Table 1 Vanadium contents in drinking water

Samples	Number of samples	Vanadium content (ng/mL)				Distribution of vanadium content[a] (number of samples)				
		Mean	GM[b]	Median	Range	< 5	5-10	10-20 ng/mL	20-50	≥ 50
Japanese tap water [c]	3	3	3	3	3-4	3	0	0	0	0
Japanese mineral water	19	15	6	5	< 1-92	8	5	2	2	2
European mineral water	21	4	3	3	1-12	15	5	1	0	0
North American mineral water	6	11	2	2	1-57	5	0	0	0	1

[a] A unit of vanadium content is ng/mL.
[b] Geometrical mean.
[c] Samples of Japanese tap water were collected in Osaka, Wakayama and Okinawa Prefectures.

50 ng/mLをこえる高バナジウム濃度を示したのは，国産ミネラルウォーターでは富士山麓産「朝霧の水」と「バナジウム天然水」の2試料，外国産ミネラルウォーターでは米国カリフォルニア州北端のシャスタ山麓産「Crystal Gyzar」の1試料であった。

3. 食品中のバナジウム濃度

Table 2に，測定した食品のバナジウム濃度をまとめた。なお，測定は食パンを除き，1食品1試料に対して実施した。乾燥重量当たりで比較した場合，1000 ng/gをこえるバナジウムが検出されたのは，海藻類（コンブとヒジキ）と貝類（アサリとシジミ）であった。しかし，魚肉のバナジウム濃度はあまり高くなかった。畜産物の中では，牛乳のバナジウム濃度が比較的高かったが，卵や肉類のバナジウム濃度は乾燥重量当たりでも100 ng/g未満だった。植物食品の中では，レタスなどの葉野菜に比較的高濃度のバナジウムが検出されたが，果実，イモ，豆，および穀物類のバナジウム濃度は乾燥重量当たりでも50 ng/g未満の低値だった。ただし穀物製品の中で，食パンには，玄麦や小麦粉のバナジウム濃度が精白米や大豆と同様に低値であるにもかかわらず，比較的高濃度のバナジウムが検出された。

4. 日本人のバナジウム摂取量の推定

食品中バナジウム濃度の測定結果と平成12年厚生労働省国民栄養調査結果[6]をもとに，日本人の食品からのバナジウム摂取量を算定したところ，日本人1人当たりのバナジウム摂取量は27 μg/日であると推定された。Fig. 1に，日本

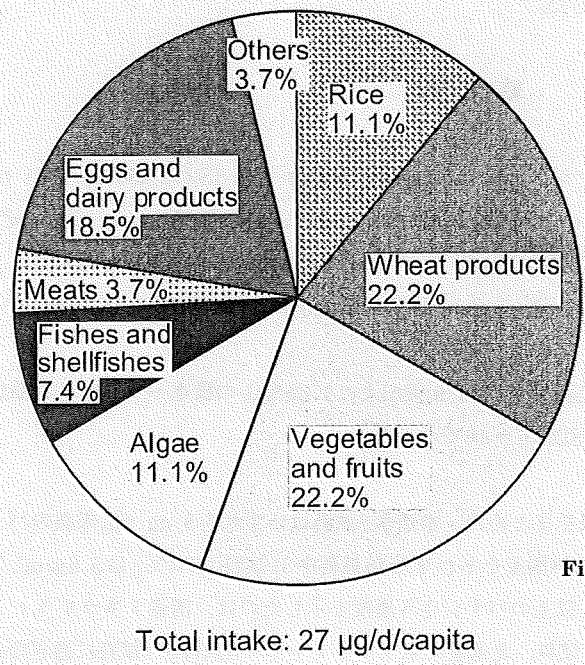

Fig. 1 Contribution of each food group to vanadium intake in Japanese.

Table 2 Vanadium contents in various foods

Foods	Vanadium content (ng/g)	
	Fresh basis	Dry basis
Cereals		
Polished rice	16	19
Whole wheat, hard, domestic	15	17
Whole wheat, hard, USA[a]	20	20
Wheat flour, hard	< 1	< 1
White table bread	132[b]	212[b]
Potatoes		
Sweet potato	13	37
Potato	4	21
Soybean, dried, domestic	1	2
Vegetables		
Japanese radish (*Daikon*)	7	128
Carrot	10	93
Cabbage	17	236
Lettuce	37	902
Spinach	25	329
Fruits		
Apple	< 1	< 1
Satsuma mandarin	< 1	< 1
Banana	5	22
Algae		
Hijiki, boiled and dried (*Hizikia fusiformis*)	1061	1228
Kombu, dried (*Laminaria* spp.)	1157	1278
Mozuku, salted, desalted (*Nemacystus decipiens*)	30	904
Wakame, blanched, salted, desalted (*Undaria pinnatifida*)	21	240
Fishes and shellfishes		
Mackerel	11	31
Horse mackerel	15	47
Salmon	30	105
Squid	22	135
Short-necked clams	148	1526
Freshwater clams	194	1659
Meats		
Beef, lean	8	22
Pork, lean	21	74
Chicken, breast without skin	17	61
Hen's egg, whole	10	42
Dairy products		
Cow's milk	33	260
Skin milk, dried	392	407

[a] Dark northern spring (DNS).
[b] Mean value of 3 samples.

人のバナジウム摂取に及ぼす各食品群の寄与を表した。バナジウム摂取への寄与が比較的大きい食品群は，小麦製品，野菜，および乳製品であると考えられた。海藻や貝類は，バナジウム含量は高いが，摂取量が少ないため，バナジウム供給源としての地位は高くなかった。

考　察

ICPMSによって標準参照試料を分析したところ，いずれの試料の測定値も保証値の範囲内にあった。また，飲料水を対象としたバナジウムの添加回収試験結果も満足のいくものであった。以上のことから，本実験におけるバナジウムの測定値は，十分に信頼できるものと判断できる。

水道水とミネラルウォーターを分析したところ，3分の2の試料が5 ng/mL未満のバナジウム濃度を示した。とくに水道水3試料のバナジウム濃度はいずれも5 ng/mL未満であった。以上のことから，特殊なミネラルウォーターを飲用し続けない限り，飲料水からのバナジウム摂取はきわめて微量であると判断できる。

特異的に50 ng/mLをこえる高バナジウム濃度を示したミネラルウォーターは富士山麓および，米国のシャスタ山山

麓で採取されたものであった。また，これら以外に20 ng/mL前後の比較的高いバナジウム濃度を示したのは，大分県日田市，および北海道黒松内で採取されたミネラルウォーターであった。これらの高バナジウム濃度のミネラルウォーターが採取された地域は，いずれも火成岩からなる地質の地域である。一方，ほとんどの試料が5 ng/mL未満の低バナジウム濃度を示した欧州産ミネラルウォーターは，産地国は様々であるが，そのほとんどがアルプス山脈内の湧水に起源を持つものであった。また，石灰岩の鍾乳洞である岩手県龍泉洞で採取されたミネラルウォーターは，全試料中最低の1 ng/mL未満のバナジウム濃度だった。これらのことから，火山地帯で採取されるミネラルウォーターは，堆積岩地質に比較して，高バナジウム濃度を示す可能性が考えられた。しかし，火山地帯で採取されたものであっても，バナジウム濃度が低い試料も多いことから，火成岩地質であることだけで高バナジウム濃度になるとはいいきれないと思われる。

コンブなどの海藻類には，乾燥重量当たり1000 ng/gをこえる高バナジウム濃度を認めた。また，植物食品の中で，レタスなどの葉野菜には，果実や穀物に比較してかなり高濃度のバナジウムが検出された。これらのことから，海水や土壌中に含有されるバナジウムは，海藻や陸上植物に容易に吸収され，海藻本体や葉に蓄積すると思われる。しかし，果実，いも，穀物のバナジウム濃度が低いことから，葉にとりこまれたバナジウムは，それ以上，植物体内を移動しないと考えられる。また，貝類は，海藻類と同程度の高バナジウム濃度を示した。貝類が高バナジウム濃度の藻類や植物プランクトンを摂取後，中腸腺などの消化器官にバナジウムを蓄積した可能性は高いと思われる。ただし，同じ水産物でも魚肉やイカ肉にはバナジウムの蓄積が認められなかったことから，消化器官から可食部である筋肉へのバナジウムの移行はわずかと考えられる。一方，畜産物では，畜肉や鶏卵のバナジウム濃度が低かったのに対して，牛乳には高濃度のバナジウムが認められた。以上のことから，牧草などの動物飼料に含まれるバナジウムは，吸収後，血漿を介して乳汁には移行するが，筋肉をはじめとする臓器には蓄積しないと推定できる。

食パンにも高濃度のバナジウムが検出された。原料のDNS小麦や強力粉のバナジウム濃度はきわめて低いことから，食パン製造工程において，バナジウムの混入が生じていると判断できる。ただし現段階では，バナジウム混入プロセスの特定にはいたっていない。

公刊されている「食品の微量元素含量表」[5]と比較すると，今回の測定値は，海藻類と貝類の数値においてはオーダー的にほぼ等しいものであった。しかし，葉野菜，乳製品，食パンなど「含量表」においてゼロ表示（10 ng/g未満）の食品に関しても，今回の測定は一定の数値を与えることができた。「含量表」の数値は，ICPMSの多元素同時測定によって得られたものであるが，標準参照試料による測定法の精度確認が行われていないことから，目安程度のものと認識すべきであろう。

今回の食品のバナジウム分析値と国民栄養調査成績[6]をもとに，日本人1人当たりの平均的なバナジウム摂取量を算定すると27 μg/日という数値が得られた。水道水に3 ng/mL程度のバナジウムが含有されることを考慮すると，日本人のバナジウム摂取量は約30 μg/日と見積もるのが適切と判断できる。高バナジウム濃度の食品は海藻類と貝類であるが，これらは日常的に摂取するものではないため，バナジウム供給源としては大きくない。実際にバナジウム供給源として寄与が大きいのは，小麦製品，野菜および乳製品であると推定できた。試算したバナジウム摂取量の数値は，米国で報告されている数値（6～18 μg/日）[7]よりもやや高い。日本人のバナジウム摂取量が米国人に比較して高いのは，日本人の海藻，貝類，葉野菜の摂取が米国人よりも多いためと考えられる。バナジウムは必須ミネラルではないため，わが国の食事摂取基準には取り上げられていない。一方，米国の食事摂取基準では，成人に対する摂取上限値のみが1.8 mg/日に設定されている[4]。今回推定した日本人の摂取量はこれを大幅に下回っており，現在の日本人のバナジウム摂取量は適正な範囲にあると推定できる。ただし，今回の推定は，食品の分析数が少ないので，あくまでも予備的なものと位置付ける必要がある。今後，食品の分析例を増やし，より厳密な摂取量の推定を行う予定である。

なお，ミネラルウォーター中で，「朝霧の水」はバナジウム濃度が最大（92 ng/mL）であった。しかし，このミネラルウォーターを1日に2 L飲用したとしても，バナジウム摂取量は200 μg/日であり，米国食事摂取基準の上限値を大幅に下回る。したがって，高バナジウム濃度のミネラルウォーターを日常的に飲用しても，健康上問題は生じないと判断

できる。

最後に，今回の摂取量推定をもとに，動物実験におけるバナジウム欠乏食について考察をこころみる。今回算定した日本人の平均的なバナジウム摂取量は約30 μg/日であった。成人1人が摂取する食事量を乾燥重量に換算して約500 g/日とすれば，食事中バナジウムの平均濃度は約0.06 μg/gということになる。したがって，ラットなどのげっ歯類を用いる場合のバナジウム欠乏食は，これよりも相当低いバナジウム濃度に設定する必要がある。

Nielsenはバナジウムなどの超微量元素の関する栄養実験を行う場合には慎重な飼料設計が必要であると述べている[8]。したがって，バナジウム欠乏食の調製にあたっては，バナジウム混入の確率が低い原料を選択しなければならない。カゼインは，動物栄養実験においてもっとも汎用されるタンパク質源であるが，牛乳中のバナジウム濃度が乾燥重量あたりで0.2 μg/gをこえる高値であることから，バナジウム欠乏食を作成する場合には不適切である可能性が高い。これに対して，穀物や豆類のバナジウム濃度は乾燥重量当たりでも50 ng/g未満の低値である。したがって，今回はバナジウム濃度を測定していないが，大豆分離タンパク質や小麦グルテンは，バナジウムをほとんど含まないタンパク質源として，バナジウム欠乏食の調製に利用できると思われる。

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参考文献

1) Nielsen FH (1987) Vanadium. in Trace Elements in Human and Animal Nutrition, 5th ed. vol. 1, ed. by Mertz W, Academic Press, New York: pp. 275-300.
2) Nielsen FH (1984) Ultratrace elements in nutrition. Ann Rev Nutr 4: 21-41.
3) Heyliger CE, Tahiliani AG, McNeill JH (1985) Effect of vanadate on elevated blood glucose and depressed cardiac performance of diabetic rats. Science 227: 1474-1477.
4) Institute of Medicine (2002) Arsenic, boron, nickel, silicon and vanadium. in Dietary Reference Intakes for Vitamin A, Vitamin K, arsenic, Boron, Chromium, copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc, National Academy Press, Washington DC: pp. 502-553.
5) 鈴木泰夫 (1993) 食品の微量元素含量表，第一出版，東京.
6) 健康・栄養情報研究会編 (2003) 平成12年厚生労働省国民栄養調査結果，第一出版，東京.
7) Pennington JA, Jones JW (1987) Molybdenum, nickel, cobalt, vanadium, and strontium in total diet. J Am Diet Assoc 87: 1644-1650.
8) Nielsen FH (1985) The importance of diet composition in ultratrace element research. J Nutr 115: 1239-1247.

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Communication

Molybdenum and Chromium Concentrations in Breast Milk from Japanese Women

Munehiro YOSHIDA,[1,†] Akiko TAKADA,[1] Junko HIROSE,[2] Mika ENDÔ,[2] Tsutomu FUKUWATARI,[2] and Katsumi SHIBATA[2]

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

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Molybdenum (Mo) and chromium (Cr) in 79 Japanese breast milk samples were measured by inductively coupled plasma-mass spectrometry. For Mo, 51 samples (64.6%) showed less than 5 ng/ml and only 12 samples (15.2%) showed more than 10 ng/ml. The range and median were <0.1 to 25.91 and 3.18 ng/ml respectively. For Cr, 38 samples (48.1%) showed less than 1 ng/ml, 20 samples (25.3%) showed 1 to 2 ng/ml, and only six samples (7.6%) showed more than 5 ng/ml. The range and median were <0.1 to 18.67 and 1.00 ng/ml respectively.

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

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 ± 46.8 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% HNO_3. Mo and Cr in the sample solutions thus prepared were measured by ICPMS with

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

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

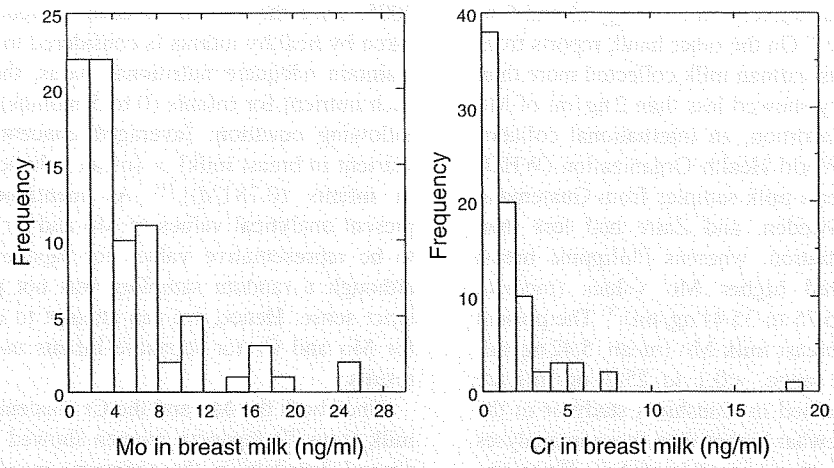

Fig. 1. Histograms of Mo and Cr Concentrations in Breast Milk from 79 Japanese Women.

direct nebulization. The ICPMS operating conditions were as follows: instrument, ICPM-8500 (Shimadzu, Kyoto, Japan); forward power, 1,200 W; coolant gas flow rate, 7.0 l/min; auxiliary gas flow rate, 1.5 l/min; nebulizer gas flow rate, 0.58 l/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 μg/g) showed values (mean ± SD) of 2.9 ± 0.6 ng/g as Cr content and 0.32 ± 0.04 μg/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 ± 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 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. 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.

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 ± 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 study[5] and in the Philippines[8] 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 ± 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] × [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 μg/d (3.18 μg/l × 0.78 l/d = 2.48 μg/d) and 0.8 μg/d (1.00 μg/l × 0.78 g/l = 0.78 μg/d) respectively.

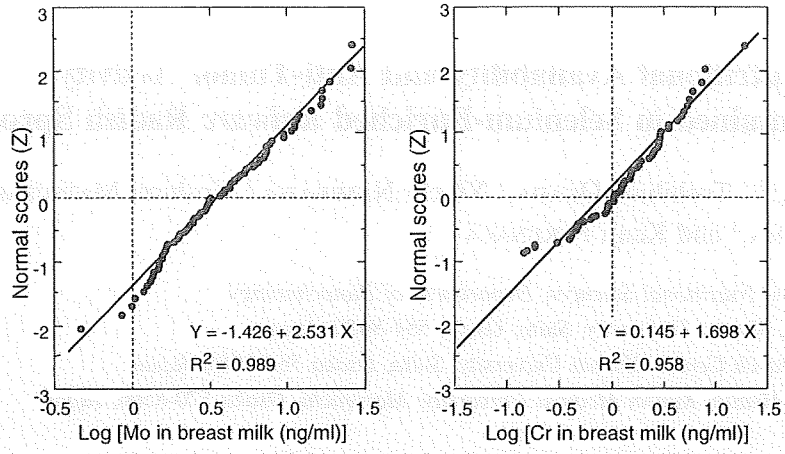

Fig. 2. Regression of Log Mo and Log Cr *versus* Normal Scores.

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References

1) Abumrad, N. N., Schneider, A. J., Steel, D., and Rogers, L. S., Amino acid intolerance during prolonged total parenteral nutrition reversed by molybdate therapy. *Am. J. Clin. Nutr.*, **34**, 2551–2559 (1981).
2) Brown, R. O., Forloines-Lynn, S., Cross, R. E., and Heizer, W. D., Chromium deficiency after long-term parenteral nutrition. *Dig. Dis. Sci.*, **31**, 661–664 (1986).
3) Ministry of Health, Labor and Welfare, "Dietary Reference Intakes for Japanese, 2005" (in Japanese), Daiichi Shuppan, Tokyo, pp. 147–155 (2005).
4) Yoshida, M., Itô, C., Hattori, H., Tsuchita, H., Yonekubo, A., and Nishimuta, M., Molybdenum contents in human milk and formula milk, and estimation of molybdenum intake of infants in Japan. *Trace Nutr. Res.* (in Japanese), **21**, 59–64 (2004).
5) Gunshin, H., Yoshikawa, M., Doudou, T., and Kato, N., Trace elements in human milk, cow's milk, and infant formula. *Agric. Biol. Chem.*, **49**, 21–26 (1985).
6) Casey, C. E., and Neville, M. C., Studies in human lactation 3: molybdenum and nickel in human milk during the first month of lactation. *Am. J. Clin. Nutr.*, **45**, 921–926 (1987).
7) Bougle, D., Bureau, F., Foucault, P., Duhamei, J.-F., Muller, G., and Drosdowsky, M., Molybdenum content of term and preterm human milk during the first 2 months of lactation. *Am. J. Clin. Nutr.*, **48**, 652–654 (1988).
8) WHO/IAEA, "Minor and Trace Elements in Breast Milk," WHO, Geneva, pp. 67–69 (1989).
9) Hattori, H., Ashida, A., Itô, C., and Yoshida, M., Determination of molybdenum in foods and human milk, and an estimate of average molybdenum intake in the Japanese population. *J. Nutr. Sci. Vitaminol.*, **50**, 404–409 (2004).
10) Yoshida, M., Ôta, S., Fukunaga, K., and Nishiyama, T., Serum molybdenum concentration in healthy Japanese adults determined by inductively coupled plasma mass-spectrometry. *J. Trace Elem. Med. Biol.*, **20**, 19–23 (2006).
11) Yamawaki, N., Yamada, M., Kan-no, T., Kojima, T., Kaneko, T., and Yonekubo, A., Macronutrient, mineral and trace element composition of breast milk from Japanese women. *J. Trace Elem. Med. Biol.*, **19**, 171–181 (2005).
12) Casey, C. E., and Hambidge, M., Chromium in human milk from American mothers. *Br. J. Nutr.*, **52**, 73–77 (1984).
13) Anderson, R. A., Bryden, N. A., Patterson, K. Y., Veillon, C., Andon, M. B., and Moser-Veillon, P. B., Breast milk chromium and its association with chromium intake, chromium excretion, and serum chromium. *Am. J. Clin. Nutr.*, **57**, 519–523 (1993).
14) Institute of Medicine, "Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc," National Academy Press, Washington, DC, pp. 197–223 (2001).
15) WHO/IAEA, "Minor and Trace Elements in Breast Milk," WHO, Geneva, pp. 32–35 (1989).
16) Ministry of Health, Labor, and Welfare, "Dietary Reference Intakes for Japanese, 2005" (in Japanese), Daiichi Shuppan, Tokyo, pp. 16–21 (2005).
17) Cohen, A. C., Simplified estimators for the normal distribution when samples are singly censored or truncated. *Technometrics*, **1**, 217–237 (1959).
18) Travis, C. C., and Land, M. L., Estimating the mean of data sets with nondetectable values. *Environ. Sci. Technol.*, **24**, 961–962 (1990).
19) Helsel, R., and Hirsch, R. M., "Statistical Methods in Water Resources (Techniques of Water-Resources Investigations of the United States Geological Survey, Book 4, Hydrologic Analysis and Interpretation, Chapter A3)," U.S. Geological Survey (http://water.usgs.gov/pubs/twri4a3/), pp. 362–364 (2002).
20) Takizawa, S., "Kankyô-Kôgakukei no Tame no Sûgaku" (in Japanese), Sûri-Kôgakusha, Tokyo, pp. 113–122 (2004).

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

Munehiro YOSHIDA,[1,2,†] Toshihide OKADA,[1] Yûichi NAMIKAWA,[1] Yoshimi MATSUZAKI,[1] Toshimasa NISHIYAMA,[3] and Kenji FUKUNAGA[1]

[1]*Laboratory of Food and Nutritional Sciences, Department of Biotechnology, Faculty of Engineering, Kansai University, Suita, Osaka 564-8680, Japan*
[2]*High-Technology Research Center, Kansai University, Suita, Osaka 564-8680, Japan*
[3]*Department of Public Health, Kansai Medical University, Moriguchi, Osaka 570-8506, Japan*

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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-

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

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	%
Torula 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

[a]KR yeast® kindly supplied by Kohjin (Tokyo). Crude protein content was 51.2%.
[b]Except 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

[a]Except 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 μg 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 NaN_3, 0.1 mM EDTA, 0.4 unit of GSH reductase, 20 μl 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 μmol 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 × 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 μl 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 ^{82}Se 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) × 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 (μg/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 ± 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 (μg/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 ± 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 (μg/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 ± 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) × 100.

the end of the experimental period, the mean ± 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 μg/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 μg/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 (μg/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 ± 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 (μg/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 ± 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 (μg/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 ± 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 μg 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 μg/g inhibited the formation of ACF irrespective of the Se source. A similar inhibitory effect of high dietary Se (1.0 μg/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 μg 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 ramps[38] at a dietary Se level of 1.0 μg/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 μg/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 μg 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 μg/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 μg/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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References

1) Driscoll, D. M., and Copeland, P. R., Mechanism and regulation of selenoprotein synthesis. *Ann. Rev. Nutr.*, **23**, 17–40 (2003).
2) Sakurai, H., and Tsuchiya, K., A tentative recommendation for the maximum daily intake of selenium. *Environ. Physiol. Biochem.*, **5**, 107–118 (1975).
3) Yoshida, M., and Yasumoto, K., Selenium contents of rice grown at various sites in Japan. *J. Food Comp. Anal.*, **1**, 71–75 (1987).
4) Yoshida, M., Selenium intake and blood selenium level in Japanese. *Nippon Eiyô Shokuryô Gakkaishi (J. Jpn. Soc. Nutr. Food Sci.)* (in Japanese), **45**, 485–494 (1992).
5) Larsson, C. L., and Johansson, G. K., Dietary intake and nutritional status of young vegans and omnivores in Sweden. *Am. J. Clin. Nutr.*, **76**, 100–106 (2002).
6) Oldfield, J. E., "Selenium World Atlas," Selenium-Tellurium Development Association, Grimbergen, pp. 3–70 (1999).
7) Rayman, M. P., The use of high-selenium yeast to raise selenium status: how does it measure up? *Brit. J. Nutr.*, **92**, 557–573 (2004).
8) Combs, G. F., Jr., and Combs, S. B., "The Role of Selenium in Nutrition," Academic Press, New York, pp. 127–177 (1986).
9) Whanger, P. D., Selenocompounds in plants and animals and their biological significance. *J. Am. Coll. Nutr.*, **21**, 223–232 (2002).
10) Combs, G. F., Jr., and Gray, W. P., Chemopreventive agents: selenium. *Pharmacol. Ther.*, **79**, 179–192 (1998).
11) Schrauzer, G. N., Anticarcinogenic effects of selenium. *CMLS, Cell. Mol. Life Sci.*, **57**, 1864–1873 (2000).
12) Clark, L. C., Combs, G. F. Jr., Turnbull, B. W., Slate, E. H., Chalker, D. C., Chow, J., Davis, L. S., Glover, R. A., Graham, G. F., Gross, E. G., Krongrad, A., Lesher, J. L. Jr., Park, H. K., Sanders, B. B., Smith, C. L., and Taylor, J. R., Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. *JAMA*, **276**, 1957–1963 (1996).
13) Surai, P. F., "Selenium in Nutrition and Health," Nottingham University Press, Nottingham, pp. 687–693 (2006).
14) Ip, C., Thompson, H. J., Zhu, Z., and Ganther, H. E., *In vitro* and *in vivo* studies of methylseleninic acid: evidence that a monomethylated selenium metabolite is critical for cancer chemoprevention. *Cancer Res.*, **60**, 2882–2886 (2000).
15) Cai, X. J., Block, E., Uden, P. C., Zhang, X., Quimby, B. D., and Sullivan, J. J., *Allium* chemistry: identification of selenoamino acids in ordinary and selenium-enriched garlic, onion, and broccoli using gas chromatography with atomic emission detection. *J. Agric. Food Chem.*, **43**, 1754–1757 (1995).
16) Yoshida, M., Sugihara, S., Inoue, Y., Chihara, Y., Kondô, M., Miyamoto, S., and Sukcharoen, B., Composition of chemical species of selenium contained in selenium-enriched shiitake mushroom and vegetables determined by high performance liquid chromatography with inductively coupled plasma mass spectrometry. *J. Nutr. Sci. Vitaminol.*, **51**, 194–199 (2005).
17) Dumont, E., Vankaecke, F., and Cornelis, R., Selenium speciation from food source to metabolites: a critical review. *Anal. Bioanal. Chem.*, **385**, 1304–1323 (2006).
18) Ip, C., Birringer, M., Block, E., Kotrebai, M., Tyson, J. F., Uden, P. C., and Lisk, D. J., Chemical speciation influences comparative activity of selenium-enriched garlic and yeast in mammary cancer. *J. Agric. Food Chem.*, **48**, 2062–2070 (2000).
19) Finley, J. W., Davis, C. D., and Feng, Y., Selenium from high selenium broccoli protects rats from colon cancer. *J. Nutr.*, **130**, 2384–2389 (2000).
20) Sugihara, S., Kondô, M., Chihara, Y., Yûji, M., Hattori, H., and Yoshida, M., Preparation of selenium-enriched sprouts and identification of their selenium species by high-performance liquid chromatography-inductively coupled plasma mass spectrometry. *Biosci. Biotechnol. Biochem.*, **68**, 193–199 (2004).

21) Reddy, B. S., Narisawa, T., and Weisburger, J. H., Colon carcinogenesis in germ-free rats with intrarectal 1,2-dimethylhydrazine and subcutaneous azoxymethane. *Cancer Res.*, **36**, 2874–2876 (1976).
22) Swenberg, J. A., Cooper, H. K., Bucheler, J., and Kleihues, P., 1,2-Dimethylhydrazine-induced methylation of DNA bases in various rat organs and the effect of pretreatment with disulfiram. *Cancer Res.*, **39**, 465–466 (1979).
23) Bird, R. P., Role of aberrant crypt foci in understanding pathogenesis of colon cancer. *Cancer Lett.*, **93**, 55–71 (1995).
24) Takahashi, M., Minamoto, T., Yamashita, N., Yazawa, K., Sugimura, T., and Esumi, H., Reduction in formation and growth of 1,2-dimethylhydrazine-induced aberrant crypt foci in rat colon by docosahexaenonoic acid. *Cancer Res.*, **53**, 2786–2789 (1993).
25) Hirose, M., Masuda, A., Ito, N., Kamano, K., and Okuyama, H., Effect of dietary perilla oil, soybean oil and safflower oil on 7,12-dimethylbenz[α]anthracene (DMBA) and 1,2-dimethylhydrazine (DMH)-induced mammary gland and colon carcinogenesis in female SD rats. *Carcinogenesis*, **11**, 731–735 (1990).
26) Paglia, D. E., and Valentine, W. N., Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med.*, **70**, 158–169 (1969).
27) Yoshida, M., Yasumoto, K., Iwami, K., and Tashiro, H., Distribution of selenium in bovine milk and selenium deficiency in rats fed casein-based diet, monitored by lipid peroxide level and glutathione peroxidase activity. *Agric. Biol. Chem.*, **45**, 1681–1688 (1981).
28) Yoshida, M., Kimura, Y., Abe, M., Ando, T., and Tachi, H., Quantitative evaluation of selenium contained in tea by high performance liquid chromatography. *J. Nutr. Sci. Vitaminol.*, **47**, 248–252 (2001).
29) Sukcharoen, B., Yanagida, K., Sonokawa, M., and Yoshida, M., Determination of selenium in domestic or imported buckwheat and barley using inductively coupled plasma-mass spectrometry. *Trace Nutr. Res.*, **21**, 97–100 (2004).
30) Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., Protein measurement with Folin phenol reagent. *J. Biol. Chem.*, **193**, 265–275 (1951).
31) Cohen, H. J., and Avissar, N., Extracellular glutathione peroxidase: a distinct selenoprotein. In "Selenium in Biology and Medicine," ed. Burk, R. F., Springer-Verlag, New York, pp. 81–91 (1994).
32) Nakamura, Y., Iwahashi, T., Tanaka, A., Koutani, J., Matsuo, T., Okamoto, S., Sato, K., and Ohtsuki, K., 4-(Methylthio)-3-butenyl isothiocyanate, a principal antimutagen in daikon (*Raphanus sativus*; Japanese White Radish). *J. Agric. Food Chem.*, **49**, 5755–5760 (2001).
33) Conaway, C. C., Yang, Y. Y., and Chung, F. L., Isothiocyanates as cancer chemopreventive agents: their biological activities and metabolism in rodents and humans. *Curr. Drug Metab.*, **3**, 233–255 (2002).
34) Davis, C. D., and Uthus, E. O., Dietary selenite and azadeoxycytidine treatment affect dimethylhydrazine-induced aberrant crypt formation in rat colon and DNA methylation in HT-29 cells. *J. Nutr.*, **132**, 292–297 (2002).
35) Baines, A. T., Holubec, H., Basye, J. L., Thorne, P., Bhattacharyya, A. K., Shriver, B., Cui, H., Roe, D., Clark, L. C., Earnest, D. L., and Nelson, M. A., The effect of dietary selenomethionine on polyamines and azoxymethane-induced aberrant crypts. *Cancer Lett.*, **160**, 193–198 (2000).
36) Finley, J. W., Ip, C., Lisk, D. J., Davis, C. D., Hintze, K. J., and Whanger, P. D., Cancer-protective properties of high-selenium broccoli. *J. Agric. Food Chem.*, **49**, 2679–2683 (2001).
37) Ip, C., Birringer, M., Block, E., Kotrebai, M., Tyson, J. F., Uden, P. C., and Lisk, D. J., Chemical speciation influences comparative activity of selenium-enriched garlic and yeast in mammary cancer prevention. *J. Agric. Food Chem.*, **48**, 2062–2070 (2000).
38) Whanger, P. D., Ip, C., Polan, C. E., Uden, P. C., and Welbaum, G., Tumorigenesis, metabolism, speciation, bioavailability, and tissue deposition of selenium in selenium-enriched ramps (*Allium tricoccum*). *J. Agric. Food Chem.*, **48**, 5723–5730 (2000).
39) Yang, G. Q., and Zhou, R. H., Further observation on the human maximum safe dietary selenium intake in a seleniferous area of China. *J. Trace Elem. Health Dis.*, **8**, 159–165 (1994).
40) Ministry of Health, Labor and Welfare, "Dietary Reference Intakes for the Japanese in 2005" (in Japanese), Daiichi Shuppan, Tokyo, pp. 184–188 (2005).

Short Communication

Assessment of Nutritional Availability of Selenium in Selenium-enriched Pumpkin

Munehiro Yoshida[1),2)], Kikue Sano[1)], Eri Ishiyuki[1)], Toshimasa Nishiyama[3)] and Kenji Fukunaga[1)]

[1)] Laboratory of Food and Nutritional Sciences, Department of Biotechnology, Faculty of Engineering, Kansai University, Osaka 564-8680, Japan
[2)] High Technology Research Center, Kansai University, Osaka 564-8680, Japan
[3)] Department of Public Health, Kansai Medical University, Osaka 570-8506, Japan

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 thioredoxine 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

Address correspondence to :
Munehiro Yoshida
Laboratory of Food and Nutritional Sciences, Department of Biotechnology, Faculty of Engineering, Kansai University, 3-3-35 Yamate Suita, Osaka 564-8680, Japan
FAX : +81-6-6388-8609
E-mail : hanmyou4@ipcku.kansai-u.ac.jp

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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 $Y = m\ X + k$, 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 $Y = m X + k$, where Y represented the parameters in mice fed the basal diet or the diet supplemented with Se at X level (μg/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) x 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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References

1) Driscoll DM, Copeland PR : Mechanism and regulation of selenoprotein synthesis. Ann Rev Nutr 23 : 17- 40, 2003.
2) Surai PF : Selenium in Nutrition and Health, Nottingham University Press, Nottingham, 2006, pp. 671-708.
3) Rayman MP : The use of high-selenium yeast to raise selenium status : how does it measure up? Brit J Nutr 92 : 557-73, 2004.
4) Finley JW : Selenium accumulation in plant foods. Nutr Rev 64 : 146-51, 2005.
5) Combs GF Jr, Combs SB : The Role of Selenium in Nutrition, Academic Press, New York, 1986, pp. 127-177.
6) Whanger PD : Selenocompounds in plants and animals and their biological significance. J Am Coll Nutr 21 : 223-32, 2002.
7) Iwashita Y, Nishi K : Cultivation of selenium-enriched vegetables in large scale. Biomed Res Trace Elem 15 : 72-5, 2004.
8) Sugihara S, Kondô M, Chihara Y, Yûji M, Hattori H, Yoshida M : Preparation of selenium-enriched sprouts and identification of their selenium species by high-performance liquid chromatography-inductively coupled plasma mass spectrometry. Biosci Biotechnol Biochem 68 : 193-9, 2004.
9) Yoshida M, Fukunaga K, Tsuchita H, Yasumoto K : An evaluation of the bioavailability of selenium in high-selenium yeast. J Nutr Sci Vitaminol 45 : 119-28, 1999.
10) Sukcharoen B, Yanagida K, Sonokawa M, Yoshida M : Determination of selenium in domestic or imported buckwheat and barley using inductively coupled plasma-mass spectrometry. Trace Nutr Res 21 : 97-100, 2004.
11) Yoshida M, Yasumoto K, Iwami K, Tashiro H : Distribution of selenium in bovine milk and selenium deficiency in rats fed casein-based diet, monitored by lipid peroxide level and glutathione peroxidase activity. Agric Biol Chem 45 : 1681-8, 1981.
12) Yoshida M, Abe M, Fukunaga K, Kikuchi K : Bioavailability of selenium in the defatted dark muscle of tuna. Food Add Contam 19 : 990-5, 2002.
13) Weiss SL, Everson JK, Thompson KM, Sunde RA : Dietary selenium regulation of glutathione peroxidase mRNA and other selenium-dependent parameters in male rats. J Nutr Biochem 8 : 85-91 1997.
14) Yoshida M, Sugihara S, Inoue Y, Chihara Y, Kondô M, Miyamaoto S, Sukcharoen B : Composition of chemical species of selenium contained in selenium-enriched shiitake mushroom and vegetables determined by high performance liquid chromatography with inductively coupled plasma mass spectrometry. J Nutr Sci Vitaminol 51 : 194-9, 2005.
15) Hondal RJ, Motley AK, Hill KE, Burk RF : Failure of selenomethionine residues in albumin and immunogloblin G to protect against peroxynitrite. Arch Biochem Biophys 371 : 29-34, 1999.
16) Burk RF, Hill KE, Motley AK : Plasma selenium in specific and non-specific forms. Biofactors 14 : 107-114, 2001.