Distribution of selenium in erythrocytes, plasma, and urine of Chinese men of different selenium status

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Blood and urine were collected from Chinese men living in areas of China with deficient, adequate, and excessive levels of selenium (Se). Se content and glutathione peroxidase (GSH-Px) activity were determined on blood fractions and total Se and trimethylselenium (TMSe) determined in the urine. There was an increase of erythrocyte and plasma Se concentration with each increase of Se status. In contrast, this was not true for the GSH-Px activity. Both erythrocyte and plasma GSH-Px activity were higher in men with adequate Se intake than in those with deficient intakes of this element, but this activity was not any higher in men with excessive Se intake than those with adequate intake. The percentage of Se associated with GSH-Px in both plasma and erythrocytes was inversely related to the Se status of the men. Gel filtration of erythrocyte lysates revealed basically one major Se peak from all men, but the amounts of Se in this peak differed greatly among the three groups. Most of this Se was associated with hemoglobin. In contrast, different patterns were obtained in the plasma from the various men. One main peak was obtained with plasma from Se- deficient men, but two Se peaks were obtained with plasma from men with adequate Se status, and up to four peaks were obtained with plasma from men with excessive Se status. The excretion of Se in urine increased proportionally with the Se status and TMSe increased from none detectable in deficient men, to 2% of total Se in men with adequate status to 7% of total Se from men with excessive Se intake.

Keywords: blood; urine; gel filtration; selenium; trimethylselenium; men; glutathione peroxidase

Introduction

An inverse relationship between the retention of selenium (Se) and Se status has been shown in several different animals.[1-3] Based on the urinary levels of Se with respect to intake the same would appear to be true in humans.[4] Not only is the retention affected, but the distribution of Se between different plasma fractions appears to be influenced by Se status. Different gel filtration patterns were observed for plasma from rats[5] and monkeys[6] of varied Se status. The distribution of Se between glutathione peroxidase (GSH-Px) and hemoglobin (Hb) in erythrocytes from rats has been shown to be influenced by their Se status.[7] Thus, differences in the distribution of Se between plasma and erythrocytes fractions might be expected in humans with varying Se status.

Trimethylselenium (TMSe) was once considered a detoxification product.[8,9] More recent observations in the rat[10] and in humans[11] suggest that under dosing conditions more relevant to nutritional intakes of humans[12] this end product metabolite constitutes only a minor component of urinary Se. A direct relationship, however, was found between the amount of the administered dose of selenite and the proportion of urinary Se excreted as TMSe in urine of rats.[13] Since populations of Chinese have been identified with markedly different Se status,[4] this offered an opportunity to investigate more thoroughly the influence of Se status on Se distribution in humans, and this was the purpose of our investigations.

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Materials and methods

Subjects

Ten men were chosen from each of three areas representing deficient, adequate, and excessive Se intakes. The men from the deficient area were from Dechang County, China, as described in the companion paper.[14] The average age of these men was 29 with a range from 18–40 years of age. The estimated Se intake of these residents is about 11 μg per day.

The men with adequate selenium intake were selected from Heping village, Shanghai County, Jiangsu Province. This village has a population of about 1,017 people. It is about 30 kilometers from the City of Shanghai, and the population of Shanghai county is about 394,130 people. Keshan disease has not been reported in this area of the country. The ages of these men ranged from 22–30 years with a mean of 25 years of age. All of these subjects were farmers. The daily intake of Se of these residents is estimated to be about 110 μg.[15]

The men with excessive Se intakes were selected from Huabei village in Enshi County, Exi prefecture, Hubei Province. Huabei village is about 50 kilometers from the city of Enshi. The primary occupation of this village is farming with some mining. The ages of these subjects ranged from 23–52 years with a mean of 35 years. Some residents of this village had previously suffered from excessive Se intakes.[15,16] The estimated daily Se intake of the residents was reported to be 750 μg in 1983.[15]

All subjects were recruited by local antiepidemic station personnel working with the village doctors. It was made known by word-of-mouth that volunteers were wanted to donate samples, and the nature and purpose of the study were explained to the men who indicated an interest. Those who appeared to be qualified were given a physical examination by the village doctor and an electrocardiogram and a chest X-ray were completed. Subjects who were considered normal and had no known chronic disease were classed as qualified.

This 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 approved subject received an amount equivalent to $10 for his participation.

Protocol

The subjects were assembled and transported to the hospitals in Enshi, Shanghai County, and Dechang before 5:00 pm the day prior to collection of samples. They were kept at the hospital for the next 24 hours and people from the local epidemic station roomed with them to assure compliance with the protocol. A 24-hr urine sample was collected from each of the subjects in plastic 2-liter containers. After the volume was measured, 100 mL urine was saved and preserved under a few drops of toluene. These sample aliquots were transported to Beijing in the liquid state and total and TMSe content determined in the laboratory of the senior author.

Blood was drawn the next morning between 0900 and 1000 hours. A tourniquet was applied to the upper arm, and a 12-mL plastic syringe with a 20-gauge disposable needle was used to draw blood. EDTA was used as an anticoagulant. The blood was centrifuged at 800*g* for 15 minutes to separate the erythrocytes from the plasma. The erythrocytes were washed once with saline and both plasma and erythrocytes frozen in separate vials with liquid nitrogen. The samples were transported to Beijing while frozen in liquid nitrogen and maintained in the frozen state at −70° C until shipped to Oregon. The samples were then packed in dry ice and shipped to Oregon State University by air express.

Analytical procedures

Aliquots of red blood cells (RBCs) were thawed, lysed (ratio 1:120) with deionized water, and Se content and GSH-Px activity determined both on this lysate and on the 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 and sodium azide.[14] All columns were standardized with purified GSH-Px and Hb. The eluted fractions were monitored for GSH-Px activity, and duplicate fractions containing the GSH-Px and Hb peaks combined, acid digested, and their Se content determined by the fluorimetric procedure.

The plasma (3 mL) was diluted with ammonium acetate buffer (ratio 1:3) and chromatographed on a column (2 × 120 cm) of Sephadex G-150 with 0.33 M ammonium acetate buffer, pH 6.8, containing EDTA and dithiothreitol (10^{-4}) at a flow rate of 12 mL per hour. About 4.5 mL were collected per fraction. These columns were standardized with purified GSH-Px. GSH-Px activity was determined on the eluted fractions, and Se content was determined directly 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.

Plasma GSH-Px from humans has been purified to homegeneity in the Oregon Laboratory[17] and shown to exhibit 28 units (μmol NADPH/min) per mg protein. This was used as the standard to calculate the percentage of Se associated with GSH-Px in the plasma of the subjects from the GSH-Px activity and Se content. The percentage of Se associated with GSH-Px in RBCs was calculated with purified bovine RBC GSH-Px as the standard[18] from the GSH-Px activity and Se content of the RBC lysate. Under our conditions of assay this enzyme oxidized 112 nmol NADPH/(min/ng/Se).

After acid digestion with nitric and perchloric acids, Se was determined on RBCs and RBC fractions, and on plasma by the semi-automated fluorimetric method[19] with an Alpchem Autoanalyzer II (Beaverton, OR, USA) as previously described.[20] GSH-Px activity was determined at 30° C in a water-jacketed cell by a coupled enzyme method[21] with t-butyl hydroperoxide (140 μM) as the substrate. The positions of the Hb peak in the RBC chromatograms and the total Hb content were determined from the absorption at 540 nm.[22]

Total Se content of the urine was determined by a modification of a previously developed method[23] in the Beijing laboratory. TMSe was measured by a modification of the two-column procedure, also by the Chinese scientists.[11] Renal clearance of Se was calculated from the concentration of this element in the plasma and in the urine.[24]

Statistical analysis

The data were subjected to statistical analysis using analysis of variance, the Student-Newman-Keuls procedure, and calculation of correlation coefficients.[25] When necessary to achieve homogeneity of variance, the data were subjected to logarithmic transformation.

Results

A summary of the blood results is presented in *Table 1*. The erythrocyte and plasma levels of Se were lowest in the Dechang subjects, higher in the Shanghai subjects, and highest in the Enshi subjects. The GSH-Px activity, however, did not follow the same trend because there was not a corresponding increase of GSH-Px activity with each increase of blood Se level.

Table 1 Selenium, glutathione peroxidase activity and percentage selenium associated with glutathione peroxidase in plasma and erythrocytes from men living in deficient, adequate, and excessive selenium areas of China

	GPx		Se		% Se with GPx	
	RBC	Plasma	RBC	Plasma	RBC	Plasma
Deficient	5.2[a]	91.4[a]	0.09[a]	17.4[a]	62.5[a]	67.1[a]
(Dechang)	±1.5	±27.0	±0.03	±5.8	±28.0	±17.0
Adequate	18.5[b]	291.0[b]	0.53[b]	96.2[b]	31.4[b]	38.1[b]
(Shanghai)	±4.0	±27.5	±0.06	±10.9	±7.7	±5.2
Excessive	20.8[b]	276.0[b]	5.8[c]	494.0[c]	3.6[c]	7.3[c]
(Enshi)	±3.2	±33.3	±2.0	±147.1	±1.3	±1.7

GPx units are expressed as nmoles NADPH oxidized/min/mg Hb (RBCs) or per mL (plasma).
Selenium units are expressed as ng Se/mg Hb (RBCs), or per mL (plasma).
[a,b,c] are respectively significantly different ($P < 0.01$) from each other.

Both RBC and plasma GSH-Px activity were higher ($P < 0.01$) in men with adequate Se intake than in those with deficient intakes, but this activity was not any higher ($P < 0.05$) in men with excessive Se intake than those with adequate intake. There was an inverse relationship between the percentage of Se associated with GSH-Px in both RBCs and plasma and the Se content in these blood fractions.

Gel filtration of RBC lysates from the three groups of men revealed very little difference in the relative distribution of Se between GSH-Px and Hb (*Figure 1*). Of course the total amounts of Se in each of these fractions differed significantly between these three groups of men. The calculated amounts of Se in GSH-Px and Hb (ng Se/mg Hb) were 0.041 and 0.069 from the Dechang subjects, 0.16 and 0.37 in the Shanghai subjects, and 0.81 and 5.0 in the Enshi residents, respectively. This was done by summing the Se in fractions 38–50 for GSH-Px and fractions 45–60 for Hb. Extrapolation of the two peaks to the base line was used to correct for the overlap (fractions 45–50) in determining the Se in each peak.

Gel filtration patterns of the plasma from men living in these three areas are shown in *Figure 2*. One main peak with a shoulder was obtained with plasma from the Dechang subjects (*Figure 2*, bottom). The main Se peak eluted slightly before the GSH-Px peak. In contrast, two Se peaks were obtained with plasma from men living in Shanghai in which GSH-Px activity eluted between these peaks (*Figure 2*, middle). Up to four Se peaks were obtained when plasma from men living in Enshi were chromatographed (*Figure 2*, top). The last peak contained the most Se. The major GSH-Px peak eluted before the last major Se peak.

Although there were marked differences in the urinary excretion of Se, no differences were found in the volumes of urine excreted among the three groups (*Table 2*). The excretion of Se in the urine was (in μg/24 hr) 3.4 ± 1.1, 24.4 ± 12, and 650 ± 384 for men living in Dechang, Shanghai, and Enshi, respectively. This excretion was significantly different ($P < 0.01$) among men from each of these areas. The TMSe with respect to total Se excreted increased from nondetectable in Dechang men to 2% in men from Shanghai and 7% of total Se in those living in Enshi.

A linear response was obtained with renal clearance on Se status of the subjects (*Figure 3*). The regression of renal clearance with plasma Se levels was calculated to be 0.83, which is statistically very highly significant ($P > 0.001$). The regression equation for this relationship is $Y = 0.001\ X + 0.1029$ ($n = 30$), where Y equals renal clearance and X equals plasma Se content.

Discussion

The results with both RBC lysates (*Figure 1*, top) and plasma (*Figure 2*, top) suggest that the predominant form of Se consumed by the subjects in Enshi was selenomethionine (Semet). The gel filtration patterns of the RBC lysate and plasma are very similar to those

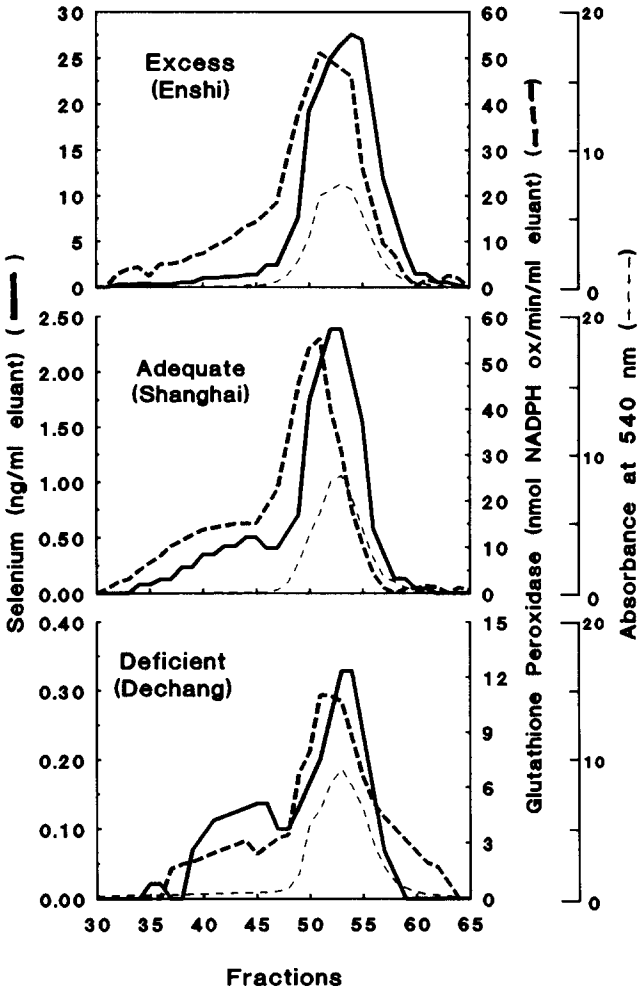

Figure 1 Gel filtration of erythrocyte lysates from Chinese men with deficient, adequate, and excessive intakes of selenium. The gel filtration conditions are described in the text. Note the marked differences in the selenium scale among the three graphs. Each graph represents the average of three determinations of pooled erythrocyte lysates from three, three, and four men.

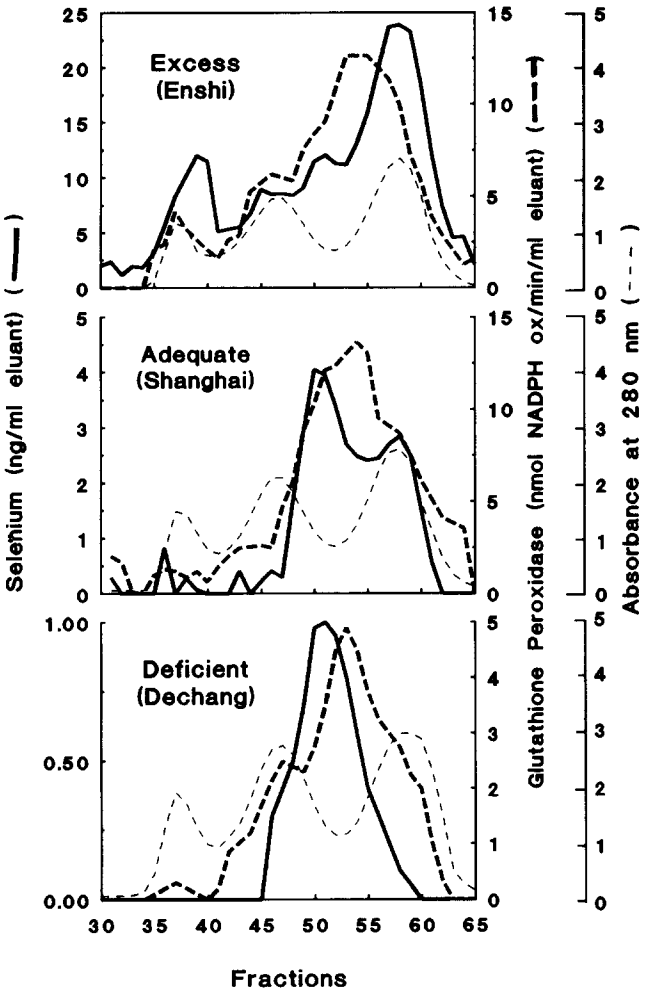

Figure 2 Gel filtration of plasma from Chinese men with deficient, adequate, and excessive intakes of selenium. The gel filtration conditions are described in the text. Note the marked differences in the selenium scale among the three graphs and the differences in the GSH-Px scale between the bottom graph and the other two. Each graph represents the average of three determinations of pooled plasma from three, three, and four men.

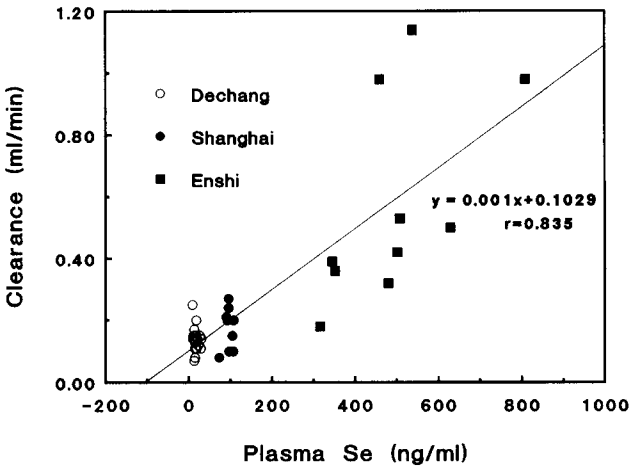

Figure 3 Renal clearance of selenium in Chinese men with deficient, adequate, and excessive intakes of selenium. The values are individual points from 30 subjects.

seen with Chinese men[14] or New Zealand women[26] who consumed Se as Semet. These patterns revealed two gel filtration Se peaks in plasma but predominantly one in the RBC lysate in subjects consuming Semet, and this is similar to the patterns observed in the present work. The extremely large amount of Se in the last peak in the plasma from the Enshi subjects (*Figure 2*, top) indicates a large intake of this seleno-amino acid by these individuals. It is has been shown that the Se content in this peak increases with high intakes of Semet by humans[27] or Rhesus monkeys.[28] The accumulation of excess Se in human Hb (*Figure 1*, top) is in agreement with work by others.[29]

The chemical form of Se in rice and corn grown in the Enshi area has recently been shown to be predominantly Semet,[31] and it is known that the Enshi residents consume large quantities of these grains. This is consistent with the blood data suggesting the major form of Se consumed by the Enshi subjects was Semet. In an earlier commentary[16] it was suggested that Se in smoke may have contributed to the high Se status of the Enshi subjects. Assuming the Se in smoke would be metabolized similarly to inorganic Se, the present results suggest that the major source of Se was instead from the diet and not from volatized Se in the smoke.

The gel filtration patterns of the plasma from the Shanghai subjects are similar to those observed for plasma from people of the United States[27] and New Zealand consuming adequate amounts of Se.[28] Increased dietary levels of Semet result in a greater Se content in the second gel filtration peak.[29] Therefore, it appears that the relative amounts of Se in these two peaks might be taken as a rough estimate of the extent of Semet intake.

Table 2 Urinary excretion of total selenium and trimethylselenium from men living in Dechang, Shanghai, and Enshi

Place	Total urine volume (mL)*	Total μg Se excreted*	μg TMSe excreted*
Dechang	1221 ± 617	3.4 ± 1.1[a]	ND†
Shanghai	988 ± 257	24.4 ± 12[b]	0.47 ± 0.30[a]
Enshi	1138 ± 158	650 ± 384[c]	42.7 ± 26.0[b]

* Values are means ± standard deviation of 24-hour excretion.
† ND, none detectable.
[a b c] are significantly ($P < 0.01$) different.

The GSH-Px activity eluted slightly ahead of the main Se peak in the RBC lysates (*Figure 1*), which is similar to the patterns observed for RBCs from New Zealand women.[26] We have no explanation for these patterns, but it should be noted that the peroxidase activity of Hb is independent of the Se content.[31] The resolution of the Se peaks in plasma in the present study was not as good as that observed for the New Zealand plasma samples.[26] This is due to the anticoagulants (heparin in New Zealand and EDTA in the present one) used in these two studies. Heparin has been shown to bind to selenoprotein p,[32] resulting in better resolution of the Se peaks upon gel filtration.[33]

The results of *Table 1* are consistent with other data showing that GSH-Px becomes saturated when adequate intakes of Se are reached.[34] There is a significant correlation of blood Se with GSH-Px activity at low levels of Se, but not at elevated levels.[34,35] The inverse relationship between the percentage of Se associated with GSH-Px in both plasma and RBCs indicates that Se is deposited with other proteins with higher Se intakes. In the Rhesus monkey, albumin is the predominant selenium-containing protein in plasma when excess levels of Semet are given.[28]

The peak of Se exposure in the Enshi subjects occurred between 1964 and 1972.[4] Several measures have been taken to reduce the Se exposure of these residents, including installation of stoves in their houses, elimination of the use of high Se coal for fertilizer, and increased consumption of dietary protein.[16] The blood and urine Se levels were reported to be 3.2 μg/mL and 2.2 μg/mL for people living in a chronic selenosis area and 0.44 μg/mL and 0.14 μg/mL for people living in a high Se area without selenosis.[4] Although all of the present subjects from Enshi had a high Se status, only two of them had previous signs of subtoxic levels of Se (loss of hair and thick and cracked fingernails). If the values for the Se area with chronic selenosis are taken, then the present results with blood (*Table 1*) and urine (*Table 2*) indicate that the measures that were taken have reduced the exposure of the subjects to Se. However, if the values for high Se without selenosis are taken, then they indicate that there has not been a reduction of exposure to Se.

The Se content of fingernails of men living in Dechang, Shanghai, and Enshi has been shown to be 0.18 ± 0.05, 0.78 ± 0.1, and 13.6 ± 3.9 μg/g,[36] respectively. This same general pattern in fingernails has also been shown for blood, hair, and urine Se levels.[4] The companion paper indicates that the chemical forms of Se also affects the Se content of tissues of humans.[14] Studies with rats indicate that the age of the animal will markedly affect these levels,[37] leading the investigators to conclude that hair and nail Se levels are of limited value. However, it should be noted that hair[4,38] and nail[36] Se levels have been used to obtain an estimate of Se status in humans. One report indicated significant correlations between Se intake and blood Se levels, blood Se levels and toenail content, and blood Se levels and fingernail content,[39] suggesting possible assessment of Se status by nail levels under some circumstances. Unfortunately, the Se levels in hair and nails of people in the western hemisphere would not be valid for this assessment because of the extensive use of polish and shampoos.

Data in *Table 2* indicate that TMSe is not a major component of urine even with excessive Se intakes. The amount of TMSe was only 7% of the total urinary excretion of Se from the subjects in Enshi. In rats, TMSe was a major component of urine only when large amounts of Se were injected.[13,10] Therefore, this rather insensitive response of TMSe to Se intake does not provide much basis for its use in assessing Se intake. The increased percentage of total urinary Se as TMSe with total urine Se (*Table 2*) is consistent with other Chinese work showing a significant correlation of urinary Se with TMSe.[40] Our positive correlation of Se status with urinary excretion of Se (*Figure 3*) is consistent with other data showing a significant correlation of daily Se intake with urinary excretion of total urinary Se output of 55%–60% over adequate range of Se intake.[41] Thus, the use of TMSe as an indication of Se toxicity does not appear very feasible.

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