Metabolism of selenate and selenomethionine by a selenium-deficient population of men in China

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Twenty young Chinese men (ages 18–40 with a mean age of 29 years) living in a selenium deficient area of China (Dechang county, Sichuan province) were selected, divided into two groups, and provided with 200 μg selenium daily for 1 year as either selenate or selenomethionine (Semet). Blood and 24-hour urine samples were collected initially, after 1 month of supplementation and subsequently at bimonthly intervals. Selenium increased in both plasma and erythrocytes at a significantly faster rate in deficient men taking Semet than in those taking selenate. Glutathione peroxidase (GSH-Px) activity increased in both plasma and erythrocytes up to 4 months where a plateau was reached, but there were no differences in this activity between these two supplemental groups. The percentage of selenium associated with GSH-Px was significantly lower in plasma and erythrocytes in men taking Semet than in those taking selenate. Gel filtration chromatography revealed two selenium-containing peaks in plasma of men taking Semet but mostly one in plasma from men taking selenate. Urinary excretion of selenium gradually increased throughout this study, but there were no differences in the excretion of total selenium or trimethylselenium (TMSe) between the two treatment groups. The TMSe content ranged between 1%–2.5% of total selenium in both groups.

Keywords: selenium deficiency; humans; Keshan disease; glutathione peroxidase; gel filtration; erythrocytes and plasma

Introduction

There are several reasons for investigating the metabolism of different forms of selenium (Se) in humans. When rats[1] rhesus monkeys[2] or women with moderately low Se status[3] were given inorganic Se there was a higher correlation of blood Se with glutathione peroxidase (GSH-Px) activity than when selenomethionine (Semet) was given. One factor responsible for this was probably the distribution of Se between the erythrocyte (red blood cells [RBC]) and plasma fractions. When selenite was given to rats[1] and monkeys[2] or selenate to women,[3] the majority of the RBC Se was associated with GSH-Px, but in contrast the majority of the Se was associated with hemoglobin (Hb) when Semet was the form of Se administered. There is only one major Se-containing peak that elutes slightly ahead of GSH-Px activity when animal plasma is subjected to gel filtration.[4] However, gel filtration of human plasma revealed two Se-containing peaks neither of which cochromatographed with GSH-Px activity. Presumably this is a reflection of the dietary Semet in humans, based on work with monkeys[2] and women with moderately low Se status.[3] Therefore, more information is needed to determine factors affecting Se metabolism in humans.

The Se status of people influences the correlation

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Presented at the 73rd Annual Meeting of the Federation of American Societies for Experimental Biology held in New Orleans, LA, and published in abstract form (The FASEB J. 3, A780).
Published with the approval of the Oregon State University Agricultural Experiment Station as Technical paper number 9203. This study was supported by Public Health Service Research grant KD 38341 from the National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases.
Received August 13, 1990; accepted October 23, 1991.

of blood Se with GSH-Px. Populations of people with adequate blood Se levels usually do not show a significant correlation between blood levels and GSH-Px activity[5] and have only 10%–15% of the RBC Se associated with GSH-Px.[6] In contrast, humans with a low Se status show a positive correlation between RBC Se and GSH-Px activity and have 30%–40% of the Se associated with GSH-Px.[6,7]

Trimethylselenium (TMSe) is the only Se compound that has been identified in urine. Although once thought to be a detoxification product of Se, its levels in urine are seldom above 5% of the total unless Se is injected.[8,9] The administered chemical forms of Se had no effect on the amount of TMSe excreted in rats,[9] but there is very little information on this in humans. A preliminary report indicated that more urinary TMSe was excreted by women of moderate Se status when they took selenate than when Semet was taken.[10] Therefore, the objectives of the present study were to investigate the influence of chemical forms of Se on its distribution between RBC and plasma protein and on its excretion as TMSe in urine from men with a very low selenium status.

Materials and methods

Subjects

The subjects for this study were male farmers (ages 18–40 with a mean of 29 years) in Dechang County of Liangshan Prefecture, in Sichuan Province, People's Republic of China. Dechang County has a population of about 150,000. The daily selenium intake was estimated to be 11 μg per person based on results of a nutrition survey conducted in 1985 by the Antiepidemic Station of Liangshan Prefecture.[11,12] There has not been any major large-scale Se supplementation in Dechang County. Previously the incidence of Keshan disease in this county has been among the highest in China,[12] but it has declined steadily since 1980.[13]

All subjects were recruited by local antiepidemic station personnel working with village doctors. It was made known by word-of-mouth that volunteers for a study were being sought. The nature and purpose of the study were explained to the men who indicated an interest. Subjects who appeared to be qualified were given a physical examination by the village doctor, including an electrocardiogram and a chest X-ray. Subjects who were considered normal and had no known chronic disease were considered qualified. Final selection, which excluded a few volunteers, was based on a prediction of subject compliance by the village doctor. The study 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. Oral consent was obtained from each subject. Each person received an amount equivalent to $70 for his participation in this study.

Protocol

This study was conducted for 1 year, starting in January of 1988. Two groups of 10 men were given one yeast tablet which contained 200 μg Se as either selenate or Se enriched yeast daily. The Se in the Se-enriched yeast has been shown by analysis to be Semet.[1] These tablets were manufactured by VitaTech International, Inc. (Tustin, CA, USA) and supplied to us by Nutrition 21 (La Jolla, CA, USA). The carrier yeast for selenate analyzed 0.2 μg Se/tablet. A designated participant in each of the treatment groups delivered a yeast tablet to each of the participants daily and watched him swallow it. On the evening before each sampling day all subjects were assembled and transported from their respective villages to an inn in Dechang County. Urine was collected for 24 hours. Blood was also collected during this time.

Blood, urine, hair, and fingernail sampling

Blood samples were taken between 0900 and 1000 hr, at the beginning of the study, 1 month after the supplementation started, and bimonthly thereafter. A tourniquet was applied to the upper arm, and a 12-mL blood sample was taken from the antecubital vein using a 20-mL plastic syringe with a 20-gauge disposable needle. EDTA was used as the anticoagulant. The blood was centrifuged at 800*g* for 15 min to separate the RBCs from plasma. The RBCs were washed once with saline and both plasma and RBCs were frozen in separate vials with liquid nitrogren, transported to Beijing while frozen in liquid nitrogen, and maintained in the frozen state at −70° C until shipped to Oregon. They were then packed in dry ice and shipped to Oregon State University in two different lots by air express. All samples through 6 months were shipped first and the remaining ones at the termination of the experiment.

Each time the subjects were brought to the laboratory, 24-hr urine samples were collected in plastic 2-liter containers. After measuring the total volume, about 100 mL was saved under toluene as a preservative. These samples were transported to Beijing in the liquid state, and total and TMSe content determined in the laboratory of Y. Xia.

After the final urine and blood samples were collected at 1 year, fingernails and hair samples were collected. The fingernails were clipped with a nailclipper. Newly grown hair was collected at the base of the skull. This was done by clipping the hair with a coarse set of clippers, and then with a fine set of clippers. The hair collected with the fine clippers was saved for analysis. The hair and fingernails were shipped by air to Oregon in the dry state. The fingernails were soaked in 0.1 N HCl for 14 hours and any remaining foreign material scraped from the nails with a knife blade before analysis. The hair was soaked in distilled water for 14 hours, washed twice with distilled water, and finally with acetone on a suction flask before analysis. The fingernails and hair were treated by these procedures to remove any Se adhering to them.

Analytical procedures

Aliquots of RBCs were lysed (ratio 1:120) with deionized water and Se content and GSH-Px activity determined both on this lysate and with plasma. The RBCs (6 mL of a 1:7 dilution with water) were chromatographed on gel filtration (Sephadex G-150) columns (2 × 100 cm) at a flow rate of 10 mL per hour with 0.05 M phosphate buffer, pH 6.8, containing EDTA (10^{-3}M) and sodium azide (10^{-4}M) as previously described.[1] About 5 mL were collected per fraction. The eluted fractions were monitored for GSH-Px activity and Hb at 540 nm. Duplicate fractions of the GSH-Px and Hb peaks were combined, acid digested and their Se content determined by the fluorimetric procedure. The overlap area (descending side of GSH-Px and ascending side of Hb) was corrected by extending the curves to the baseline. The columns were standardized with purified GSH-Px and Hb. The areas under the GSH-Px and Hb curves were determined, then divided by the total areas to obtain the percentages and the percentages multiplied by the concentration of Se in RBCs to obtain the content in each of these fractions.

Plasma was diluted with ammonium acetate buffer (ratio 1:3) and chromatographed on columns (2 × 120 cm) of Sephadex G-150 with 0.33 M ammonium acetate buffer, pH 6.8, containing

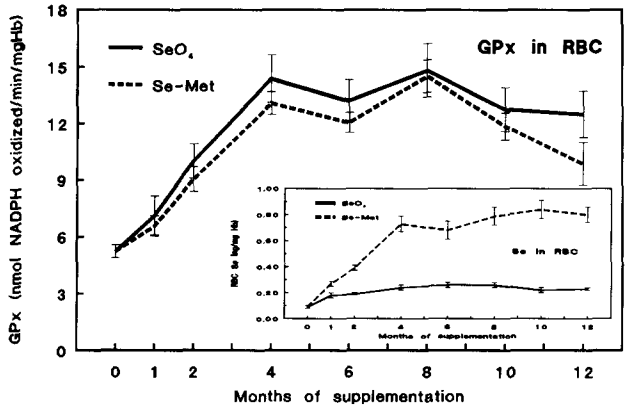

Figure 1 Accumulation of selenium (insert) and glutathione peroxidase activity in erythrocytes of Chinese men taking selenium either as selenate or selenomethionine. The bars at each point represent standard errors of 10 samples.

EDTA and dithiothreitol (10^{-4}M) at a flow rate of 10 mL per hour. About 5 mL were collected per fraction. The columns were standardized with purified GSH-Px. GSH-Px activity and absorbance at 280 nm were determined on the eluted fractions. Except for the initial samples, Se content was determined directly on the eluted fractions with a Perkin-Elmer atomic absorption spectrophotometer (Model 3030, Norwalk, CT, USA) with a Zeeman background corrector. A nickel-magnesium solution was used as the matrix modifier. The Se in the initial samples (*Figure 7*, bottom) was determined on combined fractions as was done for RBCs.

The plasma GSH-Px has been purified to homogeneity from humans in the Oregon Laboratory[14] and shown to exhibit 28 units (μmol NADPH/min) per mg protein. This calculates to be 8.15 μmol NADPH/(min.ng.Se) on the basis of 4 g atoms of Se per mole and a molecular weight of 92,000. This was used as the standard to calculate the percentage of Se associated with GSH-Px in the plasma of the subjects, using the plasma GSH-Px activity per unit Se. The percentage was multiplied by the Se content of plasma to obtain the amount of Se in GSH-Px. The difference between the percentage of Se with GSH-Px and 100 was taken as the percentage of Se in other Se-containing proteins, and this value was multiplied by the Se content of plasma to obtain the Se in other Se-containing proteins. The percentage of Se associated with GSH-Px in RBCs was calculated with purified bovine RBC GSH-Px as the standard,[15] from the GSH-Px activity and Se content of the RBC lysate. Under our conditions of assay, this enzyme oxidized 112 μmol NADPH/(min.ng.Se). After the percentage of Se associated with GSH-Px was calculated from this standard, this was multipled by the Se content of the RBCs to obtain the Se content in GSH-Px. The difference between the percentage of Se with GSH-Px and 100 was taken as the percentage of Se in Hb. This percentage was multiplied by the Se content of RBCs to obtain the Se content in Hb.

The percentage of Se with GSH-Px in plasma and RBCs was also estimated from the gel filtration chromatograms. The sum of the areas under each peak was divided into that of each peak to obtain this percentage.[2] This number was multiplied by the concentration of Se in plasma or RBCs to obtain the amount of Se per peak per mL sample.

After acid digestion with nitric and perchloric acids, Se was determined on RBCs and RBC fractions, plasma, hair, fingernails, and eluted fractions of *Figure 1* (bottom), by the semi-automated fluorimetric method[16] with an Alpchem Autoanalyzer II (Beaverton, OR, USA) as previously described.[1] GSH-Px activity was determined at 30° C in a water-jacketed cell by a coupled enzyme method[17] with t-butyl hydroperoxide (140 μmol/L) as the substrate. The positions of the Hb peak in the RBC chromatographs and the total Hb content were determined from the absorption at 540 nm.[18]

Total Se content of the urine was determined by a modification of the method of Watkinson[19] in the Beijing Laboratory. TMSe was measured in the urine samples by the two-column procedure.[20] Renal clearance of Se was calculated from the concentration of this element in the plasma and plasma fractions with that in the urine.[21]

Statistical analysis

The data were subjected to statistical analysis using analysis of variance, the Student-Newman-Keul procedure, regression analysis, and calculation of correlation coefficients.[22] Where necessary to achieve homogeneity of variance, the data were subjected to logarithmic transformation.

Results

All the subjects who started this study completed it as scheduled and were enthusiastic about it. Except for the common health problems such as colds, there were no major health difficulties with any of the participants at any time during this study. Slightly more than a two-fold increase of Se occurred in RBCs in men taking selenate where a peak appeared to be reached after about 6 months of supplementation (*Figure 1*, Insert). In contrast, about an eight-fold increase of Se occurred in RBCs in men taking Semet, where a plateau was reached at about 4 months of supplementation. The Se levels at 1 month of supplementation and afterward were significantly higher ($P < 0.01$) in RBCs of men taking Semet than in those taking selenate. Although the GSH-Px activities increased in RBCs of men taking both Se supplements, there were not significant differences between the two groups at any time of the experiment (*Figure 1*). A plateau in activity was reached in both groups at 4 months of supplementation.

The gel filtration patterns for RBC lysates from men initially and after supplementation with either selenate or Semet for one year are shown in *Figure 2*. Most of the Se was associated with Hb in the initial samples (*Figure 2*, bottom), in which the distribution was similar to that in men taking Semet for one year (*Figure 2*, top). The Se concentrations, of course, were markedly higher in these fractions after 1 year of supplementation than initially. In contrast, the Se was about equally distributed between GSH-Px (first peak) and Hb (second peak) in RBCs from men taking selenate for one year (*Figure 2*, middle).

The amounts of Se associated with GSH-Px and Hb with time of supplementation as calculated by standard GSH-Px are shown in *Figure 3*. As would be anticipated from *Figure 2*, the amount of Se increased very rapidly in Hb of RBCs from men taking Semet (*Figure 3*, right). There were no differences in the accumulation of Se in GSH-Px between the two treatment groups (*Figure 3*, left). Similar patterns were obtained when the accumulation of Se in Hb and

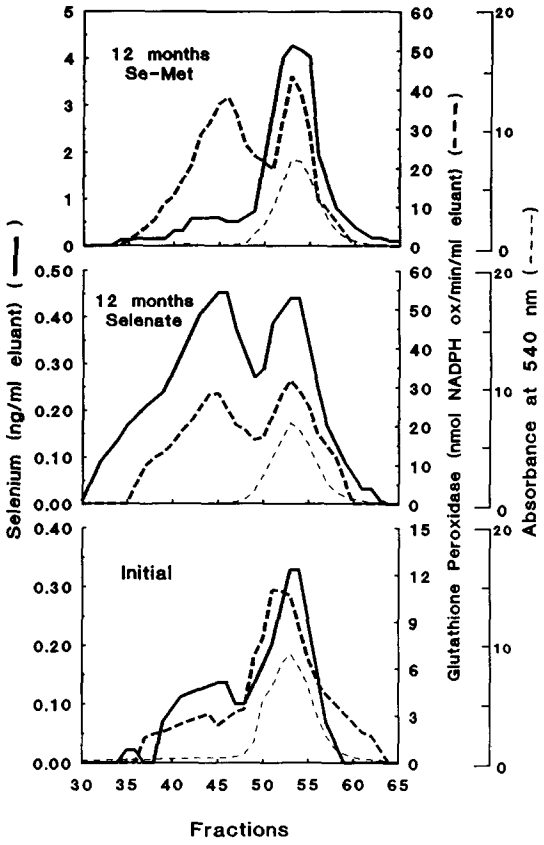

Figure 2 Gel filtration patterns of erythrocytes initially and after 1 year of supplementation with either selenate or selenomethionine. Note the marked differences in the scale for selenium among the three graphs; and the GSH-Px activity scale between the bottom graph and the other two. These graphs are the average of three determinations of pooled samples composed of RBCs from three, three, and four men.

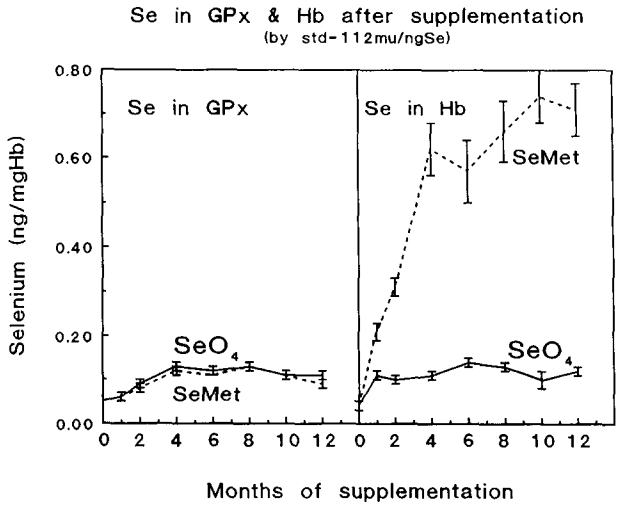

Figure 3 Amounts of selenium in hemoglobin and glutathione peroxidase as determined with the GSH-Px standard with time of supplementation with either selenate or selenomethionine. The percentage of Se with GSH-Px was determined as described in Materials and Methods. The bars at each point are standard errors of 10 samples.

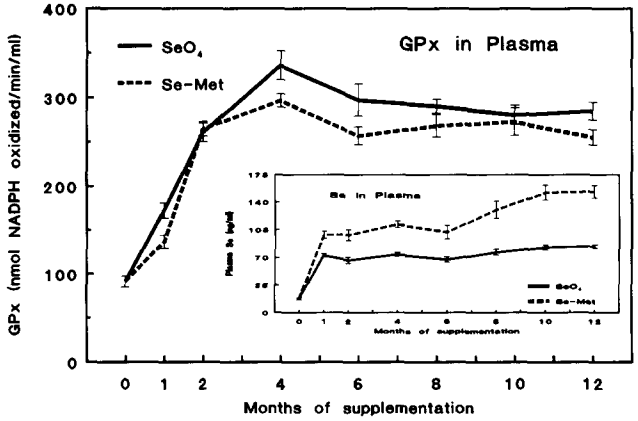

Figure 4 Accumulation of selenium (insert) and glutathione peroxidase activity in plasma of Chinese men taking selenium either as selenate or selenomethionine. The bars at each point are standard errors of 10 samples.

GSH-Px were determined from the gel filtration chromatogram (data not shown). Significantly greater accumulation of Se occurred in Hb of men taking Semet but no differences were found in the Se content of GSH-Px in RBCs between the two groups of men. The correlation coefficients for these two methods were 0.93 and 0.74 for Se in GPX, and 0.84 and 0.99 for Se in Hb, from men taking selenate and from men taking Semet, respectively.

Se levels reached a plateau in plasma much faster than in RBCs (*Figure 4*, Insert). There was a rapid increase from the original values from about 20 ng/mL to about 73 ng/mL at 1 month of supplementation with selenate, with a very slight increase throughout the remainder of the experiment. In contrast, the increase of Se in plasma of men taking Semet was much greater after the plateau was reached than in those taking selenate. At 1 month and afterwards the plasma Se levels were significantly greater ($P < 0.01$) in men taking Semet than in those taking selenate. Similar to the RBCs, the plasma GSH-Px activity increased in both groups of men but there were no significant differences between the two groups (*Figure 4*). In contrast to the Se levels, this activity in plasma did not reach a plateau until 4 months of Se supplementation.

The accumulation of Se with GSH-Px and other Se-containing proteins as calculated with the GSH-Px plasma standard is shown in *Figure 5*. Similar to the RBCs, there were no differences in the accumulation of Se with plasma GSH-Px between men taking Semet and selenate. However, significantly greater amounts of Se accumulated with the other Se-containing proteins in men taking Semet than in those taking selenate. The other Se-containing proteins represent selenoprotein P and albumin.[23]

The percentage of Se associated with RBC GSH-Px dropped from the initial values in both groups at 1 month of supplementation (*Figure 6*, top), and then gradually declined in men taking Semet through the rest of the experiment. In contrast, this percentage increased slightly after the initial drop in men taking

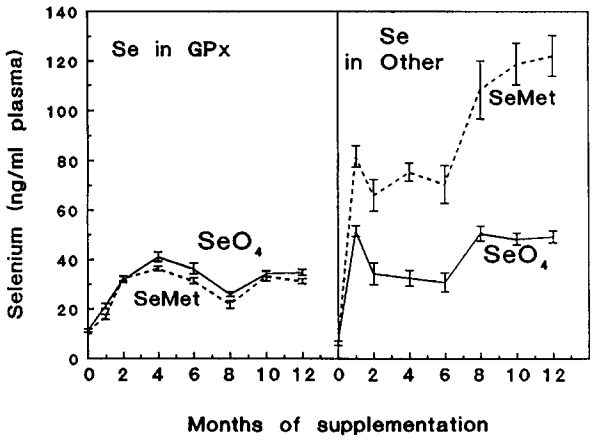

Figure 5 Accumulation of selenium in GSH-Px and other selenium-containing proteins as determined with GSH-Px standard with time of supplementation with either selenate or selenomethionine. The amounts of selenium with these selenium-containing proteins were calculated as indicated in Materials and Methods. The bars at each point are standard errors of 10 samples.

selenate. At 1 month and afterwards, the percentage of Se associated with GSH-Px was significantly higher ($P < 0.01$) in RBCs from men taking selenate than in those taking Semet. Similar to the RBCs, the percentage of selenium associated with plasma GSH-Px dropped after 1 month of supplementation in both groups (*Figure 6*, bottom). An increase was seen again in both groups of men, where a maximum was reached at 6 months in men taking selenate and at 2 months in men taking Semet. Subsequently, a slight decline in this percentage occurred throughout the rest of the study. At 1 month and afterwards the percentage of plasma Se associated with GSH-Px in men taking selenate was significantly higher ($P < 0.01$) than in those taking Semet.

Gel filtration patterns of the plasma from men initially and after 1 year of supplementation with either selenate or Semet are shown in *Figure 7*. Initially there was predominately one gel filtration peak (*Figure 7*, bottom), which eluted slightly ahead of the GSH-Px activity peak. The plasma pattern from men taking selenate for 1 year was similar to the initial pattern but, of course, the Se content was markedly higher (*Figure 7*, middle). After 1 year of Se supplementation with

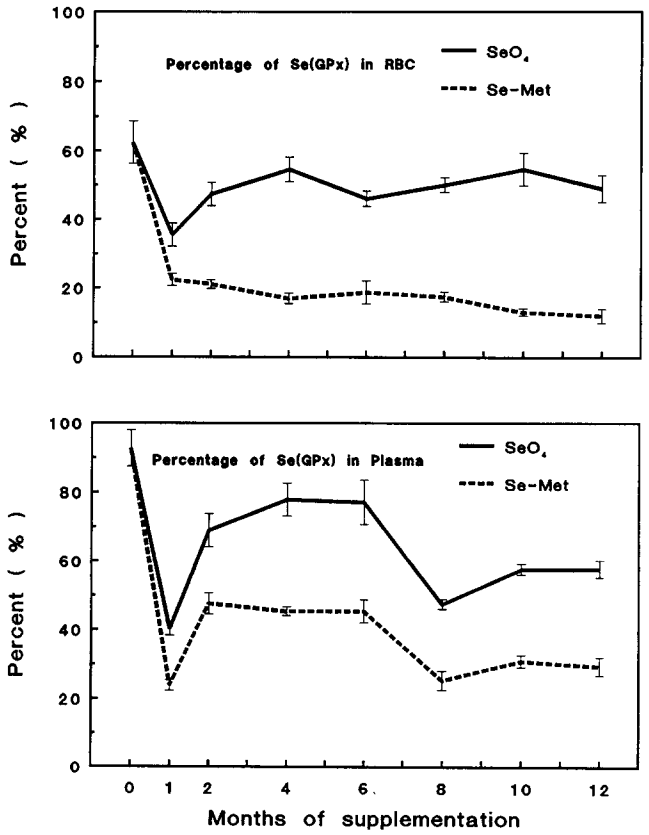

Figure 6 Percentage of erythrocyte selenium (top) and percentage of plasma selenium (bottom) associated with glutathione peroxidase in Chinese men taking selenium as selenate or selenomethionine. The bars at each point are standard errors of 10 samples.

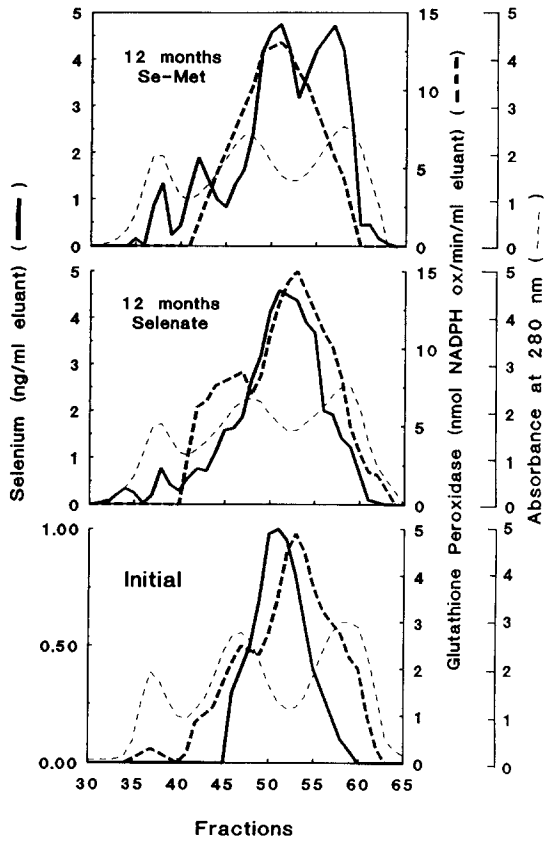

Figure 7 Gel filtration patterns of plasma taken initially and after supplementation with either selenate or selenomethionine for 1 year. Note the differences in scale for selenium and GSH-Px between the bottom graph and the top two. These graphs are the average of three determinations of pooled samples composed of plasma from three, three, and four men.

Semet, there were two gel filtration peaks (*Figure 7*, middle). The correlation of plasma Se with GSH-Px activity was similar in plasma of men taking selenate (r = 0.63) and in those taking Semet (r = 0.61). However, the correlation coefficient of RBC Se with GSH-Px activity was higher in RBCs of men taking selenate (r = 0.82) than in those taking Semet (r = 0.65).

No significant differences were found in either the total urinary Se or TMSe excretions between the two treatment groups (*Figure 8*). The total urinary Se levels increased from 3.4 ± 2.5 μg/24 hours initially to 64 ± 39 μg/24 hours for those taking selenate and to 87 ± 35 μg/24 hours for those taking Semet at 1 year. The percentage of TMSe remained low (1%–2.5%) throughout the study and no differences were found between treatments.

It took a higher total plasma Se content in men consuming Semet to result in the same renal clearance of Se as in those consuming selenate (*Figure 9*). There

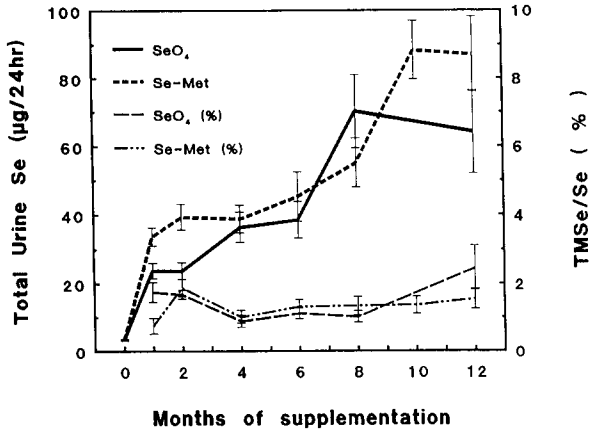

Figure 8 Urinary excretion of total and trimethylselenium in Chinese men taking selenium as either selenate or selenomethionine. The bars at each point are standard errors of 10 samples.

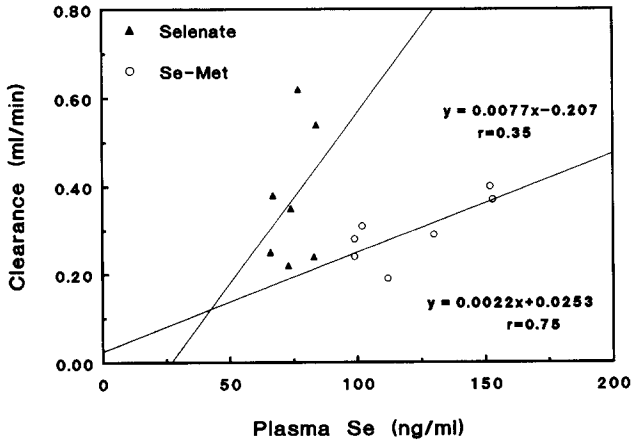

Figure 9 Renal clearance of selenium from Chinese men taking selenate or selenomethionine. These are the average values of 10 men at each sampling time.

Table 1 Effect of selenium intake as either selenate or selenomethionine on selenium content in blood, hair and fingernails in Chinese men.

Tissues	Selenate[a]	Semet[a]
Plasma	84 ± 8	153 ± 26[b]
Erythrocytes	53 ± 9	178 ± 46[b]
Hair	357 ± 43	620 ± 108[b]
Fingernails	397 ± 87	646 ± 131[b]

[a] Nanograms selenium per g tissue.
[b] $P < 0.01$.

was no overlap of any of the values for men consuming Semet in comparison with those consuming selenate. These values were statistically significant ($P < 0.1$) between the two groups of men. Se content in GPx and Se in other proteins were also plotted against renal clearance, but neither of these were as significant as that shown in *Figure 9* for total plasma Se. The regression equations and correlation coefficients for men taking selenate versus Semet were y = 0.004X + 0.20, 0.24; and y = 0.0022X + 0.099, 0.71 for renal clearance plotted against Se in other proteins; and y = 0.0007X + 0.35, 0.03; and y = 0.0019X + 0.24, 0.18 for renal clearance plotted against Se in GPx.

The Se content in plasma, RBCs, hair, and fingernails were significantly higher in men taking Semet than in those taking selenate for 1 year (*Table 1*). The initial values (mean ± standard errors) respectively for plasma, RBCs, hair, and fingernails were 17.5 ± 1.3, 17.7 ± 7.6, 134 ± 31, and 164 ± 64 ng/g. The final values represent 4.8-, 3.0-, 2.7-, and 2.4-fold increases, respectively, for plasma, RBCs, hair, and fingernails for those taking selenate, and 8.8-, 10.1-, 4.6-, and 3.9-fold increases, respectively, for those taking Semet.

Discussion

The present results indicate that the chemical form of Se has a pronounced effect upon the distribution of Se between the different proteins in RBCs and in plasma. This is consistent with the results obtained with Rhesus monkeys[2] and with New Zealand women.[3] The results with the RBCs are generally consistent with trends noted in the rat,[1] but there have been some observed differences. When rats were given selenite, over 85% of the Se in RBCs was associated with GSH-Px.[1] Even though selenate was given to New Zealand women[3] for 32 weeks or to men for 1 year in the present study (*Figure 2*), the percentage of Se in RBC GSH-Px never exceeded 50%.

Differences in plasma patterns between rats and humans have also been noted. When selenate Se was given, mostly one gel filtration Se peak was obtained in New Zealand women[3] and Chinese men (*Figure 7*, middle), but when Semet was given two Se gel filtration peaks were obtained (*Figure 7*, top). In contrast, the gel filtration patterns were very similar for plasma

from rats regardless of whether selenate or Semet was given.[24] Semet must presumably be converted to selenocysteine by the transsulfuration pathway for incorporation into GSH-Px.[25] The rat appears more efficient in this conversion, which could account for the differences between rats and higher primates.

The resolution of the Se-containing peaks upon gel filtration in the present study (*Figures 2 and 7*) was not as great as in the study with the New Zealand women.[3] This is apparently due to the anticoagulant used in the two studies. Heparin was used in the New Zealand study whereas EDTA was used in the present one. Heparin has been shown to bind selenoprotein P, resulting in better resolution of the Se-containing proteins.[26,27]

Our data indicate that the dietary forms of Se consumed by humans may affect the correlation of GSH-Px activity with Se in some blood fractions. This correlation was similar in plasma from the two groups of men, but higher in RBCs of men taking selenate than those taking Semet. This higher correlation of Se content with GSH-Px activity in RBCs when selenate was given as compared with Semet is consistent with earlier work on whole blood Se and GSH-Px activity in New Zealand women[3] and RBC Se with GSH-Px activity in Rhesus monkeys.[2] We suggest that the greater incorporation of Se from Semet into RBC Hb in comparison to selenate is the reason for these observations. The greater increase of Se in blood, plasma, and RBC with Semet than selenate is consistent with prior human studies.[3,28,29]

Supplementation of Chinese men with Semet gave gel filtration patterns of plasma Se similar to those seen in people consuming regular diets. Two gel filtration Se peaks are present in plasma from humans consuming regular diets,[3,4] whereas only one main Se gel filtration peak is seen in humans taking Se as selenate (*Figure 7*).[3] This suggests that Semet is one of the main forms of Se in human diet, which is consistent with evidence for Semet as the primary form of Se in wheat,[30] soybeans,[31] corn, and rice,[32] and Se enriched yeast.[1]

The methods used to estimate the percentage of Se associated with GSH-Px need further investigation. There was not good agreement between the use of purified GSH-Px as standard and the use of areas from a gel filtration chromatogram with plasma in the present study (data not presented). However, excellent agreement was obtained between the two methods with RBCs. This may be due to only two Se-containing proteins in RBC lysates that are fairly well resolved by gel filtration. In contrast, there are three Se-containing proteins present in plasma and these are not well resolved by gel filtration (*Figure 7*),[3] which creates a problem in estimating the areas under each curve.[2] The amount of Se associated with GSH-Px has to be obtained by extrapolation and correcting for overlap of these peaks.[2] The Oregon laboratory found about 46% of the Se associated with GSH-Px in human plasma when determined from the gel filtration chromatograms,[4] which is in close agreement with 45% by another group using calculated values.[33] However, less than 10% of the Se was calculated to be with GSH-Px when determined with purified GSH-Px as the standard.[34] In this last study, erythrocyte GSH-Px was used to calculate the percentage of Se associated with plasma GSH-Px and this low value is probably because the GSH-Px units per amount Se in plasma is now known to be less than one-tenth of that for the RBC enzyme.[33] The activity of the purified GSH-Px standards (8.15 versus 112) is consistent with this observation. However, in our study purified bovine erythrocyte GSH-Px was used to calculate the percentage of Se associated with erythrocyte GSH-Px and purified GSH-Px from human plasma was used to calculate these values for plasma GSH-Px.

Other investigators have also obtained different results depending on the method of calculation used. Based on the Se content of blood and GSH-Px activity per unit Hb, RBC GSH-Px accounted for about 57% of the RBC Se content.[33] However, when this same group precipitated erythrocyte GSH-Px with antibodies, 10%–15% of the Se was calculated to be with GSH-Px,[35] which is in close agreement with results of other researchers.[4,36] By use of antibody precipitation, 12%–15% of the Se in plasma was shown to be present with GSH-Px,[35] which is close to our values using the tandem column procedure with heparin-sepharose and reactive blue (unpublished work). Obviously, additional work is required on the factors affecting these calculations for the determination of the correct amount of Se associated with GSH-Px.

There were no significant differences observed either in the total urinary Se or the TMSe excretion between the two treatment groups (*Figure 8*). The excretion of TMSe was higher in urine of New Zealand women taking selenate than those taking Semet,[3] which is inconsistent with the present results. In the present study the urinary excretion of Se (*Figure 7*) did not reach the initial levels in the New Zealand women until after eight months of supplementation. The initial difference in Se status of people in these two studies may acocunt for this slight disagreement assuming no difference due to sex. This may not be a valid assumption because male rats have been shown to have a higher Se requirement than females.[37] However, the renal clearance of Se between men taking selenate versus Semet is almost identical to that observed in the New Zealand women.[3] The lack of any significant difference in the excretion of TMSe between men taking Semet and selenate is consistent with results with rats.[9]

The significantly higher Se content in blood, plasma, fingernails, and hair from men taking Semet than those taking selenate is consistent with results obtained with animals. Higher levels of Se were found in hair of monkeys,[2] rats,[38] and the wool of sheep[39] fed diets with Semet as compared to those fed diets with selenite. These results indicate that without knowledge of the forms of Se in the diet the Se content in tissues cannot be used to accurately assess Se intake. Thus, the higher Se content in men taking Semet is

not due to greater Se intake but instead a reflection of the forms of Se consumed. The age of a person may also be a factor based on work with rats.[38] The differences between the effects of selenate versus Semet on hair and nail levels were greater in young rats than in old ones.

The differences due to the chemical forms of Se pose problems in the assessment of the Se status of humans. Epidemiologic and intervention studies investigating possible health effects of Se, such as cancer chemoprevention, have pointed out the need for accurate indicators of Se status that would monitor the body pools of this element.[40,41] It would be beneficial in such studies to utilize tissues obtained noninvasively such as hair and nails to assess the Se status.[42] Unfortunately, the present results indicate that extreme caution must be used for this assessment. Although hair Se levels along with blood levels have been used to distinguish between populations that were deficient or adequate in Se,[42] other work indicates that there is no apparent correlation of Se concentration between hair and internal organs.[43]

Despite these limitations, hair and blood Se levels have been used to monitor Se status under some circumstances. Blood and hair Se levels have been used advantageously to assess the probability of Se deficiency in Chinese populations.[42] When the blood and hair levels were less than 0.03 ppm and 0.12 ppm, respectively, the people were at increased risk of problems associated with deficiency. Significant correlations have been reported[44] between Se intake and blood Se levels, between blood Se and hair Se, between blood Se and toenail Se, and between blood Se and fingernail Se. Recently it was reported that the Se content of toenails was lower in people with heart disease and the authors suggested a relationship between Se and cardiac problems,[45] but this was later questioned.[46] Thus, it appears that hair and nail Se levels may be used as a guide for Se status, but not for drawing firm conclusions with respect to Se and metabolic disorders.

Acknowledgments

The authors are grateful to Luzhen Gu and Yan Zhou of The Institute of Nutrition and Food Hygiene, Beijing, China, for their assistance with total selenium and trimethylselenium determinations on the urine samples. The cooperation of Xuejun Deng and Xuechao Wang of the Antiepidemic Station of Liangshan Prefecture, and Qizhong He of the Antiepidemic Station of Dechang County are also gratefully acknowledged for their assistance in organizing and supervising this study.

We wish to thank Jim Bie, Nutrition 21, La Jolla, CA, USA, for obtaining the tablets of selenium-enriched yeast and those containing selenate for this study.

Appreciation is expressed to Dr. A. Wendel, Faculty of Biology, Biochemical Pharmacology, Konstanz, Germany, for the gift of purified bovine erythrocyte GSH-Px.

We thank Dr. Ken Rowe, Department of Statistics, Oregon State University, for his assistance with the statistical analysis.

We wish to thank Dr. J. E. Oldfield, Director of the Nutrition Research Institute, Oregon State University, for his critical evaluation of this manuscript.

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