Molybdenum and Chromium Concentrations in Breast Milk from Japanese Women

Munehiro Y OSHIDA,¹ Akiko TAKADA,¹ Junko HIROSE,² Mika ENDÔ,² Tsutomu FUKUWATARI,² and Katsumi SHIBATA²

¹Laboratory of Food and Nutritional Sciences, Department of Life Science and Biotechnology, Faculty of Chemistry, Materials and Bioengineering, Kansai University, Suita, Osaka 564-8680, Japan
²Laboratory of Food Science and Nutrition, Department of Lifestyle Studies, School of Human Cultures, The University of Shiga Prefecture, Hikone, Shiga 522-8533, Japan

Received April 24, 2008; Accepted July 8, 2008; Online Publication, August 7, 2008

MO and Cr are essential trace elements in human nutrition, and deficiencies of them have been observed in patients with long-term total parenteral nutrition.¹,²) In Dietary Reference Intakes for Japanese in 2005 (DRI-J 2005), the recommended dietary allowances of Mo and Cr for adults were set at 20 to 25 µg/d and 25 to 40 µg/d respectively.³) Information on the secretion of trace elements in human milk is needed in order to estimate intake by breast-fed infants and, to establish the recommended intake for infants. In fact, adequate intake (AI) levels of several trace elements for infants (0 to 5 months) were set on the basis of the concentrations of those trace elements in breast milk of Japanese women in DRI-J 2005,³) but, AI levels for Mo and Cr were not set in DRI-J 2005 because there was no available information on the concentration of these two trace elements in breast milk from Japanese women. In the present study, we measured Mo and Cr concentrations of breast milk samples from 79 Japanese women by inductively coupled plasma-mass spectrometry (ICPMS), and attempted to estimate AI levels for these two trace elements in Japanese infants.

The study was reviewed and approved by the Ethics Committee of the University of Shiga Prefecture, and it followed the Declaration of Helsinki. Seventy-nine healthy Japanese mothers who were breast-feeding exclusively and not taking vitamin or mineral supplements were recruited in several midwife clinics in Hokkaido, Chiba, Kanagawa, Kyoto, Hiroshima, and Nagasaki Prefectures in Japan from March 2005 to December 2006. The numbers of subjects recruited in the various prefectures were as follows: Hokkaido, 12; Chiba, 10; Kanagawa, 15; Kyoto, 30; Hiroshima, 2; and Nagasaki, 10. All the subjects had given birth to infants at term (gestational age 38 to 41 weeks). The mothers were 32.0 ± 4.1 years old (mean ± SD), with a range of 19 to 39 years. There were no health problems in their babies.

Breast milk was obtained from the subjects at an intermediate time during breast-feeding, placed in a nylon bag (Kaneson, Osaka, Japan) or a polypropylene centrifuge tube (Sumitomo Bakelite, Tokyo, Japan) and stored in a freezer at −20°C until analysis. The postpartum day on which the sample was collected was 95 ± 5.6 d (mean ± SD) with a range of 5 to 191 d.

Two to 5 milliliters of breast milk was transferred to a ceramic melting pot (32φ × 24 mm), dried at 90°C for 1 h in an electric oven, and then heated in an electric furnace (As One F-B1414M, Osaka, Japan) at 550°C for 16 h. After dry incineration, the remaining ash was dissolved in 5 ml of 2% HNO₃. Mo and Cr in the sample solutions thus prepared were measured by ICPMS with

Key words: molybdenum; chromium; breast milk; dietary reference intake; inductively coupled plasma-mass spectrometry

Abbreviations: Mo, molybdenum; Cr, chromium; DRI-J 2005, Dietary Reference Intakes for Japanese in 2005; AI, adequate intake; ICPMS, inductively coupled plasma-mass spectrometry; Rh, rhodium; WHO, World Health Organization

To whom correspondence should be addressed. Fax: +81-6-6388-8609; E-mail: hanmyou4@ipcku.kansai-u.ac.jp

Footnotes:
1 To whom correspondence should be addressed. Fax: +81-6-6388-8609; E-mail: hanmyou4@ipcku.kansai-u.ac.jp


Communication
direct nebulization. The ICPMS operating conditions were as follows: instrument, ICPM-8500 (Shimadzu, Kyoto, Japan); forward power, 1.200 W; coolant gas flow rate, 7.01/min; auxiliary gas flow rate, 1.51/min; nebulizer gas flow rate, 0.581/min; sampling depth, 5.0 mm; integration time, 2.0 s; number of runs, 20; mode of analysis, pulse; isotopes monitored, $^{52}$Cr, $^{54}$Mo, $^{97}$Mo, and $^{99}$Mo. A rhodium (Rh) isotope ($^{103}$Rh) was used as the internal standard. Since the three analytical results obtained from ion intensities at 95, 97, and 98 $m/z$ were similar, the mean was used for Mo quantification. Mean values of triplicate analyses were used as Mo and Cr values for each subject. The detection limit was 0.1 ng/ml of breast milk for both elements.

Quadruplicate analyses of standard non-fat milk powder (SRM 1549, certified Cr content, $2.6 \pm 0.7$ ng/g; non-certified Mo content, $0.34 \mu g/g$) showed values (mean $\pm$ SD) of $2.9 \pm 0.6$ ng/g as Cr content and $0.32 \pm 0.04 \mu g/g$ as Mo content. On the other hand, quadruplicate analyses of pooled breast milk and a mixture of pooled breast milk with 1 ng/ml of standard Mo or Cr showed values (mean $\pm$ SD) of $5.22 \pm 0.12$ and $6.25 \pm 0.10$ ng/ml as Mo and $1.35 \pm 0.11$ and $2.26 \pm 0.13$ ng/ml as Cr respectively. In addition, quadruplicate analyses of pooled breast milk on another day showed $5.27 \pm 0.08$ ng/ml as Mo and $1.28 \pm 0.09$ ng/ml as Cr.

Among the 79 breast milk samples, only one sample had non-detectable Mo and 15 samples had non-detectable Cr. Figure 1 shows histograms of Mo and Cr concentrations in 79 breast milk samples. For Mo, 51 subjects (64.6%) showed less than 5 ng/ml, and only 12 subjects (15.2%) showed more than 10 ng/ml. This distribution of breast milk Mo is coincident with that observed in our preliminary study. Similarly, for Cr, 38 subjects (48.1%) showed less than 1 ng/ml and 20 subjects (25.3%) showed values ranging from 1 to 2 ng/ml, while only six subjects (7.6%) showed more than 5 ng/ml. Except for samples with non-detectable Mo or Cr, skewness and kurtosis were calculated to be $0.210 (z = -0.245, NS)$ and $-0.532 (z = 6.853, p < 0.001)$ for log Mo ($n = 78$) respectively, and $-0.028 (z = 0.094, NS)$ and $-0.101 (z = 5.517, p < 0.001)$ for log Cr ($n = 64$) respectively. These results indicate that both Mo and Cr show logarithmical normal distribution rather than normal distribution.

Table 1 summarizes the analytical results for Mo and Cr in 79 breast milk samples. In the calculation of these statistical values, we set all non-detectable values to 0.05 ng/ml, which was half the detection limit.

<table>
<thead>
<tr>
<th>Element</th>
<th>Mean* (ng/ml)</th>
<th>Standard deviation* (ng/ml)</th>
<th>Minimum (ng/ml)</th>
<th>Maximum (ng/ml)</th>
<th>Geometric mean* (ng/ml)</th>
<th>Median (ng/ml)</th>
<th>25 percentile value (ng/ml)</th>
<th>75 percentile value (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mo</td>
<td>5.42</td>
<td>5.33</td>
<td>&lt;0.1</td>
<td>25.91</td>
<td>3.57</td>
<td>3.18</td>
<td>1.89</td>
<td>7.16</td>
</tr>
<tr>
<td>Cr</td>
<td>1.73</td>
<td>2.57</td>
<td>&lt;0.1</td>
<td>18.67</td>
<td>0.69</td>
<td>1.00</td>
<td>0.31</td>
<td>0.90</td>
</tr>
</tbody>
</table>

*Non-detectable values were set to 0.05 ng/ml, which was half the detection limit.

Fig. 1. Histograms of Mo and Cr Concentrations in Breast Milk from 79 Japanese Women.

Table 1. Summary of Analyses of Molybdenum and Chromium Contents in Breast Milk from 79 Japanese Women
from 19 to 384 d after delivery were 24 ng/ml and 5 to 63 ng/ml respectively. On the other hand, reports from the US found that most human milk collected more than 1 month after delivery showed less than 2 ng/ml of Mo concentration. In addition, an international collaborative study by the World Health Organization (WHO) showed that most breast milk samples from Guatemala, Hungary, Nigeria, Sweden, and Zaire had less than 5 ng/ml Mo concentration, whereas Philippine breast milk samples showed higher Mo values (median, 16.36 ng/ml; range, 6.75 to 35.41 ng/ml). The present analytical values of breast milk Mo (mean, 5.42 ng/ml; median, 3.18 ng/ml; range, <0.1 to 25.91 ng/ml) are lower than those obtained in Gunshin’s study or in the Philippines, but somewhat higher than those in analyses performed in many countries outside of Asia. Since rice and soybeans are rich in Mo, the dietary Mo intake of Asian people who eat large amounts of rice and soybean products is expected to be higher than that of Western people. In fact, we confirmed that dietary Mo intake and serum Mo concentrations in Japanese is somewhat higher than in Americans or Europeans. Accordingly, it is likely that the Mo concentration in Japanese breast milk is somewhat higher than in breast milk collected in the US or Europe. The present analytical values for breast milk Mo are reasonable and representative values for Japanese breast milk, although the cause of high Mo values in breast milk in Gunshin’s study and in the Philippines is unclear.

There have also been several reports on Cr concentrations in breast milk. In Japanese subjects, values of 6.5 ng/ml and of a non-detectable level to 20.9 ng/ml were reported as the mean and range respectively for 24 Japanese subjects. Another recent Japanese study of a large number of subjects (n = 1,166) reported 59 ± 47 ng/ml (mean ± SD) as the breast milk Cr concentration, but the values in the latter study are not reliable, since no accuracy evaluation of analytical values using standard reference materials was performed. Similarly, the reliability of the former study is also insufficient, since accuracy was evaluated using only orchard leaves (SRM 1571), which contained about 1,000-fold higher amounts of Cr than breast milk. On the other hand, several recent reports indicate that the amounts of Cr in breast milk from most American mothers is less than 1 ng/ml. Accordingly, the Dietary Reference Intakes of the US has adopted a value of 0.25 ng/ml as the average Cr value in breast milk from American mothers. The present analytical values (mean, 1.73 ng/ml; median, 1.00 ng/ml; range, <0.1 to 18.67 ng/ml) were somewhat higher than the US averaged values, but are coincident with breast milk Cr values observed in an international collaborative study performed by the WHO. The present Cr values are therefore reasonable and representative values for Japanese breast milk.

The main purpose of this study was to estimate the AI values for Mo and Cr for Japanese infants. In DRI-J 2005, since the content of each nutrient in breast milk taken by healthy infants is considered to be sufficient to maintain adequate nutritional status, the AI value for each nutrient for infants (0 to 5 months) was set by the following equation: [averaged concentration of each nutrient in breast milk] × [mean volume of milk intake in infants (0.781/d)]. As mentioned above, the present analytical values of Mo and Cr are considered to be representative values for Japanese breast milk, although a random sampling was not performed in a strict sense. Hence, we can attempt to estimate the AI for Mo and Cr for Japanese infants using the present results.

Since both the Mo and the Cr concentration in breast milk from 79 Japanese women showed a logarithmical normal distribution, the geometric mean is suitable for their averaged values. However, when the data include values below detection limit, the geometric mean may vary with the way of treating them. In Table 1, we set all non-detectable values to 0.05 ng/ml, which was half the detection limit. This treatment is the most convenient and has been adopted in many studies, but the estimated geometric mean varies with the setting of the detection limit. Other approaches to estimating the geometric mean of data including non-detectable values are to use Cohen’s maximum likelihood estimator method, and the normal plot method, and the robust method. Following Cohen’s method, we calculated the geometric means of the data excluding non-detectable values and adjusted those geometric means to those of all the data using a detection limit value (0.10 ng/ml) and Cohen’s λ. According to Cohen’s method, the geometric means for Mo and Cr in the 79 breast milk samples were estimated to be 3.52 and 0.71 ng/ml respectively. Following the normal plot method, we depicted two probability plots, as shown in Fig. 2, and calculated a regression equation of log Mo or log Cr versus normal scores. Based on X intercepts in the equation, the geometric means for Mo and Cr were estimated to be 3.66 and 0.82 ng/ml respectively. Following the robust method, we substituted normal scores of the non-detectable values for Y in the regression equation of Fig. 2 to estimate extrapolated values below the detection limit. After this extrapolation, the geometric means for Mo and Cr in the 79 samples were estimated to be 3.66 and 0.82 ng/ml respectively.

These geometric means estimated by Cohen’s method, the normal plot method, and the robust method are different from those described in Table 1 (Mo, 3.57; Cr, 0.69 ng/ml). Thus, since the geometric mean of the data including non-detectable values varied with the treatment of non-detectable values, we used medians as averaged values for Mo and Cr in the 79 Japanese breast milk samples to estimate AI. When the median is used in the estimation, the AI values for Mo and Cr for Japanese infants (0 to 5 months) are 2.5 µg/d (3.18 µg/l × 0.781/d = 2.48 µg/d) and 0.8 µg/d (1.00 µg/l × 0.78 g/l = 0.78 µg/d) respectively.
Acknowledgment

This study was supported by a grant for comprehensive research on cardiovascular and lifestyle disease from the Ministry of Health, Labor, and Welfare of Japan.

References


17) Cohen, A. C., Simplified estimators for the normal distribution when samples are singly censored or truncated. Technometrics, 1, 217–237 (1959).

