Effectiveness of selenium supplements in a low-selenium area of China[1–3]

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ABSTRACT

Background: Selenium is an essential micronutrient with a recommended dietary allowance for adults of 55 μg/d. It functions as an essential constituent of selenoproteins. Although there is no evidence of selenium deficiency in the United States, people in many other areas of the world are selenium deficient, with the consequence that they are unable to express their selenoproteins fully.

Objective: We carried out a supplementation trial in a selenium-deficient population in China to assess the requirement for selenium as selenite and as selenomethionine.

Design: One hundred twenty subjects with an average selenium intake of 10 μg/d were randomly assigned and administered tablets containing no selenium or amounts as high as 66 μg Se/d for 20 wk. Plasma was sampled before supplementation and at 4-wk intervals during supplementation and was assayed for the 2 plasma selenoproteins, glutathione peroxidase and selenoprotein P.

Results: Full expression of glutathione peroxidase was achieved with 37 μg Se/d as selenomethionine and with 66 μg/d as selenite. Full expression of selenoprotein P was not achieved at the highest doses of either form.

Conclusions: Full expression of selenoprotein P requires a greater selenium intake than does full expression of plasma glutathione peroxidase. This suggests that selenoprotein P is a better indicator of selenium nutritional status than is glutathione peroxidase and that the recommended dietary allowance of selenium, which was set with the use of glutathione peroxidase as the index of selenium status, should be revised. Selenium as selenomethionine had nearly twice the bioavailability of selenium as selenite. *Am J Clin Nutr* 2005;81: 829–34.

KEY WORDS Selenium requirement, selenium deficiency, selenium bioavailability, selenomethionine, selenite, China

INTRODUCTION

Selenium is an essential micronutrient that exerts its biological functions through >25 selenoproteins (1). Nutritional deficiency of the element decreases the expression of the selenoproteins and thereby impairs selenium's biological functions. In the United States, the recommended dietary allowance (RDA) for selenium is 55 μg/d for healthy adults (2). Estimated intakes of selenium by US residents exceed that value. In Europe, however, intakes are as low as 30 μg/d, and some New Zealand residents consume <30 μg/d (3). Even lower intakes have been reported in rural regions of developing countries. China, for example, has areas with intakes of ≤10 μg/d (4).

Deficiency of selenium is necessary for the occurrence of a childhood cardiomyopathy known as Keshan disease (5). This disease is often fatal and affects small children in areas of China where daily selenium intakes are ≈10 μg. Because its incidence is low in the population of selenium-deficient children, a second stress, possibly a viral infection, is postulated to precipitate Keshan disease. No other human diseases have been proven to require selenium deficiency for their occurrence (6), although studies in animals indicate that selenium deficiency weakens the ability to withstand stresses of certain types (7, 8). Because selenoproteins exert the biological effects of selenium, it has been argued that enough selenium should be consumed to allow full expression of all selenoproteins (2).

Two selenoproteins are present in plasma and have been used as representatives of all selenoproteins. The effect of selenium supplements on plasma glutathione peroxidase activity was assessed in a 1983 study carried out in Chinese subjects with a selenium intake of 11 μg/d (9). More recently, a similar study that included selenoprotein P was carried out in New Zealand in subjects with intakes averaging 28 μg/d (3). An Institute of Medicine panel used the results of these studies to establish the selenium RDA (2). The 2 studies had several limitations, however. One was that both administered selenium only in the form of selenomethionine and thus did not assess inorganic selenium, which is commonly used for supplements. Another was that the New Zealand study began from such a high basal selenium intake that distinctions between the levels of selenium administered could not always be made.

The present study was undertaken to determine the amount of selenium needed to optimize the 2 plasma selenoproteins. Subjects with a very low selenium intake were supplemented with

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selenomethionine, selenite, or placebo. The study provides additional data on which selenium requirements can be based and allows conclusions about the relative bioavailability of these forms of the element.

SUBJECTS AND METHODS

Study location

The study was carried out in Mianning County of Liangshan Prefecture in Sichuan Province. Mianning County (population 320 202 in 2001) is in a selenium-deficient region of China where Keshan disease is endemic. This county was the site of the 1974–1976 placebo-controlled trial that demonstrated the effectiveness of selenium in prevention of Keshan disease (5). Between mid-1983 and 1993, local salt was supplemented with selenite, which provided a calculated average intake of 50 μg Se/d (10). The number of cases of Keshan disease decreased from 98 in 1983 to 7 in 1984 and 3 or fewer each year thereafter through 1993 (11). In 1987 we used subjects from Mianning County as the selenium-replete control subjects for a selenium supplementation study performed in Liangshan Prefecture (12, 13).

Selenite addition to salt consumed in Mianning County was discontinued after 1993, and Keshan disease reappeared. There were 4–9 cases annually from 1994 to 1998. Thirteen cases were reported in 1999 and 7 in 2000 (11). Thus, residents of Mianning County have become selenium deficient once again. The present study was carried out between February and August of 2001 under these selenium-deficient conditions.

Subjects

Male and female farmers residing in Huiping village (population 12 377), which is located 2 km from Mianning City, were notified that a selenium supplementation study would be performed in healthy subjects and that study participants would be paid. Screening of the 174 subjects aged $\geq$18 y who responded consisted of a physical examination (performed by a doctor from the Anti-Epidemic Station of Mianning), an electrocardiogram, and a chest X-ray. The study protocol was explained in detail to the 155 subjects found to be healthy, and 120 subjects volunteered for the study.

Dietary selenium intake

A nutrition survey was carried out in 2000 in Huiping to determine its suitability for the study. Food-frequency questionnaires were administered, and selenium intakes were calculated by using a table of the selenium contents of foods from the Mianning area. The average selenium intake was determined to be 10 μg/d for adults.

The selenium intakes of the study subjects were calculated from whole blood and hair selenium concentrations by using formulas developed by Chinese investigators (14). The blood and hair samples were taken on the first day of the study before supplementation began.

Selenium supplements

Placebos and tablets containing sodium selenite and L-selenomethionine (henceforth referred to as selenomethionine) were compounded by T Bader of College Pharmacy (Colorado Springs, CO). All tablets contained microcrystalline cellulose, lactose 310 granulated (monohydrate), and stearic acid on a base composed of magnesium stearate, Di Tab (Rhodia Inc, Cranbury, NJ), and Primogel (FMC Corp, Philadelphia, PA). Selenomethionine was a gift of V Badmaev of Sabinsa Corp (Piscataway, NJ), and sodium selenite was purchased by College Pharmacy from Spectrum Pharmacy Products (Tucson, AZ). Tablets were specified to contain no added selenium or 15, 30, 45, 60, or 75 μg Se in each form. Thus, there were 11 batches of tablets. Ten tablets from each batch were assayed for selenium content in our lab, and 10 from each batch were assayed in an outside lab (Analytic Services Laboratory at South Dakota State University, Brookings, SD) by the method of Koh and Benson (15). Both analyses yielded similar results and were averaged. Tablet selenium contents ($\pm$SD; $n = 20$) for each batch were as follows (in μg): selenomethionine, 13 ± 0.8, 24 ± 1.7, 37 ± 4.9, 48 ± 4.0, and 61 ± 7.5; selenite, 15 ± 9.0, 31 ± 23, 52 ± 21, 47 ± 16, and 66 ± 23. Selenium was undetectable (<0.05 μg) in the placebo tablets.

Five bottles containing 28 tablets each were prepared for each subject. The placebo group was assigned 20 subjects, and each selenium group was assigned 10 subjects. Each set of 5 bottles was given a number from 1 to 120 by using shuffled cards. After the analyses had been finished and the code had been broken, some characteristics of the randomly assigned groups were examined. With the use of one-way analysis of variance with Scheffe's multiple-comparison procedure, no significant differences were found among the randomly assigned groups with respect to sex ($P = 0.88$), age ($P = 0.92$), body mass index ($P = 0.19$), plasma glutathione peroxidase ($P = 0.81$), selenoprotein P ($P = 0.76$), or plasma selenium ($P = 0.47$).

All data analyses used the determined tablet selenium values. Because 2 of the selenite groups (52 ± 21 and 47 ± 16 μg) were indistinguishable, they were combined for analysis. This results in selenite having one less group than selenomethionine. After all analyses had been completed and before the code was broken, the initial plasma selenium concentration of one subject was noted to be >2 SDs higher than the mean of the group. That subject was deleted from the analysis. The deleted subject was a male in the placebo group.

Protocol

The subjects were brought from their village to the Anti-Epidemic Station in Mianning City for initiation of the study and 5 more times at 28-d intervals for blood sampling. A hair sample was obtained at the initial visit. Subjects were assigned numbers 1 through 120 and were administered the tablets that had been assigned to the same number. The tablets were administered under the supervision of a staff member of the Anti-Epidemic Station. This staff member supervised 4 residents of Huiping village, who each personally administered the appropriate tablets to 30 subjects each morning.

Blood (20 mL) was sampled by venipuncture at each visit before the daily study tablet was administered. Blood was treated with 1 mg disodium EDTA/mL to prevent coagulation, and plasma was separated by centrifugation at $2000 \times g$ for 15 min at 4 °C. Plasma samples were frozen at liquid nitrogen temperature until shipment to Nashville, TN, on dry ice. On receipt in Nashville, the samples were stored at -70 °C until analyzed. The protocol was approved by the Vanderbilt University Institutional Review Board and the Ethical Review Committee of the Institute of Nutrition and Food Hygiene, Chinese Academy of Preventive Medicine in Beijing.

Assays

Plasma selenoprotein P was measured by radioimmunoassay with a reference plasma as the standard (13). The same reference plasma was later assayed by using a newly developed enzyme-linked immunosorbent assay and was determined to contain 5.8 ± 0.8 mg selenoprotein P/L plasma (n = 8; results not shown), which agrees well with results reported elsewhere (16). This allowed the selenoprotein P concentrations in the plasma of the subjects to be reported in mg selenoprotein P/L plasma.

Plasma glutathione peroxidase activity was measured by using hydrogen peroxide as the substrate (12). One unit is 1 μmol NADPH oxidized · min^{-1} · mL^{-1}. Selenium was measured fluorometrically (15, 17).

Sample size

The sample size required to detect a statistically significant difference at 20 wk was predetermined on the basis of our previous work (12). We estimated that in the participants with the lowest dose that provided saturation of plasma selenium, the mean would be 68 μg/L with an SD of 12 μg/L. For the next lowest dose, we were interested in detecting plasma selenium concentrations ≤50 μg/L. A sample size of 9 in each group was required to detect a statistically significant difference with a two-tailed alpha of 0.05 and 80% statistical power. To account for a potential dropout rate of 10%, we estimated that a total of 10 participants would be needed in each of the 6 arms of this study. These sample size calculations were performed with the software package NQUERY ADVISORY version 3.0 (18).

Statistical analysis

A Pearson chi-square test or Fisher's exact test was used to assess categorical comparisons among groups. Differences in the mean values of glutathione peroxidase activity, selenium concentration, and selenoprotein P concentration were compared among the randomly assigned groups by using a one-way analysis of variance with Scheffe's multiple-comparison procedure. Before-after comparisons were analyzed with Wilcoxon's signed-rank test. A general linear model repeated-measures analysis of variance was used to assess changes from baseline between groups. To determine whether there was a plateau for glutathione peroxidase and selenoprotein P, we tested for nonlinearity in the relation between the dose of the supplement and the square of these terms. For those with a significant nonlinearity, a general linear model with a Helmert contrast was used. This contrast compares level 1 (placebo) with the mean of all higher doses, then level 2 with the mean of all higher doses. The breakpoint was identified as the first dose for which the mean values of the higher doses was no longer statistically significant. P values < 0.05 were considered significant, and all tests were two-tailed. Statistical analyses were performed on a personal computer with the statistical package SPSS for WINDOWS (version 11.5; SPSS Inc, Chicago) and the statistical language R (Internet: www.r-project.org; accessed 18 June 2004).

RESULTS

Initial selenium status

Some characteristics of the subjects are shown in **Table 1**. Body mass index averages were in the normal range for both men and women. Selenium biomarker values in the initial plasma sample are shown in **Table 2**. The mean plasma selenium concentration of all study subjects, 22 ± 7 μg/L, was 18% of the US mean serum selenium concentration (19). Glutathione peroxidase activity was ≈40% of values in the United States (RF Burk and KE Hill, unpublished observations, 2004), and the selenoprotein P concentration was 23% of the US standard. Both selenoprotein measurements were slightly higher in men than in women.

TABLE 1
Characteristics of the study subjects[1]

	Women (n = 55)	Men (n = 64)
Weight (kg)	53.3 ± 8.5	58.4 ± 5.5
BMI (kg/m^2)	22.4 ± 3.5	21.4 ± 2.0

[1] All values are $\bar{x}$ ± SD. Values for weight ($P < 0.001$) but not BMI ($P = 0.133$) differed significantly between the men and the women (Mann-Whitney U test).

Whole blood and hair selenium concentrations were measured (data not shown) so that dietary intake of selenium could be estimated (14). That estimate was 9 μg/d for women and 11 μg/d for men, which agrees with the results of the nutrition survey carried out in Huiping in 2000. Thus, the subjects were selenium deficient because they ate low-selenium food.

Response of plasma selenoproteins to selenium supplements

The 2 plasma selenoproteins were assessed at intervals of 4 wk during the 20 wk of supplementation. The response of glutathione peroxidase activity to the selenomethionine supplements is shown in **Figure 1**. Glutathione peroxidase activity responded briskly for 12 wk in the 3 groups receiving the highest selenium supplements and then appeared to reach a common plateau that was equivalent to values obtained in US subjects. The values of the 2 groups receiving lower amounts of selenium appeared to drift upward during the entire 20 wk but remained at lower levels. Values in the placebo group did not change significantly during the study.

Making the assumption that the 16-wk and 20-wk values would approach a steady state that could be expected for each level of supplementation, we averaged those values for each supplementation group to construct dose-response curves for the 2 plasma selenoproteins. The response of plasma glutathione peroxidase activity to the selenium supplements is shown in **Figure 2**. When selenium was supplemented in the form of selenomethionine, maximum enzyme activity was reached with a supplement dose of 37 μg/d. When the supplement was selenite, a dose of 66 μg was required to reach the same maximum

TABLE 2
Initial plasma selenium status of the study subjects[1]

	Women (n = 55)	Men (n = 64)
Selenium (μg/L)	21.0 ± 6.8	23.2 ± 7.7
Glutathione peroxidase (U/L)	36.7 ± 18.7	49.1 ± 21.7
Selenoprotein P (mg/L)	1.2 ± 0.4	1.5 ± 0.5

[1] All values are $\bar{x}$ ± SD. Values for both selenoproteins ($P < 0.001$) but not for the plasma selenium concentration ($P = 0.133$) differed significantly between the men and the women (Mann-Whitney U test).

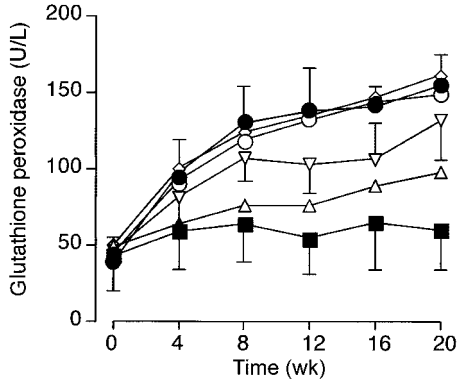

FIGURE 1. Mean (±SD) plasma glutathione peroxidase activity in subjects supplemented with selenomethionine for 20 wk. Groups are as follows: placebo, ■, $n = 19$; 13 μg Se, △, $n = 10$; 24 μg Se, ▽, $n = 10$; 37 μg Se, ○, $n = 10$; 48 μg Se, ◇, $n = 10$; and 61 μg Se, ●, $n = 10$.

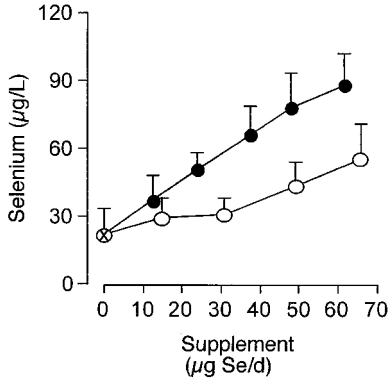

FIGURE 3. Response of the plasma selenium concentration to selenium supplementation. Values are the mean (±SD) of the 16-wk and 20-wk selenium concentrations of each group: placebo group, circle with x, $n = 19$; selenomethionine groups, ●, $n = 10$ per group; and selenite groups, ○, $n = 10$ per group except $n = 20$ in the 49 μg group.

level. Thus, selenium in the form of selenomethionine was almost twice as effective as selenium in the form of selenite in supporting plasma glutathione peroxidase activity.

The response of the selenoprotein P concentration to selenium supplementation, which is also shown in Figure 2, differed in nature from the response of glutathione peroxidase activity. Whereas the response of glutathione peroxidase activity was direct and proportional to selenium intake, the response of selenoprotein P was sigmoidal, with a steep response being flanked by more gradual responses. The steep rise gave way to a gradual rise above 30 μg Se as selenomethionine. However, the response curve appeared to continue to rise with subsequent supplement increases, suggesting that a maximal value had not been reached. Moreover, the highest average selenoprotein P concentration reached in this study was only 75% of the standard US value. Therefore, optimization of selenoprotein P will require either a longer period of supplementation or higher doses of selenium than were used in this study. As with glutathione peroxidase, selenium in the form of selenomethionine was more effective than selenium in the form of selenite in causing an increase in selenoprotein P.

Response of plasma selenium to selenium supplements

Plasma selenium concentrations, averaged for weeks 16 and 20, are shown in **Figure 3**. Selenomethionine supplementation raised plasma selenium concentrations more than did selenite supplementation. This result was expected because selenomethionine raises selenoproteins better than does selenite, as shown in Figure 2, and is also randomly incorporated into proteins at methionine sites (20). The latter property complicates the use of the plasma selenium concentration as a biomarker, because selenomethionine is the major form of selenium in most diets.

DISCUSSION

The present study showed that the 2 plasma selenoproteins exhibit different patterns of response to selenium supplementation of deficient subjects. Glutathione peroxidase activity rose in direct proportion to the amount of selenium supplemented until it reached its optimal value (Figure 2). Selenoprotein P, on the other hand, rose in a sigmoidal pattern and never reached its optimal value (Figure 2). Thus, a selenium requirement can be

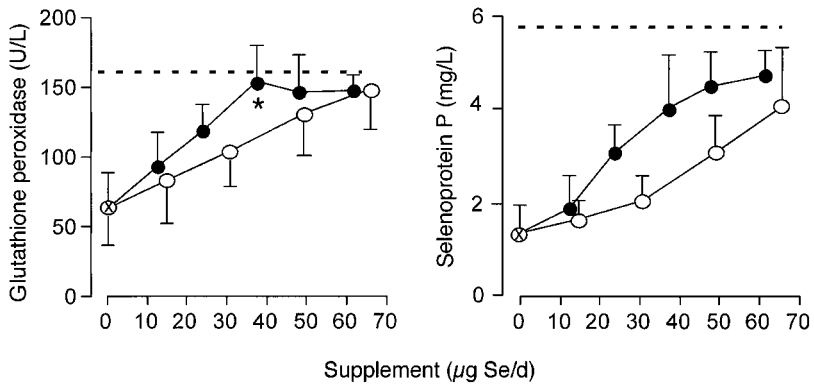

FIGURE 2. Response of plasma selenoproteins to selenium supplementation. Values are the mean (±SD) of the 16-wk and 20-wk values of each group: placebo group, circle with x, $n = 19$; selenomethionine groups, ●, $n = 10$ per group; and selenite groups, ○, $n = 10$ per group except $n = 20$ in the 49 μg group. The asterisk indicates the highest dose that is significantly different from the immediately lower dose. The broken lines indicate the glutathione peroxidase (left panel) and selenoprotein P (right panel) US (selenium-replete) values for our laboratory.

calculated by using the glutathione peroxidase data but not by using the selenoprotein P data.

The optimization of plasma glutathione peroxidase activity by selenomethionine agrees well with the study carried out in 1983 in China (9) and with a reinterpretation of the data of the New Zealand study (2). The present study achieved optimization with 47 μg Se/d, whereas 41 μg/d was required in the earlier Chinese study and 38 μg/d in the New Zealand study. Thus, use of the present glutathione peroxidase results would require little adjustment of the US RDA, which was based on those 2 earlier studies.

Because glutathione peroxidase and selenoprotein P responded differently to selenium supplementation (Figure 2), factors that influence their plasma concentrations must be considered. The source of plasma glutathione peroxidase is almost entirely kidney tubule cells (21). Therefore, plasma glutathione peroxidase appears to be a biomarker for selenium in the kidney.

Plasma selenoprotein P has many sources (22). Work in rats suggested that ≈75% originates in the liver (23; T Kato and RF Burk, unpublished data, 1991) with the rest originating in other tissues, including muscle. Thus, it seems likely that the complex shape of the selenoprotein P dose-response curve (Figure 2) is related to the sequential filling of selenium pools in different tissues of the body. Moreover, it suggests that not all pools were filled in the present study, which used 20 wk of supplementation. The other study in which selenoprotein P was measured achieved optimization at 20 wk with 40 μg Se/d (3). However, the baseline selenoprotein P in that study was 60% of the optimal value instead of 23% as in the present study, which indicates that selenium repletion should be more easily accomplished in the New Zealand subjects than in the deficient Chinese subjects. On the basis of these considerations, a study of greater length that also uses higher-selenium supplements is needed to determine the amount of selenium required to optimize selenoprotein P in selenium-deficient subjects. It appears likely that a higher selenium intake will be required to optimize selenoprotein P than to optimize glutathione peroxidase. Such a result would indicate that selenoprotein P is a better biomarker for whole-body selenium than is glutathione peroxidase and should lead to a reconsideration of the US RDA for selenium.

An important finding of the present study is that the bioavailability of selenium supplemented as selenomethionine, the major form of selenium found in food, is almost twice that of selenium supplemented as selenite (Figure 2). This finding explains our observation in a 1987 study that subjects in Mianning receiving ≈50 μg Se/d, most of which was selenite added to salt, did not have optimized glutathione peroxidase (12, 13). We conclude that selenium supplements given for nutritional purposes need to be larger when selenite is given than when selenomethionine is given.

Plasma selenium concentrations as shown in Figure 3 are often used to assess selenium nutritional status. Plasma selenium comprises the selenium in the 2 selenoproteins and the selenium present as selenomethionine (20). When optimized, the 2 selenoproteins contain ≈80 μg Se/L plasma (13). Therefore, plasma selenium concentrations below that value indicate that the selenoproteins are not optimized. Values >80 μg/L indicate that selenomethionine is present in proteins such as albumin. The subjects receiving selenite in the present study never achieved optimization of selenoprotein P, and, consequently, their plasma selenium concentration reached a maximum of 52 μg Se/L (Figure 3). Those receiving selenomethionine reached 88 μg Se/L despite not achieving optimization of their selenoprotein P. The reason for this false positive result of the plasma selenium concentration is that administration of selenomethionine led to incorporation of that form of selenium into albumin and other plasma proteins, raising the plasma selenium content. This demonstrates that knowledge of the form of selenium ingested is needed to assess plasma selenium values.

In conclusion, the present study showed that the 2 plasma selenoproteins are not equivalent as biomarkers of selenium nutritional status. Selenoprotein P appears to be better than glutathione peroxidase for this purpose. Although additional work is needed to establish a precise quantity, it appears almost certain that use of selenoprotein P to determine the selenium requirement of humans would result in the present values being raised. Also, our study shows that the bioavailability of organic and inorganic forms of selenium vary by almost 2-fold. This fact must be considered when nutritional supplements of selenium are being formulated.

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YX participated in the design of the study and organized and managed the study. KEH participated in the design of the study and supervised the laboratory analyses in Nashville. DWB provided statistical assistance in the design of the study and in its interpretation. JX supervised the study in Mianning. RFB participated in the design of the study and the preparation of the manuscript. None of the authors had any financial or personal relations with the National Institutes of Health other than as grantees and none has such relations with the Sabinsa Corporation.

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