

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

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 , ^{95}Mo , ^{97}Mo , and ^{98}Mo . A rhodium (Rh) isotope (^{103}Rh) was used as the internal standard. Since the three analytical values 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 ± 0.7 ng/g; non-certified Mo content, $0.34 \mu\text{g/g}$) showed values (mean \pm SD) of 2.9 ± 0.6 ng/g as Cr content and $0.32 \pm 0.04 \mu\text{g/g}$ 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 ± 0.12 and 6.25 ± 0.10 ng/ml as Mo and 1.35 ± 0.11 and 2.26 ± 0.13 ng/ml as Cr respectively. In addition, quadruplicate analyses of pooled breast milk on another day showed 5.27 ± 0.08 ng/ml as Mo and 1.28 ± 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.⁴⁾ 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

Table 1. Summary of Analyses of Molybdenum and Chromium Contents in Breast Milk from 79 Japanese Women

	Mo (ng/ml)	Cr (ng/ml)
Mean*	5.42	1.73
Standard deviation*	5.33	2.57
Minimum	<0.1	<0.1
Maximum	25.91	18.67
Geometric mean*	3.57	0.69
Median	3.18	1.00
25 percentile value	1.89	0.31
75 percentile value	7.16	2.32

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

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. The arithmetical means for Mo and Cr in the 79 samples were 5.42 and 1.73 ng/ml respectively. Since both elements showed logarithmical normal distribution, their geometric mean and median values were lower than their arithmetical mean values. The ranges of geometric mean \pm geometric standard deviation were 1.33 to 9.57 ng/ml for Mo and 0.17 to 3.34 ng/ml for Cr. There was no significant association between Mo or Cr concentrations and days postpartum on which samples were collected. In addition, no regional variation was observed in Mo or Cr.

There have been several reports on Mo in breast milk. Gunshin *et al.* found that the mean and range of Mo concentration in breast milk from 24 Japanese women

from 19 to 384 d after delivery were 24 ng/ml and 5 to 63 ng/ml respectively.⁵⁾ 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.^{6,7)} 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).⁸⁾ 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,⁹⁾ 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.^{9,10)} 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 study⁵⁾ and in the Philippines⁸⁾ 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.⁵⁾ Another recent Japanese study of a large number of subjects ($n = 1,166$) reported 59 ± 47 ng/ml (mean \pm SD) as the breast milk Cr concentration,¹¹⁾ 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.^{12,13)} 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.¹⁴⁾ 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;¹⁵⁾ 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] \times [mean volume of milk intake in infants (0.78 l/d)].¹⁶⁾ 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,¹⁷⁾ the normal plot method,¹⁸⁾ and the robust method.¹⁹⁾ 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 .²⁰⁾ 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 μ g/d ($3.18 \mu\text{g/l} \times 0.78 \text{ l/d} = 2.48 \mu\text{g/d}$) and 0.8 μ g/d ($1.00 \mu\text{g/l} \times 0.78 \text{ l/d} = 0.78 \mu\text{g/d}$) respectively.

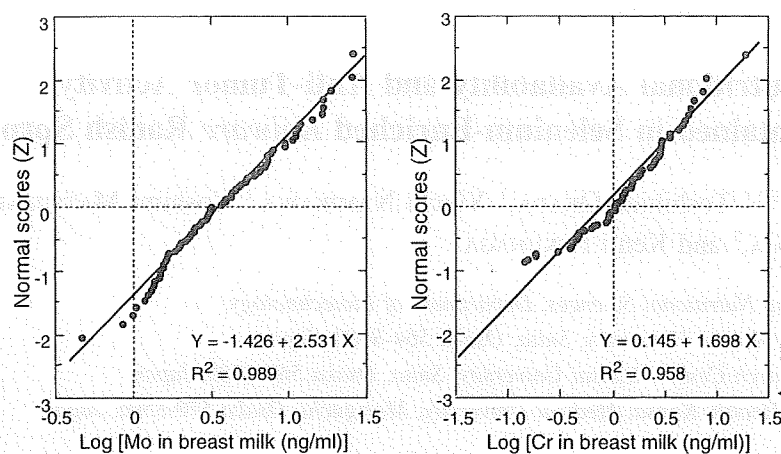


Fig. 2. Regression of Log Mo and Log Cr versus Normal Scores.

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Evaluation of Nutritional Availability and Anti-Tumor Activity of Selenium Contained in Selenium-Enriched *Kaiware* Radish Sprouts

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We estimated the nutritional availability of selenium (Se) in Se-enriched *Kaiware* radish sprouts (SeRS) by the tissue Se deposition and glutathione peroxidase (GPX) activity of rats administered the sprouts, and examined the effect of SeRS on the formation of aberrant crypt foci (ACF) in the colon of mice administered 1,2-dimethylhydrazine (DMH) to evaluate anti-tumor activity. Male weanling Wistar rats were divided into seven groups and fed a Se-deficient basal diet or the basal diet supplemented with 0.05, 0.10, or 0.15 µg/g of Se as sodium selenite or SeRS for 28 d. Supplementation with Se dose-dependently increased serum and liver Se concentrations and GPX activities, and the selenite-supplemented groups showed a higher increase than the SeRS-supplemented groups. The nutritional availability of Se in SeRS was estimated to be 33 or 64% by slope ratio analysis. Male 4-week-old A/J mice were divided into seven groups and fed a low Se basal diet or the basal diet supplemented with selenite, SeRS, or selenite + non-Se-enriched radish sprouts (NonSeRS) at a level of 0.1 or 2.0 µg Se/g for 9 weeks. After 1 week of feeding, all mice were given six subcutaneous injections of DMH (20 mg/kg) at 1-week intervals. The average number of ACF formed in the colon of mice fed the basal diet was 4.3. At a supplementation level of 0.1 µg Se/g, only SeRS significantly inhibited ACF formation. At a supplementation level of 2.0 µg Se/g, both selenite and SeRS significantly inhibited ACF formation. The addition of NonSeRS to the selenite-supplemented diets tended to inhibit ACF formation, but this was not statistically significant. These results indicate that SeRS shows lower nutritional availability but higher anti-tumor activity than selenite.

Key words: selenium; selenium-enriched sprouts; nutritional availability; cancer prevention; aberrant crypt foci

Selenium (Se) is an essential trace element in human and animal nutrition, and it plays several important roles in the form of selenoproteins, including the families of glutathione peroxidases (GPXs), deiodinases and thioredoxine reductases.¹⁾ The average Se intake in the Japanese population is about 100 µg/d/capita.^{2–4)} This estimated value is obviously higher than the Recommended Dietary Allowance (RDA) of Se for adults, but since foods with high Se content are limited to particular food groups such as fish, eggs, meats, and US hard wheat,⁴⁾ a severely unbalanced diet may cause low Se status. It has been pointed out that vegetarians and vegans are most at risk from low Se intakes.⁵⁾ Worldwide, there are some low Se areas, such as New Zealand and Finland.⁶⁾ To prevent low Se status, the preparation of various types of high Se food is useful to increase daily Se intake.⁷⁾

The utilization of dietary minerals including Se is the net result of several physiological and metabolic processes that convert a portion of ingested minerals to certain metabolically critical forms that are necessary for normal physiological function. As for mineral nutrition, it is necessary to show the extent of biological utilization of dietary minerals in their critical or functional forms quantitatively. The quantitative description of biological utilization of dietary minerals has come to be called their bioavailability.⁸⁾ More strictly, the term bioavailability must be replaced by nutritional availability, since there exists the impression that bioavailability includes not only nutritional but also pharmaco-

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Abbreviations: Se, selenium; SeRS, selenium-enriched *Kaiware* radish sprouts; NonSeRS, non selenium-enriched *Kaiware* radish sprouts; GPX, glutathione peroxidase; ACF, aberrant crypt foci; MeSec, Se-methylselenocysteine; ITC, isothiocyanate; DMH, 1,2-dimethylhydrazine; RDA, Recommended Dietary Allowance; HPLC, high performance liquid chromatography; ICPMS, inductively coupled plasma mass spectrometry; LOAEL, lowest observed adverse effect level; UL, tolerable upper intake level

logical activities. The nutritional availability of dietary Se varies with the chemical species of Se in foods.⁸⁾ Since the compositions of Se species in Se-enriched foods are diverse,⁹⁾ their nutritional availability is thought to vary with the kind of foods. Accordingly, Se-enriched foods should be evaluated for its nutritional availability.

Besides nutritional roles, Se is thought to be associated with cancer prevention, judging by the results of epidemiological studies.^{10,11)} In particular, a recent finding, that overall cancer morbidity and mortality were nearly 50% lower with daily supplementation with Se at a level of 200 µg/d, is of great interest.¹²⁾ The anti-tumor activity of Se has also been confirmed in numerous animal experiments,¹³⁾ and a monomethylated Se metabolite is critical in Se chemoprevention.¹⁴⁾ The metabolic conversion rate of monomethylated selenoamino acids such as Se-methylselenocysteine (MeSec) and γ -glutamyl-Se-methylselenocysteine to the monomethylated Se metabolite is probably higher than that of selenite, selenate, or selenocystine. These monomethylated selenoamino acids have been identified in several Se-enriched vegetables.^{9,15-17)} The anti-tumor activities of these Se-enriched vegetables have also been evaluated, and have been found to be higher than that of selenite.^{18,19)} Hence, the applicability of Se-enriched vegetables to cancer prevention is to be expected.

In previous studies, we prepared Se-enriched sprouts of various plant species including *Kaiware* radish and identified the main chemical species of Se in these Se-enriched sprouts as MeSec.²⁰⁾ In the present study, we estimated the nutritional availability of Se in Se-enriched *Kaiware* radish sprouts (SeRS) by tissue Se deposition and GPX activity, and also evaluated anti-tumor activity of SeRS.

In animal experiments to examine the anti-tumor activity of natural products, various chemical carcinogens have been used to induce tumors in liver, colon, and mammary gland. Among these chemicals, 1,2-dimethylhydrazine (DMH) has often been used to induce colon cancer.²¹⁾ Since DMH injected is excreted in the bile after conversion to an active metabolite in various organs, the colon is most exposed to the active carcinogenic metabolite.²²⁾ Consequently, the active metabolite causes alkylation of DNA mainly in the colon, and induces colon cancer specifically.

On the other hand, it has been proposed that aberrant crypt foci (ACF) are preneoplastic lesions and that those with a crypt multiplicity of more than 4 continue growing to tumors.²³⁾ Since the experimental period for formation of ACF is short and the identification of ACF formed is done readily, ACF has been used as an index of precancerous lesions in the colon.²⁴⁾ Hence we therefore scored the number of ACF with a crypt multiplicity of more than 4 as an index for the risk of colon cancer in the present study.

Table 1. Composition of Basal Se-Deficient Diet Used in Experiment 1

Ingredients	%
<i>Torula</i> yeast ^a	35.2
Sucrose	51.8
Soybean oil	8.0
AIN93G salt mixture ^b	3.5
AIN93G vitamin mixture	1.0
Choline bitartrate	0.2
DL-Methionine	0.3

^aKR yeast[®] kindly supplied by Kohjin (Tokyo). Crude protein content was 51.2%.

^bExcept for sodium selenate.

Materials and Methods

Preparation of SeRS. Seeds of *Kaiware* radish (a type of Japanese white radish (*daikon*), the sprouts of which are eaten (scientific name, *Raphanus sativus*)) were purchased from a local retail shop in Osaka, Japan. SeRS were prepared by hydroponics using 10 µg Se/ml of sodium selenite solution, as described previously.²⁰⁾ Non-Se-enriched radish sprouts (NonSeRS) were also prepared using deionized water. SeRS and NonSeRS were freeze-dried and milled. The Se contents of the SeRS and NonSeRS were 110 and 0.03 µg/g dry weight respectively.

Animal feeding. The experimental protocol was reviewed and approved by the Animal Ethics Committee of Kansai Medical University and followed the "Guide for the Care and Use of Experimental Animals" of the Prime Minister's Office of Japan. Experimental animals were fed in a room under a controlled 12 h light (8:00 to 20:00) and dark cycle at a temperature of 22 to 24 °C respectively and a humidity of 60%. The animals were given experimental diets and deionized water *ad libitum* during the entire experimental period.

In experiment 1, 42 male weanling Wistar rats were divided into seven groups and fed a *Torula* yeast-based Se-deficient basal diet or the basal diet supplemented with 0.05, 0.10, or 0.15 µg/g of Se as sodium selenite or dried powder of the SeRS for 28 d. The composition of the basal Se-deficient diet is shown in Table 1. Analysis showed the basal Se-deficient diet to contain less than 0.01 µg Se/g. After feeding for 28 d, the rats were anesthetized with diethyl ether, blood was collected from the aorta abdominalis, and the liver was excised, washed, blotted, and weighed.

In experiment 2, 84 male 4-week-old A/J mice were divided into seven groups and fed a casein-based low Se basal diet or the basal diet supplemented with selenite, dried powder of the SeRS, or selenite + dried powder of NonSeRS at a level of 0.1 or 2.0 µg Se/g for 9 weeks. The supplementary level of NonSeRS was equal to that of SeRS; when the supplementary Se levels were 0.1 or 2.0 µg/g, supplementary amounts of both sprouts were 0.91 or 18.2 mg/g respectively. The composition of the

Table 2. Composition of Basal Low Se Diet Used in Experiment 2

Ingredients	%
Casein	20.0
α -Corn starch	13.2
β -Corn starch	39.75
Sucrose	10.0
Corn oil	7.0
AIN93G salt mixture ^a	3.5
AIN93G vitamin mixture	1.0
Choline bitartrate	0.25
Cellulose	5.0
L-Cystine	0.3

^aExcept for sodium selenate.

basal low Se diet is shown in Table 2. Since α -linolenic acid inhibits the development of mammary gland and colon cancer induced by DMH,²⁵⁾ we used corn oil, which contains a lower level of this polyunsaturated fatty acid than soybean oil. Analysis showed the low Se basal diet to contain 0.035 μ g Se/g. After 1 week of feeding, all mice were given six subcutaneous injections at 1-week intervals of saline containing DMH (20 mg/kg body weight). After feeding for 9 weeks, the mice were anesthetized with diethyl ether, blood was collected by heart puncture, the liver was removed, washed with saline, blotted, and weighed, and the colon was removed, opened longitudinally, washed with saline, and fixed flat between paper towels in a Formalin Neutral Buffer Solution (containing 4% formaldehyde, pH 7.4; Wako Pure Chemical Industries, Osaka, Japan).

Assays. The blood was kept at room temperature and serum was obtained. The liver was homogenized with 9 volumes of saline in a Teflon-glass homogenizer.

GPX activities in the serum and liver homogenate were assayed by a modification of the method of Paglia and Valentine,²⁶⁾ with *tert*-butyl hydroperoxide as the peroxide substrate.²⁷⁾ The standard assay medium contained 0.13 mM NADPH, 2 mM GSH, 0.27 mM *tert*-butyl hydroperoxide, 1 mM NaN₃, 0.1 mM EDTA, 0.4 unit of GSH reductase, 20 μ l of the serum and the liver homogenate, and 50 mM of sodium phosphate buffer (pH 7.0, 37°C), in a final volume of 3.0 ml. The reaction was started by adding the hydroperoxide to the assay medium, previously equilibrated at 37°C. Units of enzyme activity were defined as μ mol NADPH oxidized per min.

Se in the sprouts and diets was analyzed by high performance liquid chromatography (HPLC) with a fluorometric detector.²⁸⁾ Up to 1 g of the diets or sprouts was carefully digested with 10 ml of nitric acid for 30 min. After that, the mixture was further digested with 5 ml of perchloric acid until the appearance of white fumes of perchloric acid. The volume of the digest was made up to 10 ml with water, and the diluted digest was heated with 1 ml of 10% HCl in a boiling water bath for 30 min. The pH of the mixture was then adjusted to 1.0

to 1.5 with 7 M of ammonium solution, and 1 ml of 0.1% 2,3-diaminonaphthalene dissolved in 0.1 M HCl was added. The volume of the mixture was made up to 30 ml with 0.1 M of HCl, and the mixture was incubated at 50°C for 30 min. Then the 4,5-benzopiazselenol formed was extracted with 10 ml of cyclohexane and quantified by HPLC. The conditions of HPLC was as follows: column, TSKgel Silica-60 (250 \times 4.6 mm i.d., Tosoh, Tokyo); mobile phase, cyclohexane/2-propanol (w/w = 99/1); flow rate, 1.0 ml/min; detection, a fluorescence detector (excitation 378 nm, emission 520 nm).

Se in the serum and liver homogenate was determined by inductively coupled plasma mass spectrometry (ICPMS).²⁹⁾ In the case of serum, 200 μ l of the sample was heated with 0.5 ml of nitric acid in a boiling water bath until the disappearance of insoluble components. The volume of the digest was made up to 5.0 ml with water. In the case of liver, up to 10 ml of the homogenate was heated with 5 ml of nitric acid in a boiling water bath until the disappearance of insoluble components. The volume of the digest was made up to 25 ml with water. To determine Se, these diluted digests were directly nebulized to ICPMS and the ion intensity of ⁸²Se was monitored.

Protein was measured by the method of Lowry *et al.*,³⁰⁾ with bovine serum albumin as a standard. In experiment 1, serum biochemical tests, including total protein, albumin, alanine aminotransferase, aspartate aminotransferase, total lipid, total cholesterol, urea nitrogen, and creatinine, were also performed by a commercial service (Japan Medical Laboratory, Osaka, Japan).

Analysis of ACF in colon of mice in experiment 2. The fixed colon of mice was stained with 0.02% methylene blue for 3 min and then washed with saline. An operator who was unaware of the dietary treatment scored the number of ACF in the stained colon under a dissecting microscope. In the present study, we scored the number of ACF with a crypt multiplicity of more than 4 as described above.

Assessment of nutritional availability of Se. In experiment 1, the nutritional availability of Se from SeRS was assessed using sodium selenite as reference Se. The deposition of Se and the increase in GPX activity in the liver and serum were used as responses to increasing amounts of dietary Se. Since the responses (*R*) to increasing amounts of dietary Se (*X*) were assumed to be described by the general equation $R = mX + k$, the relative nutritional availability of Se from SeRS was estimated by the slope-ratio technique, which compares the slope of dose-response plots to the slope observed for selenite Se. Nutritional availability was defined as {(slope of SeRS)/(slope of selenite) \times 100}.⁸⁾

Statistics. Experimental data were assessed by one-way analysis of variance. When the *F* value was

Table 3. Se Deposition of Rats in Experiment 1

Supplemented Se		Se deposition	
Source	Level ($\mu\text{g/g}$ diet)	Liver (ng/g tissue)	Serum (ng/ml)
None	0	28 ± 5^a	73 ± 3^a
Selenite	0.05	98 ± 10^{ab}	185 ± 10^b
SeRS	0.05	57 ± 7^{ab}	141 ± 7^b
Selenite	0.10	254 ± 31^c	265 ± 27^c
SeRS	0.10	102 ± 10^{ab}	185 ± 7^b
Selenite	0.15	344 ± 35^d	342 ± 10^d
SeRS	0.15	130 ± 21^b	249 ± 16^c

Values are the means \pm SE ($n = 6$). Means not sharing a common superscript in the same column differ significantly at $p < 0.05$.

Table 4. GPX Activities of Rats in Experiment 1

Supplemented Se		GPX activity	
Source	Level ($\mu\text{g/g}$ diet)	Liver (unit/g protein)	Serum (unit/ml)
None	0	40 ± 2^a	1.24 ± 0.08^a
Selenite	0.05	109 ± 5^{ab}	2.80 ± 0.18^{bc}
SeRS	0.05	71 ± 5^a	2.15 ± 0.18^{ab}
Selenite	0.10	225 ± 35^c	3.59 ± 0.35^{cd}
SeRS	0.10	100 ± 7^{ab}	2.50 ± 0.16^b
Selenite	0.15	446 ± 38^d	4.19 ± 0.31^d
SeRS	0.15	179 ± 24^{bc}	3.13 ± 0.25^{bc}

Values are the means \pm SE ($n = 6$). Enzyme units expressed as μmol NADPH oxidized per min. Means not sharing a common superscript in the same column differ significantly at $p < 0.05$.

Table 5. Regression Analysis between Supplementary Se Levels and Tissue Se Contents and GPX Activities in Experiment 1

Dependent variables	Slope (m)		Constant (k)		Correlation coefficient (r)	
	Selenite	SeRS	Selenite	SeRS	Selenite	SeRS
Se content						
Liver	2.21×10^3	0.71×10^3	16	27	0.91	0.81
Serum	1.77×10^3	1.14×10^3	84	76	0.94	0.95
GPX activity						
Liver	2.66×10^3	0.89×10^3	5	31	0.90	0.84
Serum	19.3	12.1	1.51	1.35	0.87	0.85

Regression was fitted to the equation $R = mX + k$, where R represented tissue Se content or GPX activity in rats fed the basal diet or the diet supplemented with Se at level X ($\mu\text{g/g}$). Units of dependent variables were the same as described in Tables 3 and 4.

significant ($p < 0.05$), the Tukey-Kramer multiple range test was performed to determine which pairs of the means were significantly different ($p < 0.05$). These statistical tests were performed using a personal computer (eMac, Apple Computer, Cupertino, CA) with operating system Mac OS 9.2 and statistical program package StatView-J version 5.0 (Abacus Concept, Berkeley, CA).

Results

Experiment 1

During the entire feeding period of 28 d, no significant differences were observed in body weight or animal growth irrespective of dietary Se status. At the end of the experimental period, the mean \pm SE of the body weight (g) of the various groups were as follows: basal, 269 ± 9 ; + 0.05 μg Se/g as selenite, 271 ± 5 ; + 0.05 μg Se/g as SeRS, 260 ± 7 ; + 0.10 μg Se/g as selenite, 268 ± 13 ; + 0.10 μg Se/g as SeRS, 265 ± 10 ; + 0.15 μg Se/g as selenite, 270 ± 3 ; + 0.15 μg Se/g as SeRS, 260 ± 8 . Similarly, the effect of Se supplementation was least significant on liver weight and serum biochemistry (data not shown).

The response of Se deposition and GPX activities in the liver and serum are summarized in Tables 3 and 4. Se deposition and GPX activities both increased gradually with increasing of supplementary levels of Se, regardless of source or of the tissue monitored. These

responses did not tend to level off within the range of Se levels tested in the present study, but responses varied with Se source. Selenite Se led to a higher accumulation of Se and a higher elevation of GPX than did sprout Se. In particular, the four parameters in rats supplemented with selenite showed significantly higher values than those in rats supplemented with SeRS, at supplementary levels of 0.10 and 0.15 μg Se/g. This difference in the Se source was more remarkable in the liver than in the serum.

Table 5 summarizes the results of regression analysis made between supplementary Se levels and either Se deposition or GPX activities. In either combination, a strong correlation was observed when the regression was fitted to the general equation $R = mX + k$; the assumption that the responses can be described by this general equation was adequate. Table 6 shows the relative nutritional availability of Se in SeRS as assessed by comparing the regression slope with that of selenite Se. The estimated availability was always lower than 100%, but varied according to which response data, serum or liver parameters, were used in the assessment. Although the serum parameters gave relative nutritional availability measures averaging 64%, the liver parameters gave lower measures averaging 33%.

Experiment 2

There was no significant difference in body weight or animal growth irrespective of the dietary condition. At

Table 6. Nutritional Availability of Se Contained in SeRS

Parameters	Availability (%)
Se deposition	
Liver	32.1
Serum	64.4
GPX activity	
Liver	33.5
Serum	63.0

Nutritional availability was estimated using the slope of the regression line described in Table 5: (slope of SeRS)/(slope of selenite) \times 100.

the end of the experimental period, the mean \pm SE of body weight (g) of the groups were as follows: basal, 26.9 ± 0.6 ; + 0.1 μg Se/g as selenite, 27.6 ± 0.6 ; + 0.1 μg Se/g as SeRS, 26.6 ± 0.5 ; + 0.1 μg Se/g as selenite and NonSeRS, 26.7 ± 0.5 ; + 2.0 μg Se/g as selenite, 26.8 ± 1.0 ; + 2.0 μg Se/g as SeRS, 25.9 ± 1.3 ; + 2.0 μg Se/g as selenite and NonSeRS, 26.3 ± 0.5 .

Tables 7 and 8 summarize Se deposition and GPX activities in the liver and serum of the mice. The response patterns for the four parameters were similar. When the supplementary Se level was 0.1 $\mu\text{g/g}$, responses by selenite Se were higher than those of Se in the SeRS irrespective of supplementation with NonSeRS. On the other hand, when the supplementary level was 2.0 μg Se/g, the responses in mice supplemented with selenite alone were higher than those in other mice;

supplementation with high amounts of NonSeRS inhibited the elevation of Se deposition and GPX activities.

Table 9 shows the number of ACF formed in the colon of mice injected with DMH. The average number of ACF formed in the colon of mice fed the basal low Se diet was 4.3. Supplementation with Se to the basal diet showed an inhibitory effect on the formation of ACF, but the extent of inhibition varied with the Se source and the supplementary level. When the supplementary Se level was 0.1 $\mu\text{g/g}$, a significant inhibitory effect on the formation of ACF was observed only in mice supplemented with SeRS. On the other hand, when the supplementary level was 2.0 μg Se/g, both selenite and SeRS showed a significant inhibitory effect on the formation of ACF, but an increase in the supplementary level of SeRS to 2.0 μg Se/g did not cause a further decrease in ACF formation. The addition of NonSeRS to the selenite-supplemented diets tended to inhibit the formation of ACF, but this inhibition was not statistically significant ($p > 0.05$).

Discussion

One purpose of the present study was to assess the nutritional availability of Se in SeRS quantitatively. As the Table 6 shows, Se from SeRS was less bioavailable than equivalent amounts of selenite Se within the tested

Table 7. Se Deposition of Mice in Experiment 2

Supplemented Se		Supplemented sprouts		Se deposition	
Source	Level ($\mu\text{g/g}$)	Type	Level (mg/g)	Liver (ng/g tissue)	Serum (ng/ml)
None	0	—	—	151 ± 14^a	155 ± 7^a
Selenite	0.1	—	—	328 ± 50^{bc}	234 ± 11^{bcd}
SeRS	0.1	SeRS	0.91	240 ± 20^{ab}	185 ± 6^{ab}
Selenite	0.1	NonSeRS	0.91	234 ± 18^{ab}	209 ± 10^{abc}
Selenite	2.0	—	—	512 ± 36^d	275 ± 27^d
SeRS	2.0	SeRS	18.2	412 ± 23^{cd}	264 ± 7^d
Selenite	2.0	NonSeRS	18.2	327 ± 21^{bc}	248 ± 5^{cd}

Values are the means \pm SE ($n = 12$). Means not sharing a common superscript in the same column differ significantly at $p < 0.05$.

Table 8. GPX Activities of Mice in Experiment 2

Supplemented Se		Supplemented sprouts		GPX activity	
Source	Level ($\mu\text{g/g}$)	Type	Level (mg/g)	Liver (unit/g protein)	Serum (unit/ml)
None	0	—	—	229 ± 30^a	1.80 ± 0.06^a
Selenite	0.1	—	—	723 ± 84^c	2.51 ± 0.18^b
SeRS	0.1	SeRS	0.91	427 ± 35^{ab}	1.60 ± 0.06^a
Selenite	0.1	NonSeRS	0.91	820 ± 55^c	1.83 ± 0.09^a
Selenite	2.0	—	—	827 ± 63^c	2.95 ± 0.20^b
SeRS	2.0	SeRS	18.2	521 ± 37^b	2.77 ± 0.17^b
Selenite	2.0	NonSeRS	18.2	480 ± 45^b	2.54 ± 0.17^b

Values are the means \pm SE ($n = 12$). Enzyme units expressed as μmol NADPH oxidized per min. Means not sharing a common superscript in the same column differ significantly at $p < 0.05$.

Table 9. Effect of Supplementation with Se and Sprouts on Formation of ACF in Colon of Mice Administered DMH

Supplemented Se		Supplemented sprouts		ACF (number/colon)
Source	Level ($\mu\text{g/g}$)	Type	Level (mg/g)	
None	0	—	—	4.3 ± 0.5^b
Selenite	0.1	—	—	3.8 ± 0.6^b
SeRS	0.1	SeRS	0.91	2.3 ± 0.6^a
Selenite	0.1	NonSeRS	0.91	3.1 ± 0.3^{ab}
Selenite	2.0	—	—	2.3 ± 0.3^a
SeRS	2.0	SeRS	18.2	2.3 ± 0.3^a
Selenite	2.0	NonSeRS	18.2	2.0 ± 0.2^a

Values are the means \pm SE ($n = 12$). Means not sharing a common superscript in the same column differ significantly at $p < 0.05$.

range of supplementation, regardless of the response measures employed for nutritional assessment. This was also confirmed in experiment 2 (Tables 7 and 8). Supplementation with SeRS gave a lower elevation of tissue Se deposition and GPX activities than selenite did in mice.

We have identified the main Se species in SeRS as MeSec.²⁰⁾ Since dietary Se must be metabolized to selenide before incorporation into selenoproteins,¹⁾ a lower elevation of GPX in tissues of rats or mice given SeRS indicates that demethylation of MeSec is negligible, but occurs to a certain extent in the tissues. The estimated availability varied according to which response data were used in the assessment; the availability values of the serum parameters were two times higher than the liver parameters. The molecular species differs between liver and serum GPX; while the former is called classical GPX (GPX1), the latter, called extracellular GPX (GPX3), is synthesized in the kidney and secreted into the plasma.³¹⁾ Accordingly, the difference in responses, as between liver and serum might be caused by differences in demethylation ability between the liver and the kidney.

Supplementation with a high amount of NonSeRS inhibited the elevation of Se deposition and GPX activities caused by $2.0 \mu\text{g}$ Se/g of selenite in experiment 2. This indicates that components in radish sprouts lowered the nutritional availability of Se. Cruciferous vegetables, including *Kaiware* radish, contain several isothiocyanates (ITCs) as pungent taste substances, and the major ITC in Japanese white radish has been identified as 4-(methylthio)-3-butenyl isothiocyanate.³²⁾ Since ITCs are highly reactive with the thiol group,³³⁾ it is likely that this inhibition was caused by ITCs in the sprouts.

Another purpose of the present study was to evaluate the anti-tumor activity of SeRS. As described in Table 9, Se added to the low Se diet at a level of $2.0 \mu\text{g/g}$ inhibited the formation of ACF irrespective of the Se source. A similar inhibitory effect of high dietary Se ($1.0 \mu\text{g/g}$ or more) on ACF formation has been reported for selenite,³⁴⁾ selenomethionine,³⁵⁾ high Se broccoli,¹⁹⁾ and high Se broccoli sprouts.³⁶⁾ Hence, it is

possible that dietary Se at high levels inhibits ACF formation in the colon and prevents colon cancer.

At a supplementary level of $0.1 \mu\text{g}$ Se/g, only SeRS showed significant inhibition of the formation of ACF. This indicates that Se in SeRS has higher anti-tumor activity than selenite Se. Similar higher anti-tumor activity in Se-enriched vegetables has been reported for broccoli,^{19,36)} garlic,³⁷⁾ and ramps³⁸⁾ at a dietary Se level of $1.0 \mu\text{g/g}$ or more. Since the main Se species in these Se-enriched vegetables, including SeRS, has been identified as MeSec^{9,15-18,20)} and a monomethylated Se metabolite is critical in Se chemoprevention,¹⁴⁾ the higher anti-tumor activity in these Se-enriched vegetables is thought to be derived from MeSec.

Although an anti-tumor effect of Se has been found at a dietary level of $1.0 \mu\text{g/g}$ or more even in the case of Se-enriched vegetables in previous studies,^{19,36-38)} SeRS inhibited ACF formation at a dietary level of $0.1 \mu\text{g}$ Se/g in the present study. The anti-tumor activity of SeRS is considered to be higher than that of other Se-enriched plant foods. Since about 90% of Se species in the SeRS used in the present study were identified as MeSec,²⁰⁾ the inhibitory effect caused by lower level of SeRS may be associated with the high ratio of MeSec in Se species of SeRS.

On the other hand, the addition of NonSeRS to the selenite-supplemented diets tended to inhibit the formation of ACF. This leads to the possibility that NonSeRS also inhibits ACF formation. It has been reported that ITCs in cruciferous vegetables inhibit the development of several types of tumors.³³⁾ Because the Se concentration of SeRS ($110 \mu\text{g/g}$ dry weight) used in the present study was lower than that in the high Se broccoli and broccoli sprouts used in previous reports,^{19,36)} more Se-enriched vegetables were added to the diet to adjust the dietary Se level to $0.1 \mu\text{g/g}$ in the present study than in previous reports. Hence the ITC content in the diet supplemented with SeRS must have been higher than in the diets with high Se-broccoli or Se-broccoli sprouts at an equivalent Se level, and this high level of ITCs may have elevated the inhibitory effect of SeRS on ACF formation at the low supplementary level.

The present experimental results indicate that SeRS has lower nutritional availability but higher anti-tumor activity than selenite. This high anti-tumor activity of SeRS indicates the use of this high-Se plant food in the diet for cancer prevention. However, careful consideration is necessary as to the use of SeRS, since Se is a highly toxic element. Based on the lowest observed adverse effect level (LOAEL) of Se (913 µg/d),³⁹⁾ the dietary reference intake for Japanese in 2005 indicated 350 to 450 µg/d as the tolerable upper intake level (UL) of Se for adults.⁴⁰⁾ Since the energy content of the basal diet used in experiment 2 was estimated to be 3.86 kcal/g, 0.1 and 2.0 µg Se/g correspond to 0.026 and 0.52 µg Se/kcal respectively. Thus, based on the estimation that the energy intake of Japanese adults is 2,000 kcal/d, the intake of a diet with 0.1 or 2.0 µg Se/g in mice is considered to correspond to a human Se intake of 52 or 1,040 µg/d respectively. This indicates that a diet containing Se at a level of 2.0 µg/g causes a high Se intake, which exceeds not only the UL but also the LOAEL for Se. Accordingly, the inhibitory effect of Se on ACF formation at a level of 2.0 µg/g cannot be applicable to the human diet regardless of the Se source.

Since an additional Se intake of 52 µg/d probably causes no adverse effects on human health, the inhibitory effect of SeRS on ACF formation at 0.1 µg Se/g appears to be applicable to the human diet. However, the present results indicate only that supplementation with SeRS of a low Se diet at a level of 0.1 µg Se/g was effective at inhibiting the formation of DMH-induced ACF in the colon of mice. Moreover, supplementation with SeRS at 2.0 µg Se/g did not show a higher inhibitory effect than that at 0.1 µg Se/g. Since the average daily Se intake of the Japanese has been estimated to be about 100 µg/d,²⁻⁴⁾ additional Se intake from SeRS may not be effective for cancer prevention.

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Short Communication

Assessment of Nutritional Availability of Selenium in Selenium-enriched Pumpkin

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Abstract

The nutritional availability of selenium (Se) in Se-enriched pumpkin (Se-pumpkin) was assessed by comparing with selenite and Se-enriched *Kaiware* radish sprouts (Se-sprouts). Male weanling ddY mice were fed a *Torula* yeast-based Se-deficient diet. After feeding for 3 weeks, mice were divided into 7 groups and fed the basal diet or a diet supplemented with 0.05 or 0.25 µg/g of Se as either sodium selenite, Se-pumpkin or Se-sprouts for further one week. Supplementation of Se dose-dependently increased serum and liver Se concentrations and glutathione peroxidase (GPX) activities. In serum Se and GPX, the increases by Se supplementation did not significantly vary with Se source, but in the liver Se and GPX, the increases by selenite supplementation were significantly higher than those by supplementation with Se-pumpkin or Se-sprouts. A difference between the effect of Se-pumpkin and that of Se-sprouts was found in the elevation of liver Se concentration; supplementation with Se-pumpkin caused significantly higher elevation of liver Se than that with Se-sprouts. When liver Se was used, the nutritional availabilities of Se from Se-pumpkin and that Se-sprouts were estimated to be 97% and 65% to selenite Se, respectively. However, when liver GPX was used for the estimation, the availability of Se from either Se-pumpkin or Se-sprouts was less than 50% to selenite Se.

Keywords : selenium, nutritional availability, glutathione peroxidase, selenium-enriched vegetables, selenium-enriched radish sprouts, selenium-enriched pumpkin

Introduction

Selenium (Se) is an essential trace element in human and animal nutrition, and plays several important roles in the form of selenoproteins that include the families of glutathione peroxidase (GPX), deiodinases or thioredox-

ine reductases [1]. Besides the nutritional roles, Se is thought to be associated with cancer prevention from the results of many epidemiological studies and animal experiments [2]. To prevent a low Se status, various Se-enriched foods were prepared and used to increase daily Se intake [3, 4]. In particular, several Se-enriched plant foods have been developed since their anti-tumor activities are expected to be higher than those of selenite, selenate or high Se yeast [4].

The utilization of dietary minerals including Se is the net result of several physiological and metabolic processes that converted a portion of ingested minerals to certain metabolically critical forms that are necessary for normal physiological function. In the view of mineral nutrition, it is necessary to show an extent of the biological utilization of dietary minerals for their critical or functional forms quantitatively. The quantitative description of biological utilization of dietary minerals has come to

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be called their "bioavailability" or "nutritional availability" [5]. There are three factors contributing to the nutritional availability ; physiological characteristics of host animals, dietary components ingested with minerals and chemical species of minerals. Since the composition of Se species in the Se-enriched plant foods is diverse [6], their nutritional availabilities are thought to vary with the kinds of plant species. In the present study, we attempted to compare the nutritional availability of Se in Se-enriched pumpkin (Se-pumpkin) to those in selenite and Se-enriched *Kaiware* radish sprouts (Se-sprouts) by using tissue Se deposition and GPX activity of mice given these Se sources.

Materials and Methods

Se-pumpkin was purchased from PhytoSelenium Research Laboratories (Kumamoto, Japan) [7], and Se-sprouts were prepared by hydroponics, which used 10 µg Se/ml of sodium selenite solution as described previously [8]. Both Se-enriched vegetables were freeze-dried and milled. Se contents of Se-pumpkin and Se-sprouts were 45 and 121 µg/g dry weight, respectively.

The protocol of the animal experiment was reviewed and approved by the Animal Ethics Committee of Kansai Medical University. Fifty-six male weanling ddY mice were fed a *Torula* yeast-based Se-deficient basal diet [9]. After feeding for 3 weeks, mice were divided into 7 groups and fed the basal diet or the basal diet supplemented with 0.05 or 0.25 µg/g of Se as either sodium selenite, Se-pumpkin or Se-sprouts for a further week. Serum and liver were then collected, and their Se contents

and GPX activities were measured. Se was determined by inductively coupled plasma mass spectrometry [10] and GPX activities were assayed using *tert*-butyl hydroperoxide as the peroxide substrate [11].

In the present study, the nutritional availability of Se from Se-pumpkin or Se-sprouts was assessed using sodium selenite as reference Se. The concentration of Se and GPX activity in liver and serum were used as the responses to increasing amounts of dietary Se. As the responses (*Y*) to increasing amounts of dietary Se (*X*) can be described by the general equation $Y = mX + k$, the relative nutritional availability of Se from Se-enriched vegetables was estimated by a slope-ratio technique that compares the slope of dose-response plots to the slope observed for selenite Se [12].

Results

No significant difference was observed in the body weight among groups. At the end of the feeding period, the mean ± SD of body weight for all mice (n=56) was 33.2 ± 1.9 g.

Se concentration and GPX activities in the liver and serum are summarized in Tables 1. The Se concentration and GPX activities both increased gradually with an increase of the supplementary level of Se, regardless of its source or the tissue monitored. In the serum Se and GPX, the increases by Se supplementation did not vary with the Se source, but in the liver Se and GPX, the increases by selenite supplementation were higher than those supplemented with Se-pumpkin or Se-sprouts. In the elevation of liver Se concentration, supplementation with Se-

Table 1 Se concentration and GPX activities in serum and liver of rats fed experimental diets

Se supplemented to diet		Se concentration		GPX activity	
Source	Level (µg/g)	Serum (ng/ml)	Liver (ng/g)	Serum (unit/ml)	Liver (unit/g protein)
None	-	37 ± 5 ^a	54 ± 3 ^a	0.17 ± 0.01 ^a	17 ± 3 ^a
Selenite	0.05	162 ± 10 ^b	210 ± 20 ^b	0.36 ± 0.07 ^{ab}	75 ± 11 ^a
Se-pumpkin	0.05	113 ± 6 ^b	98 ± 3 ^{ab}	0.22 ± 0.03 ^a	24 ± 6 ^a
Se-sprouts	0.05	102 ± 4 ^{ab}	92 ± 3 ^{ab}	0.20 ± 0.03 ^a	16 ± 5 ^a
Selenite	0.25	449 ± 20 ^c	657 ± 43 ^d	0.81 ± 0.12 ^c	442 ± 35 ^c
Se-pumpkin	0.25	428 ± 24 ^c	598 ± 36 ^d	0.81 ± 0.13 ^c	212 ± 42 ^b
Se-sprouts	0.25	411 ± 23 ^c	463 ± 48 ^c	0.72 ± 0.13 ^{bc}	217 ± 42 ^b

Values are the means ± SEM (n=8). GPX units expressed as µmol NADPH oxidized per min. Means in the same column not sharing a common superscript differ significantly (*p* < 0.05) by analysis of variance followed by Tukey-Kramer multiple range test.

Table 2 Regression of supplementary Se level (X) with parameters of Se status (Y) and nutritional availability of Se from Se-pumpkin or Se-sprouts.

Parameters	Source of Se supplemented	Regression	Correlation coefficient (r)	Nutritional availability (%)
Serum Se	Selenite	$Y = 1592 X + 56$	0.973	-
	Se-pumpkin	$Y = 1568 X + 36$	0.975	98.5
	Se-sprouts	$Y = 1362 X + 36$	0.911	85.6
Serum GPX	Selenite	$Y = 2.46 X + 0.20$	0.767	-
	Se-pumpkin	$Y = 2.67 X + 0.14$	0.815	108.5
	Se-sprouts	$Y = 2.06 X + 0.14$	0.711	83.7
Liver Se	Selenite	$Y = 2356 X + 71$	0.954	-
	Se-pumpkin	$Y = 2267 X + 23$	0.967	96.2
	Se-sprouts	$Y = 1529 X + 38$	0.858	64.9
Liver GPX	Selenite	$Y = 1735 X + 5$	0.952	-
	Se-pumpkin	$Y = 819 X + 3$	0.804	47.2
	Se-sprouts	$Y = 757 X + 2$	0.746	43.6

Regression was fitted to the equation $Y = mX + k$, where Y represented the parameters in mice fed the basal diet or the diet supplemented with Se at X level ($\mu\text{g/g}$). Units of parameters are the same as in Table 1. Nutritional availability was estimated using the slope of the regression ; (slope of Se-pumpkin or Se-sprouts)/(slope of selenite) $\times 100$.

pumpkin caused a higher elevation of liver Se than that with Se-sprouts.

The regression analyses of supplementary Se with the Se concentration or GPX activities are summarized in Table 2. As the increases of Se concentration and GPX activity in mouse serum or liver were significantly correlated with supplementary levels of each Se source, linear regression could be calculated in each combination. Accordingly, as also described in Table 2, the relative nutritional availability of Se from Se-pumpkin or Se-sprouts can be estimated by the slope ratio analysis, which uses sodium selenite as a reference. Based on the serum parameters, the availability of Se from either Se-pumpkin or Se-sprouts was more than 80% to selenite Se. However, based on the liver GPX, the availability of Se from either Se-pumpkin or Se-sprouts was less than 50% to selenite. When liver Se was used for analysis, the nutritional availability of Se from Se-pumpkin and that from Se-sprouts was estimated to be 97% and 65% to selenite Se, respectively.

Discussion

In the estimation of nutritional availability, each parameter of Se status gave difference values. Among the responses of parameters in mice supplemented with selenite, serum GPX gave the lowest correlation coefficient. Compared to liver GPX activity, serum GPX activity

reaches a plateau level at lower dietary Se level [13]. Thus, this low correlation indicates a possibility that the response of serum GPX reached a plateau level in the tested range of Se supplemented. When comparison between liver GPX and Se concentration is made, GPX has been thought to be superior to Se concentration as an index for Se status because GPX is one of the functional forms of Se in tissues [5]. Accordingly, nutritional availability based on the liver GPX is the most reliable among the four parameters, and the availability of Se either from Se-pumpkin or Se-sprouts is less than 50% to selenite Se.

We have already identified the main Se species in Se-pumpkin and Se-sprouts as protein-bound selenomethionine (SeMet) [14] and Se-methylselenocysteine (MeSec) [8], respectively. Dietary Se must be metabolized to selenide before incorporation to selenoproteins [1]. The low nutritional availability of Se from Se-pumpkin or Se-sprouts estimated in the present study indicates that the formation of selenide either by demethylation of MeSec or transsulfuration of SeMet was not sufficient for the synthesis of selenoprotein including GPX.

When liver Se was used in the estimation of nutritional availability, Se from Se-pumpkin gave higher availability values than that from Se-sprouts. Since SeMet is a non-specific form of Se that is metabolized as a constituent of methionine pool, SeMet can be non-specifically incorporated into body proteins [15, 16]. Accordingly, this result

may indicate that SeMet in Se-pumpkin was nonspecifically incorporated into liver protein. The SeMet incorporated to liver protein can take part in the amino acid pool, be metabolized to selenide *via* the transsulfuration pathway gradually and then be incorporated into selenoproteins. Thus, with the long-term administration of Se-pumpkin, the nutritional availability of Se from Se-pumpkin may be higher than that obtained in the present study.

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広範囲 血液・尿化学検査 免疫学的検査

—その数値をどう読むか—

[第 7 版]

(2)

IV. 生化学的検査[2] G. 金属

モリブデン

吉田宗弘

IV. 生化学的検査[2] G. 金属

モリブデン

Molybdenum

吉田宗弘

Key words : モリブデン, 微量元素, 血清濃度, 肝機能, 腎機能

1. 概 説

モリブデン(Mo)は、ヒトを含む哺乳動物ではキサンチンオキシダーゼ、アルデヒドオキシダーゼ、亜硫酸オキシダーゼの補酵素(Mo 補欠因子)として機能し、栄養上必須の微量元素である。遺伝的な Mo 補欠因子欠損症では、亜硫酸蓄積による重度の脳障害や水晶体異常、およびキサンチン代謝異常による血清尿酸濃度異常などが生じる。Mo をほとんど含まない高カロリー輸液を長期間投与した一例では、亜硫酸オキシダーゼ活性の低下に伴う神経過敏、昏睡、頻脈、頻呼吸などが発生している¹⁾。これらの症状がモリブデン酸塩投与で消失したため、この症例は Mo 欠乏と考えられている。しかしヒトの Mo 欠乏はこの一例のみであり、食事性欠乏症は知られていない。Mo が穀物や豆類に豊富に含まれ、Mo の摂取量が推奨量(25-30 $\mu\text{g}/\text{日}$)²⁾をはるかに超える 150 μg 以上であるためと考えられる³⁾。

Mo の毒性はほかの重金属よりも低い。ヒトの中毒事例は、いずれも産業現場における Mo ダスト吸入によるもので、血清尿酸の増加、セルロプラスミンの減少などが報告されている⁴⁾。経口的な Mo 摂取による中毒事例は皆無といつてよく、我が国の摂取の耐容上限量(9.0 $\mu\text{g}/\text{kg}/\text{日}$)²⁾は米国や欧州と同様に動物実験の結果

を外挿したものである。

2. 検査の目的

血清と尿の Mo は、Mo 摂取量⁵⁾や職業性の Mo 曝露⁶⁾を反映し、これらの定量的指標になる。特に Mo 曝露の証明を目的に、血清や尿の Mo 測定を行うことは有効である。

Mo の恒常性が尿排泄によって制御されること、肝臓に高濃度の Mo が存在することから、腎機能や肝機能異常によって血清や尿の Mo が変動する可能性がある。ゆえに血清や尿の Mo は腎および肝機能検査の一項目として使用できる可能性がある。

3. 試料の採取方法、保存条件

試料採取と保存に関して特別な注意はない。血液や尿などは乾燥に注意して凍結(-20℃)すれば、長期間保存できる。

4. 測 定 法

生体試料中の Mo は、試料灰化後、フレイムレス原子吸光法(AAS)、または誘導結合プラズマ質量分析法(ICPMS)で測定する。AAS は高濃度試料が混在した場合に機器汚染が生じ、測定値が高くなる場合がある。ICPMS は安定した結果が得られるため汎用されている。ICPMS では、ロジウムなどの内部標準物質の使用が必

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表 1 血清と全血の Mo 濃度に関する報告のまとめ

	対象者数	年齢	濃度 (μg/L) ^a	分析法 ^d	文献
血清/血漿					
日本	22M, 33F	20-59	0.70 (0.27-1.81) ^b	ICPMS	6)
ベルギー	27M, 23F	18-75	0.55±0.21	NAA	7)
米国	2M	不明	0.5±0.1	ICPMS	8)
全血					
英国	44	不明	0.62±0.29	ICPMS	9)
ドイツ	50M, 80F	18-70	0.43 (0.14-1.1) ^c	ICPMS	10)
ベネズエラ	244M, 174F	18-27	2.66±0.66	AAS	11)

^a特記しないかぎり数値は平均値 ± 標準偏差を示す。
^b数値は幾何平均値 (括弧内は幾何平均値 ± 幾何標準偏差の範囲) を示す。
^c数値は平均値 (括弧内は 5 パーセンタイルから 95 パーセンタイルの範囲) を示す。
^dICPMS: 誘導結合プラズマ質量分析法, NAA: 放射化分析法, AAS: フレームレス原子吸光法。

須である。

硝酸を用いた湿式灰化の適用は肝臓など Mo 濃度 100ppb 以上の試料に限定される。血清や母乳など Mo 濃度が 5ppb 未満の試料は電気炉を用いて 550℃で乾式灰化し、少量の 1-2 % 硝酸に溶解後、測定する。尿は 1-2 % 硝酸で 10 倍程度に希釈し測定する。

5. 基準値

表 1 に血清と全血の Mo の測定結果をまとめた⁶⁻¹¹⁾。ベネズエラの報告以外は血清と全血の Mo 濃度に大きな差がなく、1.0 μg/L 未満の人が大半を占めることを示している。米国人を対象にした出納実験の結果⁹⁾に基づく、Mo 摂取量 (X, μg/日) と血清 Mo 濃度 (Y, μg/L) との間には図 1 のように $Y=0.00264X+0.41$ の回帰式で示される強い相関があると推定できる。そこで血清 Mo の基準値設定にはこの回帰式を用いる。Mo 摂取量は大豆製品の大量摂取などによって 350 μg/日程度に高まる場合があるが、500 μg/日を超えることは考えられない。食事からの Mo 摂取の最高値を 500 μg/日として先の回帰式に代入すると 1.73 μg/L となり、更に 95 % 信頼区間の上限を算定すると 2.25 μg/L となる。この値を血清 Mo 濃度の正常上限と判断する。健常人でも測定限界である 0.1 μg/L 未満の場

合があるため⁹⁾下限は設定しない。また、全血が血清とほぼ同じ濃度を示すので、全血の基準は血清と同じとする。

日本人女性を対象にした出納試験では、見かけ上、摂取 Mo の約 70 % が尿に排泄されている¹²⁾。日本人の平均的な Mo 摂取量は欧米よりも多く、150-350 μg/日と推定されるので^{2,12)}、尿への Mo 排泄量は 100-250 μg/日となる。欧州の 2 つの研究では、随時尿の Mo 濃度の範囲として 4-183 μg/L、平均値として約 50 μg/L が示されている^{13,14)}。1 日の尿量を考慮すると、この値は日本人にも適用できる。以上より、随時尿と 1 日尿の Mo の基準値は、それぞれ 10-200 μg/L と 50-250 μg/日とする。

表 2 に以上の基準値をまとめた。

6. 生理的変動 (測定に影響を及ぼす因子)

血液および尿の Mo 濃度に影響を及ぼすのは食事からの Mo 摂取である。ただし、通常の食生活において、血清と尿の Mo 濃度に食事に伴う日内変動があるか確認されていない。しかし、Mo 曝露や腎機能検査の目的で尿 Mo を測定する場合は、1 日尿を用いることが望ましい。

7. 臨床的意義 (異常値を示す疾患)

食事中 Mo の 90 % 以上は消化管から吸収さ

IV

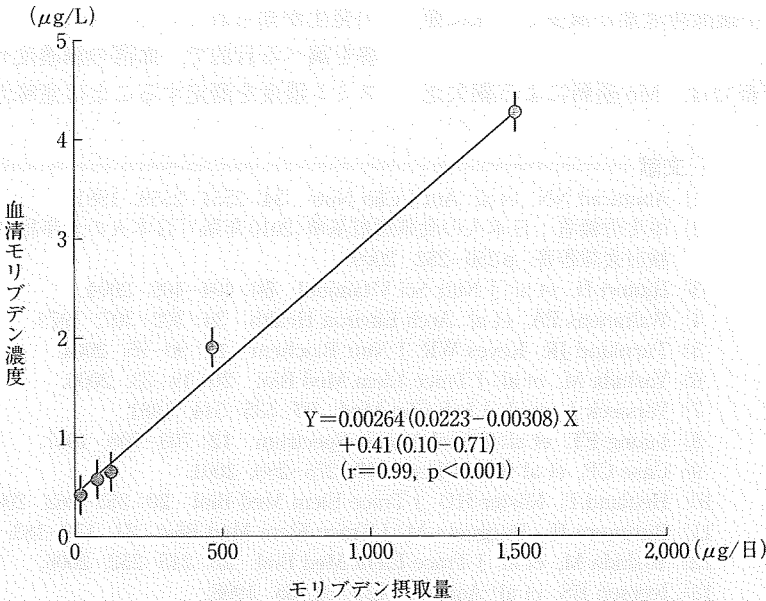


図1 モリブデン摂取量と血清モリブデン濃度の関連
(文献⁹⁾より作図)

回帰式中の括弧は95%信頼区間の下限-上限を示している。

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表2 血液および尿のMoの基準値

項 目	基準値
全血	2.25μg/L未満
血清または血漿	2.25μg/L未満*
尿：随時尿	10-200 μg/L
1日尿	50-250 μg/日

*日本人のMo摂取量の最大値を500 μg/日とした場合の値。実際に500 μg/日となる可能性はほとんどなく、大豆製品大量摂取によるMo摂取の最大値としては400 μg/日が現実的である。仮に400 μg/日とすれば、血清Mo濃度の基準値は1.94 μg/L未満となる。

れ速やかに尿に排泄される¹²⁾。したがって、腎の濾過機能が低下すると、Mo尿排泄が阻害され、血清Mo濃度が上昇する。事実、人工透析患者では血清Moが著しく高値を示す¹⁵⁾。しかし、臨床症状がなく、血清BUN濃度が基準値を超えた人の血清Mo濃度に異常は認められないので、腎臓が原因で血清Moが上昇するのは、人工透析が必要なほどに腎機能の低下した場合なのかもしれない。

肝臓はMoを高濃度に蓄積しており、肝臓障害時には肝臓から血清へのMo逸脱が生じると考えられる。事実、ウイルス性肝炎、肝硬変などの肝臓疾患、胆管疾患において、血清Mo濃度が高値を示している⁷⁾。このような血清Mo濃度の上昇は、臨床症状がなく血清ALTまたはAST活性が基準値を超えるのみの軽度肝機能低下者においても観察されている⁶⁾。したがって、血清Mo濃度が基準値を上回る場合は腎臓障害ではなく、肝臓障害を想定するのが妥当と思われる。

Mo補欠因子欠損症は、Moを利用できない遺伝性疾患であるが、血清や尿中Mo濃度に異常が生じるかは不明である。

8. 関連検査項目

Mo酵素であるキサンチンオキシダーゼ活性の変動は尿酸生成量に影響を及ぼす。ゆえに、Moに関連した代謝異常を検出するためには血清尿酸濃度の測定が必要であろう。

Mo酵素の亜硫酸オキシダーゼは、Mo欠乏の臨床症状と深く関連する。この酵素の活性が

低下すると、尿中硫酸排泄量が減少し、尿に亜硫酸が出現する。

ウシなどの家畜では、Mo 過剰による銅欠乏

の発生が知られている。ゆえに、Mo 曝露の影響を調べる目的で、血清の銅濃度やセルロプラスミン濃度を測定することは意味がある。

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