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Effect of dietary selenium restriction on selected parameters of selenium status in men with high life-long intake

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The influence of selenium (Se) restriction on disposition in plasma and urine fractions of infused ^{74}Se (selenite) was studied when adult males (Enshi City, Hubei Province, PRC) whose habitual daily Se intake is approximately 480 μg per day were transferred to Lichuan County, where the daily intake is approximately 30 μg. The subjects received an infusion (106 μg Se) on the day before consuming foods low in Se and a second infusion (113 μg Se) 63 days later. Blood and 24-hour urine samples were collected each day for 7 days after the first infusion and on days 22, 43, and 62 following the first infusion. Urine and blood were also collected daily for the next 7 days after the second infusion. Plasma total Se concentration increased for 7 days after each of the two infusions and urine Se decreased exponentially following both the first and second infusions. The excretion of trimethylselenonium followed the same pattern as the total urinary Se. Surprisingly, there was not a significant difference in selenite retention between the two infusion periods, and the data indicated that, regardless of the chemical form of Se present in various organs, its catabolism leading to excretion in urine followed the same pathway as that of selenite. Labeled Se was incorporated predominantly in the plasma selenoprotein P fraction and the half-life of Se in this fraction was determined to be 1.9 to 2.9 days. Thus, a longer depletion period is required in these subjects to obtain more significant changes. (J. Nutr. Biochem. 10:564–572, 1999)

Keywords: high selenium intake; selenium depletion; stable isotopes of selenium; urine selenium; plasma selenoprotein P; selenite exchangeable pool; Chinese men

Introduction

Long-term selenium (Se) supplementation is under consideration for humans as a potentially effective modality for cancer chemoprevention.[1,2] Although the optimum level of Se supplementation for this purpose has not yet been established, various studies suggest that this level is several-fold higher than its habitual intake of residents in the U.S.,[2] which is approximately 100 μg/day for adults.[3] Even though the current Se status of U.S. adults meets nutritional needs,[3] the level may need to be higher to provide maximum protection against certain cancers.[1]

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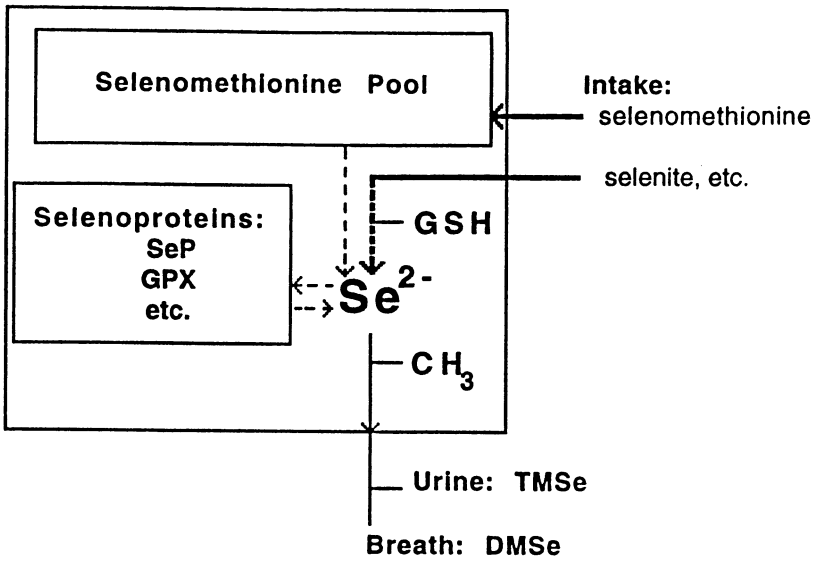

Figure 1 Simplified scheme of metabolism of dietary selenomethionine and selenite in subjects with adequate selenium (Se) status. SeP, selenoprotein P; GSH, reduced glutathione; GPX, glutathione peroxidase; Se^{2-}, selenide; CH_3, methyl; TMSe, trimethyl selenonium chloride; DMSe, dimethyl selenide.

The extent to which total body Se is increased during long-term supplementation is not known with any accuracy for humans, but is expected to depend strongly on the chemical form of Se in the supplement, the level and length of supplementation, and possibly other factors.[1,4] Supplements containing Se as selenomethionine are expected to increase total body Se to a much greater extent than inorganic salts of Se or selenocysteine-containing proteins.[3,4] Se present in body proteins as selenomethionine is thought to be a means of storage for Se.[5] Continued ingestion of selenomethionine could lead to large increases in total body Se because of nonspecific substitution for methionine.[4,6] Although this could be beneficial, if it accumulates over extended periods of time it could cause toxicity.[1] Therefore, a sufficient understanding of the quantitative relationship between parameters of long-term Se supplementation and changes in total body Se content is essential.

Because direct measurements of tissue, organ, or whole body Se^{2-} flux in humans are not experimentally feasible, suitable surrogates must be found. *Figure 1* shows some of the potentially useful markers where various selenocompounds can be converted to selenide or a closely related form.[5–8] Accessible selenoproteins [plasma glutathione peroxidase (GPX) or plasma selenoprotein P (SeP)] are not likely to be useful because they respond only over the nutritional range of Se intake, but the methylated derivatives dimethyl selenide (DMSe) in breath[9] and trimethyl selenonium chloride (TMSe) in urine[10] may provide useful markers of whole body Se^{2-} flux if the quantitative relationship between rate of generation of Se^{2-} and excretion of DMSe and/or TMSe could be established over the relevant range of parameters of Se supplementation.

Here we report the results of an investigation conducted with men who are life-long residents of the village of Luojiaba (Jianshi County, Hubei Province, PRC), an area with one of the highest known Se content of food in the world.[11,12] The focus of the investigation was to examine the quantitative relationship between life-long high intake of Se and selected parameters of its metabolism relevant to the issue of tissue Se^{2-} flux resulting from this high life-long intake.

Experimental methods

Protocol

Ten adult male volunteers who had been life-long residents of the village of Luojiaba (estimated daily Se intake 197–1,230 μg/d; *Table 1*) were recruited for this investigation. Only data on plasma from five of these subjects are presented. The protocol was approved by the Oregon State University Committee for the Protection of Human Subjects and by a special institutional review board convened at the Chinese Academy of Preventive Medicine in Beijing. All subjects gave written informed consent prior to participation in the study and were paid an equivalent of $300 (USD) for their participation. After baseline samples were obtained (day 0), the men were moved to Changping Village (Lichuan County, Hubei Province), which is located approximately 300 km from their native village, where they stayed for 70 days. The latter village is in the Keshan disease area, which during the 1969 to 1983 period had incidence and mortality rates of 103/100,000 and 45.2%, respectively. During their 70-day stay, they consumed locally grown food, which provided 43.3 μg Se/d based on a 3-day dietary survey. Anthropometric and Se baseline data for the subjects are summarized in *Table 1*.

Table 1 Anthropometric and baseline selenium (Se) parameters for the volunteers*

Subject	Age (y)	Weight (kg)	Hemato-crit (%)	Estimated Se intake (μg/d)	Urine Se (μg/d)	Plasma Se (ng/mL)
1	50	67	45.7	507	356	379
2	45	69	44.4	363	199	309
3	49	65	47.7	439	325	347
4	19	59	43.9	402	218	329
5	44	64	46.2	358	255	306
6	41	61	45.7	348	185	301
7	19	57	47.8	197	89	212
8	25	60	46.2	463	270	359
9	22	65	47.8	492	244	372
10	29	71	43.9	1230	433	654

*Se intake was calculated as: Log [Se intake] = 1.624 Log [plasma Se] + 3.389, in which units are μg/d and μg/mL for intake and plasma concentrations, respectively[12].

On day 1 of the protocol, each subject was infused with 106 μg Se labeled with the stable isotope ^{74}Se, which was prepared specifically for intravenous administration in the form of selenite (see below) by adding the stable isotope solution to a 500-mL bag of 5% intravenous glucose solution. This infusion was performed before the subjects were placed on the low Se diet. Infusion rate was adjusted (by gravity) to last for 5 hours (7:00 AM to 12:00 PM).

Complete urine collections were obtained for each 24-hour period for 7 days after start of infusion, and thereafter on days 22, 43, and 62. Blood samples (15 mL) were obtained at 7:00 AM each day; plasma was separated by centrifugation and each fraction frozen immediately until used. An identical infusion procedure was repeated on day 64, with the exception that the total amount of Se infused was 113 μg. Following the second infusion, seven 24-hour urine collections were obtained and blood was collected at the end of each urine collection period similar to the first infusion period. The subjects were closely monitored in both infusion periods by the medical/research staff during the course of the investigations.

Preparation of stable isotope for infusion

Elemental Se (13.6 mg) enriched with respect to ^{74}Se (% by weight: ^{74}Se 54.4; ^{77}Se 4.406; $R_{74/77}$ 12.35; the corresponding values for unenriched Se were 0.82, 7.4, and 0.11, respectively) was dissolved in saline solution. It was then filtered through a 0.45-μm filter and 2.0 mL portions were dispensed into sterile glass ampules. All ampules were then sterilized by autoclaving at 120°C for 20 minutes. Three of the ampules were analyzed and dissolved in 2 to 3 mL concentrated HNO_3. The resulting solution was heated gently until almost dry. The remaining liquid was transferred with deionized water into an acid-washed plastic container and the volumes adjusted with deionized water. This solution had a Se concentration of 99.8 μg/mL and pH of 3.0. A measured portion of this solution was prepared for infusion as follows. Solution pH was adjusted with NaOH to 7.4 and diluted with 0.9% saline solution (52.5 μg/2 mL ampule). Freedom from pyrogenicity was tested in five rabbits with three randomly selected ampules using standard procedures. A similar batch was prepared for the second infusion with a final Se concentration of 56.6 μg/2 mL ampule.

Analytical procedures

Urine samples. The volume of each 24-hour urine collection was determined and measured aliquots were processed for the determination of total Se (urine-Se:Se_u) using $HNO_3/HClO_4$ digestion.[13] In addition, measured aliquots of urine were processed for the isolation of TMSe using the KOH-thermolysis procedure.[10] Each resulting solution was then analyzed for Se content with the fluorometric method[14] using the equipment noted previously,[15] and the isotope ratio for $^{74}Se/^{77}Se$ ($R_{74/77}$) was determined with hydride generation-inductively coupled plasma mass spectrometry (HG/ICP-MS).[13]

Plasma samples. A measured volume of each plasma sample was fractionated into three Se-containing fractions using tandem-column chromatography as described previously.[16] Each resulting fraction was then processed for the measurement of Se (fluorometric method) and $R_{74/77}$ (HG/ICP-MS).

Calculations

Experimental data of each sample for Se and $R_{74/77}$ were combined to calculate that portion of Se in the sample that originated from the enriched infusate, according to equations 1, 2, and 3.

Table 2 Baseline (day 0) values of selenium (Se)

Parameter	Values (±SEM)	
	All subjects	Subjects 1–5
Intake (μg/d)	480 ± 88	414 ± 28
Se_u (μg/d)	257 ± 31	270 ± 30
Se_{TMSe} (μg/d)	10.7 ± 2.9	10.0 ± 2.0
% of Se_u	3.9 ± 1.0	4.0 ± 1.2
Se_{pl} (ng/mL)	357 ± 36	334 ± 14
Se_{SeP} (ng/mL)		147 ± 11
% of Se_{pl}		44 ± 4
Se_{GPX} (ng/mL)		100 ± 17
% of Se_{pl}		30 ± 4
Se_{Alb} (ng/mL)		135 ± 25
% of Se_{pl}		36 ± 10

u–urine. TMSe–trimethyl selenonium chloride. pl–plasma. SeP–selenoprotein P. GPX–glutathione peroxidase. Alb–albumin.

$$R_{74/77} = [^{74}Se° + {}^{74}Se^*]/[^{77}Se° + {}^{77}Se^*]$$
$$= [0.00814\ Se° + 0.544\ Se^*]/$$
$$[0.0738\ Se° + 0.0441\ Se^*] \quad (1)$$

$$Se° = Se - Se^* \quad (2)$$

where $R_{74/77}$ is the measured isotope ratio in sample (on weight/weight basis); Se is the Se content of sample, measured fluorometrically; Se° is the Se content of sample of unenriched origin; Se* is the Se content of sample of enriched origin; and constants are appropriate weight fractions of each isotope in either unenriched Se or infusate Se.

Simultaneous solution of the above two equations yields the desired quantity:

$$Se^* = [(0.0738\ R_{74/77} - 0.00815)\ Se]/$$
$$(0.02974\ R_{74/77} + 0.53585) \quad (3)$$

Results and discussion

Parameters of Se

Baseline (day 0) values of Se are summarized in *Tables 1 and 2*. Because data on metabolism of Se* and plasma fractions were available for only five subjects (subject code 1–5, *Table 1*), we have compared the corresponding available data between the 10-subject universe and the subset of five subjects in *Table 2*. The average values for the subset are comparable to the 10-subject universe so that on a group basis the subset reflects the universe reasonably well.

The estimated life-long Se intake varied over a wide range among the 10 subjects (*Table 1*). The lowest estimated intake was 197 μg/d (subject 7) and the highest was 1,230 μg/d (subject 10). This corresponds to approximately 2 to 10 times the average daily intake of U.S. adults. To reduce day-to-day variability of urine Se in the observed relationship between the estimated long-term Se intake and urine excretion (*Figure 2*), the average values of daily urine excretion for days 1 through 7 were plotted (*Figure 3A*). Error bars (±1 SEM; $n = 7$) given for the ordinate show the observed daily variability during days 1 through 7 for each subject. The best-fit linear equation ($r = 0.95$) relating urine Se to long-term intake is $y = 99.9 + 0.24x$ (*Figure 2*). The

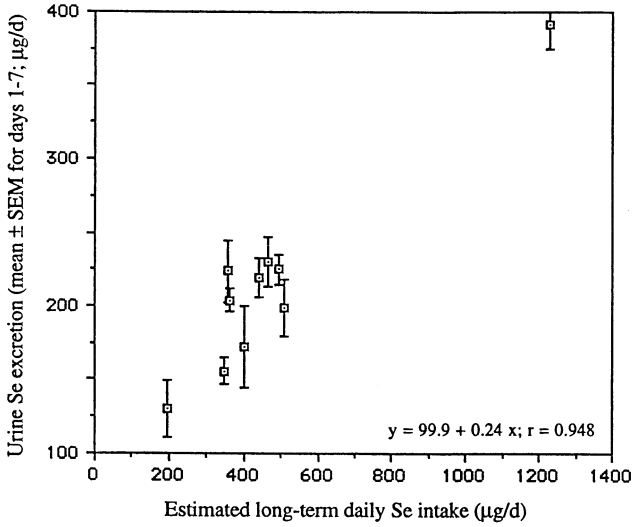

Figure 2 Observed correlation between the estimate of long-term selenium (Se) intake and urine Se excretion for 10 subjects. Data are mean ± 1 SEM for days 1 through 7 for each of the subjects.

estimated average Se intake during days 1 through 7 (including Se from infusate) was 58 µg/d [(43.3 × 7 + 105.5)/7], which is in the range of 15 to 29% of the 7-day average for urine Se for these subjects. Because only a fraction of daily intake is expected to contribute to daily urine excretion,[17,18] the measured urine excretion data probably are a reasonable estimate of urine Se excretion resulting from catabolic turnover of body Se for these subjects. Se content of the TMSe fraction of urine (Se_{TMSe}) was only 3.94 ± 0.99% of daily urine excretion for the 10 subjects and 4.03 ± 1.16% for the subset of 5 subjects. Although data about the contribution of Se_{TMSe} to Se_u are limited, the obtained values are similar, on a percentage basis, to those reported for healthy U.S. adults who consume much lower life-long intakes of Se.[10] Because biological methylation is expected to play an increasing role in regulating body Se status as Se intake is increased,[1,7,8] the present data suggest that other factors may limit production or excretion of TMSe as catabolic flux of body Se is increased for subjects consuming such high intakes of Se. Such a potential factor may be an increase in the elimination of DMSe via breath, thus removing tissue DMSe as a precursor of TMSe production.

Average plasma Se concentrations were similar for the 10-subject universe and the 5-subject subset (*Table 2*). Compared with typical values of approximately 100 ng/mL for U.S. residents,[3] the very high plasma Se concentrations for these subjects reflect the expected large increase in total body Se in subjects consuming such high amounts of Se from natural sources for life. The values reported in the present investigation are consistent with plasma values reported for similar groups.[15,19] In the present study, equilibrium distribution of plasma Se corresponded to (as % of plasma Se): 43.5 ± 4.3 for SeP, 29.6 ± 4.3 for GPX, and 36.1 ± 9.6 for albumin (Alb; *Table 2*). Using the same method as in the present investigation, Deagen et al.[16] reported a range of 58 to 60% for SeP, 17 to 23% for GPX, and 17 to 32% for Alb in Chinese men with varying levels

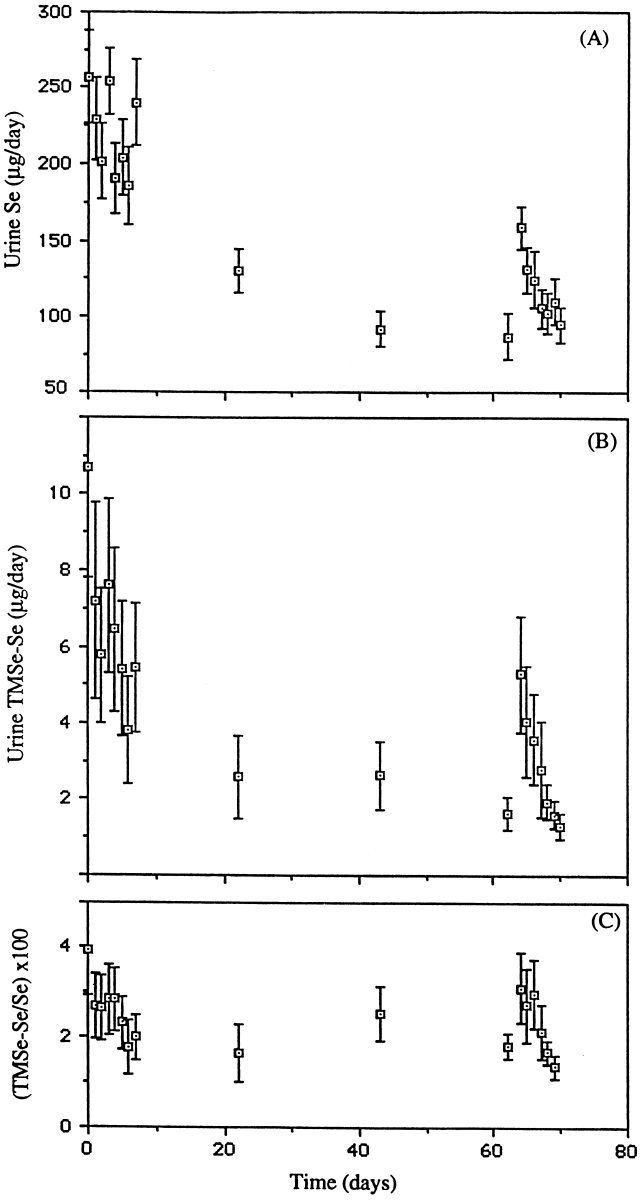

Figure 3 Time course of (A) urine selenium (Se) and (B) urine trimethyl selenonium chloride (TMSe), and (C) their ratio during the experiment period. Data are mean ± 1 SEM for 10 subjects.

(21–162 ng/mL) of plasma Se. Similarly, in Rhesus monkeys that consumed a commercial diet and whose plasma Se concentration was 166 ng/mL, Deagen et al.[16] found the following distribution: 74% for SeP, 11% for GPX, and 15% for Alb. When the monkeys were given selenite in drinking water, similar distributions were observed. In contrast, when selenomethionine was added to drinking water, a considerably larger fraction of plasma Se was found in Alb (43%) with a commensurate decrease in SeP (34%). When selenomethionine was removed from the drinking water, plasma distributions, 1 month later, reverted to values observed for the commercial diet. Because selenomethionine is known to cause a false high Se content in the GPX fraction from the column,[16] the high percentage (29.6%) of Se in this fraction of these subjects is no doubt due to the

Table 3 Magnitude of changes in selenium (Se) parameters due to 62 days of Se restriction*

Parameter	Day 1	Day 62	% Decrease
Se_u (μg/d)	229 ± 28	87 ± 15	62
Se_{TMSe} (μg/d)	7.2 ± 2.6	1.6 ± 0.4	78
Se_{pl} (ng/mL)	372 ± 17	299 ± 15	20
Se_{SeP} (ng/mL)	164 ± 27	106 ± 10	35
% of Se_{pl}	44 ± 6	35 ± 3	
Se_{GPX} (ng/mL)	101 ± 8	57 ± 8	44
% of Se_{pl}	28 ± 3	19 ± 3	
Se_{Alb} (ng/mL)	167 ± 17	98 ± 18	41
% of Se_{pl}	47 ± 3	35 ± 8	

*Urine data are for 10 subjects; plasma data for 5 subjects. u–urine. TMSe–trimethyl selenonium chloride. pl–plasma. SeP–selenoprotein P. GPX–glutathione peroxidase. Alb–albumin.

high selenomethionine content of their diets.[20] By use of a more specific method (i.e., immunoassay), other workers found only 14% of the plasma Se of humans in GPX.[21] Therefore, the data of the present study are in agreement with literature which indicate that selenomethionine is a component of plasma albumin,[22] whereas in both GPX and SeP selenocysteine is the sole form of Se.[6,23]

Effect of Se restriction on parameters of Se

The effect of Se restriction on Se_u and Se_{TMSe} and their ratio is shown in *Figure 3*. Data are for 10 subjects, and error bars correspond to ±1 SEM. As expected, both Se_u and Se_{TMSe} decreased dramatically during the 2 months of Se restriction, with a transient increase after the second infusion. Despite this dramatic decrease in urinary excretion of Se from 229 ± 28 μg/d (day 1) to 87 ± 15 μg/d for day 62 (*Table 3*), there was little change in the Se_{TMSe}/Se_u ratio (*Figure 3C*).

Figure 4 shows the observed changes in plasma Se concentration and its three fractions for the entire study period. Plasma Se concentrations increased markedly during the initial 7 days following the first infusion (372 ± 17 ng/mL on day 1; 479 ± 14 ng/mL on day 7). Thereafter, there was a consistent decrease until day 62 (299 ± 15 ng/mL). There was a 20% decrease in plasma Se concentration between days 1 and 62 (*Table 3*). Plasma Se concentration for both SeP and GPX fractions decreased steadily during the restriction period (*Figure 4B and 4C*), whereas data for the Alb fraction (*Figure 4D*) were similar with that for whole plasma (*Figure 4A*). When the sum of the Se concentrations of the three fractions recovered from the column (*Figure 4E*) is compared with plasma Se measured in whole plasma (*Figure 4A*), the rapid increase in plasma Se concentration observed during the initial 7 days following the first infusion seems to largely disappear. Column recoveries (amount of Se recovered in the three fractions relative to the amount applied to columns) were within the range of 74 ± 5% to 95 ± 6% (data not shown). Thus, we conclude that the apparent discrepancy between data in *Figure 4A* and *Figure 4E* is related to the presence of selenospecies other than those accounted for in the three (SeP, GPX, and Alb) fractions. It is postulated that a measurable fraction of plasma Se in these subjects is in

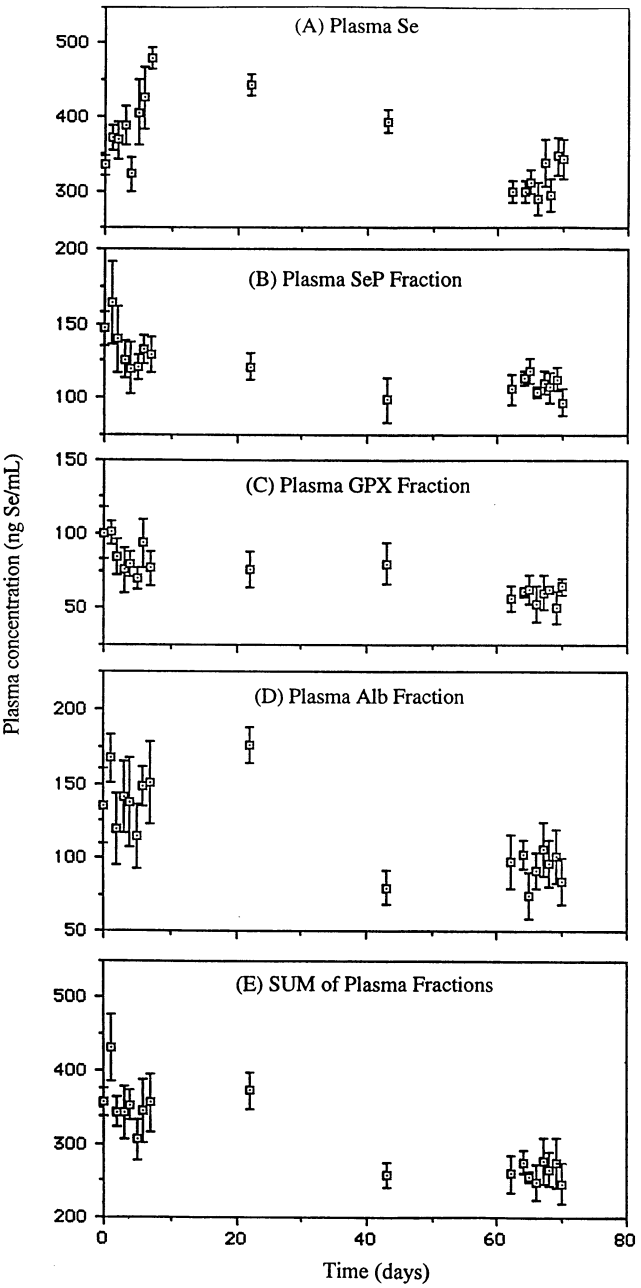

Figure 4 Time course of plasma selenium (Se) and its three fractions during the experiment. Data are mean ± 1 SEM for five subjects. *(A)* Whole plasma, *(B)* selenoprotein P (SeP) fraction, *(C)* glutathione peroxidase (GPX) fraction, *(D)* albumin (Alb) fraction, and *(E)* sum of all fractions.

chemical form(s) not recovered by the column fractionation method employed here. In support of this, when plasma samples from deficient or adequate subjects were subjected to this chromatographic procedure, recoveries from 95 to 102% were obtained (data not shown). Further efforts will be made to identify this selenospecies, which because it appears to be present at high Se intakes, may be a biomarker for excess Se.

Even though the total plasma Se decreased by 20% (*Table 3*), the levels were still higher than the values for

subjects receiving adequate Se. Because the subjects were apparently still saturated with Se, it is surprising that there were decreases in Se in the GPX and SeP fractions, which are selenoproteins that are saturated with high amounts of this element.[24,25] Therefore, this suggests that decreases of Se in these two plasma fractions and the Alb one are selective and that the unidentified selenospecies did not decrease markedly during this depletion period. The sum (432) of the Se in the three fractions is 60 ng/mL less than the total plasma Se content at day 1 (*Table 3*) and is 38 ng/mL less than the total at 62 days. We postulate that the reason for this disagreement is the presence of this unknown selenospecies, which was not accounted for by the method employed. This factor also is postulated to account for the lack of agreement between the sum of plasma fractions (*Figure 4E*) and the total plasma Se content (*Figure 4A*). Furthermore, if the subjects were not completely in a steady state condition, this could also contribute to the problem.

There was a 62 to 78% decrease in urine excretion of Se and its methyl derivative during the 2 months of Se restriction, but in contrast, plasma Se concentrations decreased by only 20% (*Table 3*). An estimate can be made of total Se lost in urine of these subjects during the 2 months of Se restriction by integration of the single-exponential equation fitted to the experimental data ($Se_u = 228\ e^{-0.01793t}$; $r = 0.94$). This leads to total urine Se loss of 8.5 mg. Total body losses must have been larger by the unmeasured amounts lost through the gastrointestinal tract and lungs. Total body Se content of these subjects is not known, but must have been considerably higher than the approximately 15-mg loss that is expected in U.S. adults.[26] Despite this large loss, urine Se excretion on day 62 was 87 ± 15 μg/d, still considerably higher than the value expected in U.S. residents,[17,18] who consume nutritionally adequate amounts of Se during their entire lives.

*Effect of Se restriction on parameters of Se**

Data presented above indicate that 2 months of Se restriction resulted in loss of more than 8.5 mg Se from the total body pool. This marked decrease in body Se content was reflected in decreases in the rate of urine excretion of Se, TMSe formation, and decreases in plasma concentrations of both SeP and GPX. Changes in the latter three parameters, which are expectedly derived from the Se^{2-} pool, should reflect a decrease in the rate of generation of Se^{2-} in various tissues (especially liver). If this decrease was sufficient compared with the need, or capacity, for de novo synthesis of various selenoproteins, we would expect initiation of conservation mechanisms to maintain body Se status at sufficient levels to generate the requisite tissue concentrations of Se^{2-}. This would in turn result in appropriate changes in metabolism of infused selenite.[17,18] Data related to urine excretion of the labeled selenite for both infusion periods are shown in *Figure 5*. In *Figure 5A*, daily excretion rates for isotopically labeled urine Se are compared for the two infusion periods. *Figure 5B* shows the kinetics of labeled TMSe excretion, and in *Figure 5C* the ratio of labeled TMSe to labeled Se is plotted. There were no significant differences in daily excretion of the label for any of the 7 days between the two infusion periods ($P > 0.05$).

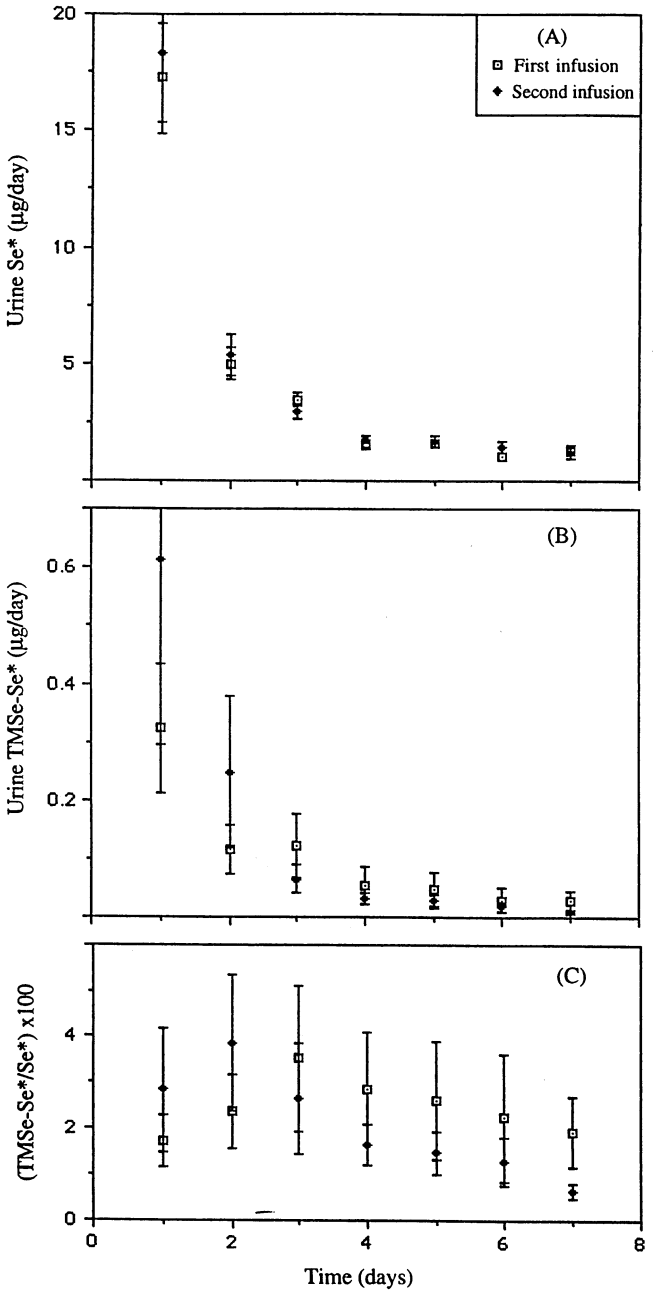

Figure 5 Observed rates of urine excretion of *(A)* labeled selenium (Se*) and *(B)* its trimethyl selenonium chloride (TMSe) component and *(C)* their ratio for the two infusion periods. Data are mean ± 1 SEM for five subjects.

The same was also true for rate of excretion of labeled TMSe ($P > 0.05$). Finally, their ratio was also similar for the corresponding days of the two infusion periods. Seven-day cumulative excretion in urine for Se* was 31.3 ± 3.4 (±1 SEM) μg for the first infusion period and 32.6 ± 3.8 μg for the second period ($P > 0.05$). Similarly, the corresponding values for labeled TMSe were 0.72 ± 0.29 μg and 1.01 ± 0.44 μg ($P > 0.05$), respectively. Therefore, the seemingly large decreases in total body Se brought about as a result of 2 months of Se restriction were not sufficient to alter the quantitative aspects of retention of a

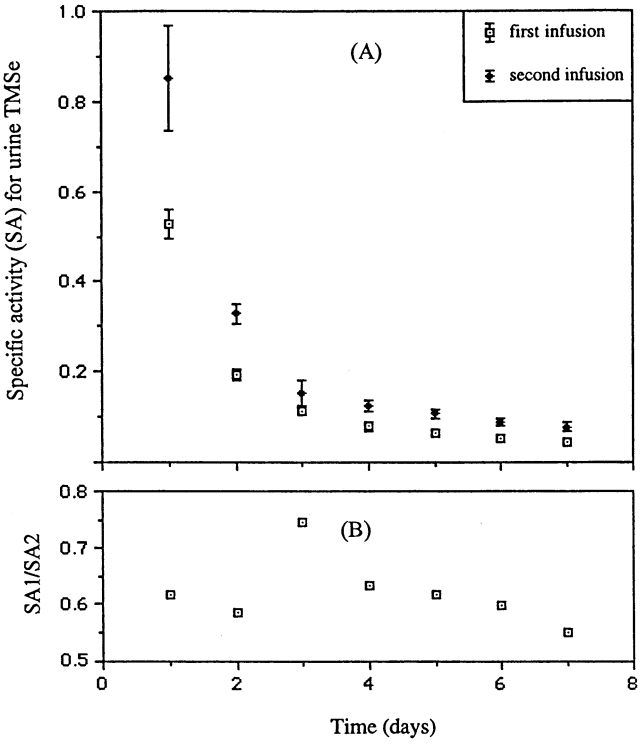

Figure 6 Daily changes in specific activity (SA; $^{74}Se^*/^{77}Se^\circ$) in urine-trimethyl selenonium chloride (TMSe) during the two infusion periods. *(A)* SA data for each infusion period (mean ± 1 SEM; $n = 5$). *(B)* Ratio of mean of SA for the second infusion period to (SA2) that for the corresponding day of the first (SA1) infusion period.

single dose of infused labeled selenite or its conversion to TMSe. This is very different from data obtained in U.S. adults in whom short-term Se restriction has a dramatic effect on retention of a dose of labeled selenite.[17,18] An explanation for this difference is not readily apparent but the fact that the subjects in the present study were so much more saturated with Se may be a contributing factor.

That significant decreases in tissue concentrations of Se^{2-} had actually occurred as a result of the 62-day restriction protocol is illustrated by the specific activity (SA) data for the TMSe portion of urine Se for the two infusion periods (*Figure 6*). SA was calculated for each urine sample as $R_{74/77} - R^\circ_{74/77}$, which corresponds to $^{74}Se^*/^{77}Se^\circ$ (see equations 1 through 3 for definitions). Clearly, the corresponding SA for the first infusion period is always smaller than that for the second (*Figure 6B*), with an overall average of 0.62 ± 0.02 (*Figure 6B*). Because the same amount of labeled selenite was infused for both periods, this indicated that the precursor pool of endogenous Se from which TMSe was produced had decreased to 62.0 ± 2.3% of its initial value as a result of the 62 days of restriction. However, this decrease was apparently not sufficient to affect retention kinetics of labeled selenite (*Figure 5*).

The time course of incorporation of the infused Se into the three plasma fractions is shown in *Figure 7*. For both infusion periods, the label was incorporated predominantly into the SeP fraction. Incorporation into the GPX fraction was always higher than that for the Alb fraction, but neither

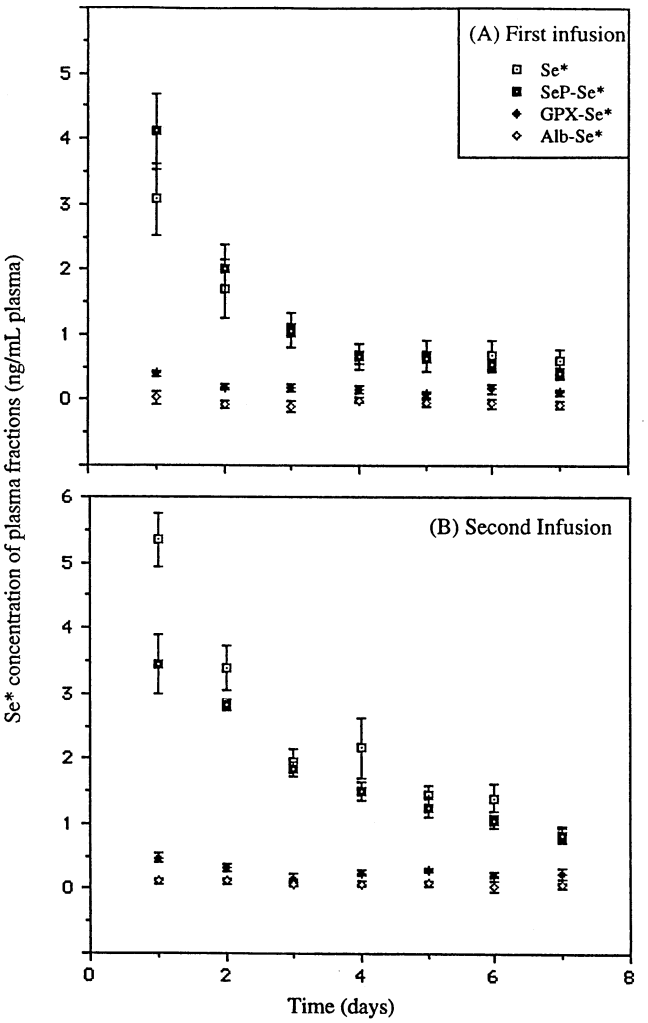

Figure 7 The time course of concentration of labeled selenium (Se*) in the three plasma fractions during each of the two infusion periods. Data are mean ± 1 SEM for five subjects. SeP, selenoprotein P; GPX, glutathione peroxidase; Alb, albumin.

fraction accounted for much of the circulating plasma label. Data summarized in *Table 4* show the percentage distribution of plasma Se* among the three fractions for selected

Table 4 Incorporation of selenite label into plasma fractions

	SeP	GPX	Alb
Time (d)	(% of plasma Se*)		
First infusion			
1	134 ± 19	13 ± 2	1 ± 2
5	94 ± 10	14 ± 6	−7 ± 9
7	67 ± 13	20 ± 7	−10 ± 10
Second infusion			
1	64 ± 8	9 ± 1	2 ± 1
5	87 ± 11	20 ± 2	6 ± 3
7	100 ± 13	29 ± 9	7 ± 6

Error bars are 1 SEM ($n = 5$).
SeP–selenoprotein P. GPX–glutathione peroxidase. Alb–albumin. Se*-labelled selenium.

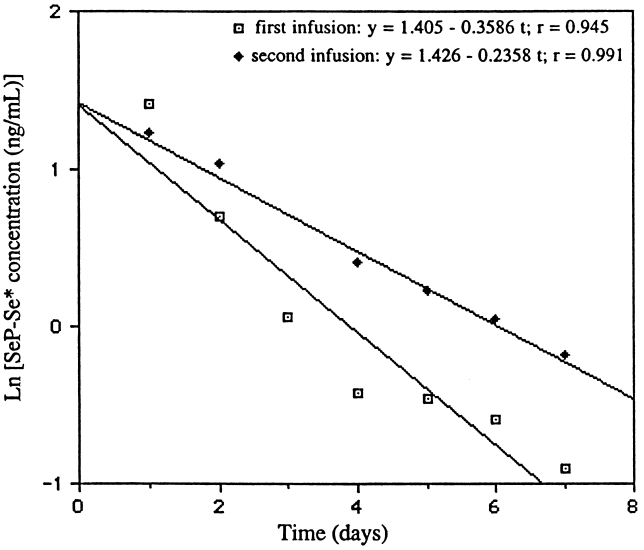

Figure 8 Plot of natural logarithm of labeled selenium (Se*) in selenoprotein P (SeP) versus time for the two infusion periods. Each data point is the mean of five subjects.

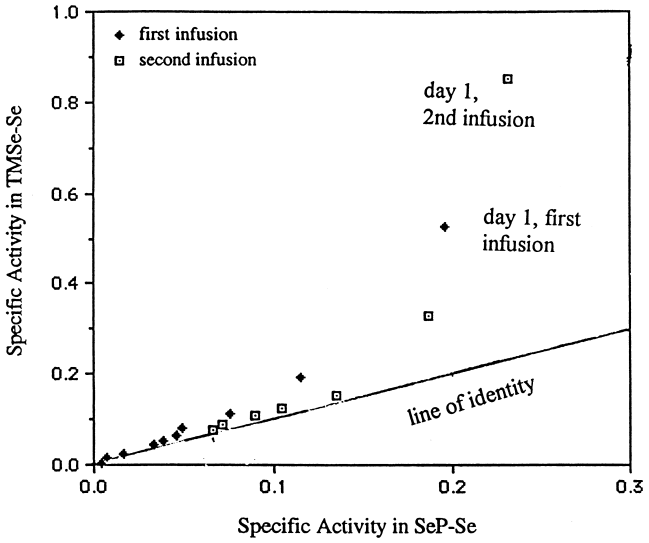

Figure 9 Observed correlation between specific activity (SA) of urine trimethyl selenonium chloride (TMSe) and plasma selenoprotein P (SeP) for the two infusion periods. Data are group averages (n = 5) for each day after an infusion (for first infusion period, days 1–62; for second period, days 64–70).

time points. For any time, the sum should be 100% only if there is no other selenospecies in plasma, but this is postulated to not be the case especially in subjects with high total body Se. The error in each measurement is relatively high because errors of analysis and chromatography are magnified. Even with these limitations, the data clearly indicate that infused selenite is promptly incorporated into SeP, but much more slowly into GPX. As expected, there is essentially no incorporation into the Alb fraction because selenite cannot be converted to selenomethionine, which is the form of Se in Alb.[22] This is dramatically different from the distributions of Se (*Table 3*), which is likely due to the differences in the turnover kinetics of SeP and GPX, and presence of selenomethionine in plasma Alb in subjects consuming natural sources of Se. The main form of Se consumed by residents of Enshi[20] has been shown to be selenomethionine.

No information is currently available about turnover kinetics of plasma SeP in humans.[27,28] The natural logarithm of the concentration of Se* in the SeP fraction has been plotted against time for the two infusion periods (*Figure 8*). These data yield a value in the range of 1.9 to 2.9 days for the half-life of Se in this protein. The reasons for any differences in the measured half-life between the two periods is not likely related to increased re-utilization of the label in de novo synthesis of the protein during the second period because the expectedly higher body retention of the label was not observed during the second period (*Figure 5A*). Any re-utilization of Se* would be diluted out by the body Se in the synthesis of SeP and thus the half-life values for Se* reflect fairly accurate ones.

That both TMSe and SeP derive their Se from a common precursor pool (presumably the Se^{2-} pool) is further supported by the SA curves for the two selenospecies (*Figure 9*). A common precursor pool from which Se for both TMSe and SeP is derived would require that both end products show identical specific activities at any time after each infusion, which the data of *Figure 9* indicates as the case. First, because labeled SeP is diluted with a large amount of unlabeled SeP initially present in the plasma pool and labeled TMSe is not, the former should initially show a much lower SA than the latter. The SA data for the two end products merge at later times as unlabeled plasma SeP and make an ever smaller contribution to the dilution of the labeled SeP due to normal degradation. For instance, with a half-life of 2 to 3 days (*Figure 8*) unlabeled plasma SeP should decrease to 50% of its initial amount 2 to 3 days after each infusion. By day 7 of each infusion, unlabeled background should have decreased to approximately 15% of its initial value. Second, SA data for TMSe for the second infusion period reached the line of identity (*Figure 9*) sooner than for the first infusion period because 2 months of Se restriction reduced plasma concentration of unlabeled SeP by a significant amount (*Table 3*).

Implications for long-term supplementation

If long-term Se supplementation is to be a useful option for chemopreventive purposes,[1,2] it is imperative that the relationship between dietary factors of supplementation and the tissue levels of specific selenospecies with chemopreventive potential be understood. Current knowledge about the efficacy of Se supplementation for chemopreventive purposes is meager, but the need for continuous tissue generation of high levels of Se^{2-} appears to be a key issue.[7,8] Because Se^{2-} is labile, the supplementation regime must focus on a mechanism for its continuous generation in the target tissue at levels consistent with chemopreventive efficacy.[29]

Lack of an increase in retention of labeled selenite for the second infusion period compared with the first may be indicative that the body's capacity to retain Se^{2-} generated from turnover of presumably selenomethionine-containing proteins was still at saturation in these subjects even after

dietary Se restriction for 2 months. If this were the case, any additional increase in total body selenomethionine content would not add to chemopreventive benefit. The relationship between catabolic flux of Se^{2-} and excretion of TMSe is certain to be complicated by the as yet poorly understood quantitative relationship between tissue flux of Se^{2-} and other excretory end products. DMSe excreted in expired air may be of particular relevance to this issue as its production is known to be quantitatively significant under conditions of high chronic intake of Se,[9] and future studies should include the measurement of this methylated selenocompound.

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