age for Sr, and infant's birth wt for S are consistent in both of the studies, however, others are not. The latter "inconsistent" results of statistical analyses might be evidence that the selection of these factors are not the reflection of causal relationships. Regarding the former "consistent" results, the rate of contribution of the significant independent variable(s) to the total variation in element concentration in milk, expressed as R^2 in Table 5, was only 13%. From these results, it can be concluded that elemental concentrations in human milk can vary according to some of the biological attributes of mother and infant, but they can explain only a minor portion of the total variation. Although the rate of contribution may be small, the possible biological mechanism(s) underlying these relations must be clarified for the understanding of the biology of human milk.

In our previous study, the variable "days postpartum" was selected as highly significant independent variable to explain Se and S variation with negative coefficients (*1*). From this result, we concluded that Se and S concentrations in transitory milk concurrently decreased with the days postpartum probably because of the similarity of the chemical property of the two elements. However, the result of the present study, again, was not consistent. This could be attributed to the absence of causal relationship as mentioned earlier, but another interpretation can be made. The variable "hospital for delivery," which was not included in the present analysis (*see* Methods), was selected as a significant independent variable for milk Se variation (*1,16*), indicating that Se concentration in milk can vary according to the factor(s) associated with the difference of hospital (this variable was not selected for S variation). The relation between "days postpartum" and milk Se concentrations might be weakened by exclusion of the variable "hospital for delivery" from the independent variable. One of the factors to be considered is the difference in dietary element intake during the hospitalization for delivery (about 1 wk), according to the hospitals. The studies, so far, generally agree that intake of elements does not correlate with elemental concentrations in milk (*6*). On the other hand, however, the existence of geographical variation in the concentrations of some elements (*10,11*) suggests the possible relation between the two. The influence of the food habits or element intakes of mothers on the elemental concentrations in milk is thus worthy to be reexamined. Work along these lines is in progress in our laboratory.

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