Effects of Chemical Form of Selenium on Plasma Biomarkers in a High-Dose Human Supplementation Trial

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Abstract

Intervention trials with different forms of selenium are under way to assess the effects of selenium supplements on the incidence of cancer and other diseases. Plasma selenium biomarkers respond to selenium administration and might be useful for assessing compliance and safety in these trials. The present study characterized the effects of selenium supplementation on plasma selenium biomarkers and urinary selenium excretion in selenium-replete subjects. Moderate (–200 μg/d) to large (–600 μg/d) selenium supplements in the forms sodium selenite, high-selenium yeast (yeast), and l-selenomethionine (selenomethionine) were administered. Subjects were randomized into 10 groups (placebo and three dose levels of each form of selenium). Plasma biomarkers (selenium concentration, selenoprotein P concentration, and glutathione peroxidase activity) were determined before supplementation and every 4 weeks for 16 weeks. Urinary selenium excretion was determined at 16 weeks. Supplementation with selenomethionine and yeast raised the plasma selenium concentration in a dose-dependent manner. Selenite did not. The increased selenium concentration correlated with the amount of selenomethionine administered. Neither glutathione peroxidase activity nor selenoprotein P concentration responded to selenium supplementation. Urinary selenium excretion was greater after selenomethionine than after selenite, with excretion after yeast being intermediate and not significantly different from either of the other two. We conclude that plasma selenium concentration is useful in monitoring compliance and safety of selenium supplementation as selenomethionine but not as selenite. Plasma selenium seems to reflect the selenomethionine content of yeast but not the other yeast selenium forms. As judged by urinary selenium excretion, selenium in the form of selenomethionine is better absorbed than selenite. (Cancer Epidemiol Biomarkers Prev 2006;15(4):804–10)

Introduction

In 1996, Clark et al. reported that a selenium supplement of 200 μg/d, administered to prevent a second nonmelanoma skin cancer, reduced the risk of cancers of the prostate, lung, and colon (1). In response to that report, additional intervention trials have been initiated to assess the effectiveness of selenium as a cancer chemoprevention agent (2).

Several forms of selenium are available for use in chemoprevention trials. l-Selenomethionine (selenomethionine), which was chosen for use in the Selenium and Vitamin E Cancer Prevention Trial, is synthesized by plants and consequently is the major form of selenium in the human diet. High-selenium yeast, a type of which was used in the Clark et al. study, is produced by growing yeast in a high-selenium medium. Analyses of high-selenium yeast indicate that the major chemical form of selenium in it is selenomethionine, with several other compounds making up the remainder (3). Inorganic selenium in the form of selenite or selenate has been used successfully in animal chemoprevention studies.

The most frequently used dose of selenium in intervention trials is 200 μg/d. North Americans taking such a supplement will not exceed the safe upper limit of 400 μg/d set by the Institute of Medicine (4). However, higher selenium doses that might be toxic are sometimes employed in trials (5) and monitoring for selenium toxicity and compliance is needed in all studies of selenium administration regardless of the selenium dose.

Three biomarkers in plasma are used to assess selenium nutritional status and to predict toxicity. Two of them are plasma selenoproteins (selenoprotein P and glutathione peroxidase-3). Plasma levels of these selenoproteins are used primarily as nutritional biomarkers of the element. Their concentrations are depressed in selenium deficiency but increase with increasing selenium supplementation until reaching plateaus at levels determined by genetic and environmental factors (6-8). The rationale for their status as nutritional biomarkers is that they serve as representatives of all the selenoproteins in the body.

The third biomarker is plasma selenium. It consists of selenium in the forms of selenocysteine in the two selenoproteins and selenomethionine present at methionine positions in all proteins plus small-molecule forms that contribute <3% of the total (9, 10). Selenomethionine substitution for methionine seems to be a random process that is dependent on the availability of selenomethionine (9). Thus, these three biomarkers all respond to selenium intake, but each responds differently and yields different information.

At present, the recommended dietary allowance for North American adults is 55 μg selenium/d based on the intake required to optimize plasma glutathione peroxidase activity (4). Typical selenium intakes in North America are 80 to 140 μg/d. Intakes in many parts of the world, however, are lower largely due to low soil selenium levels. The lowest reported intakes of selenium are in some regions of China where people consume ≤10 μg/d (8, 11). Intakes in Europe and New Zealand range from ≤30 to 60 μg/d. Clearly, people in some parts of the world do not meet their selenium requirements.

To use plasma biomarkers for assessing selenium status and to maintain safety during selenium supplementation trials, knowledge of how the different supplements affect the biomarkers is needed. We conducted a randomized, placebo-controlled trial in which healthy North Americans were
supplemented with organic and inorganic forms of selenium in daily selenium doses up to ~600 µg. The effects of this supplementation on the plasma selenium biomarkers are reported here.

**Subjects and Methods**

**Subjects.** Volunteers ages ≥18 years were recruited at Vanderbilt University Medical Center between February and July 2003. Respondents to advertisements were eligible if they were in good health, were neither pregnant nor planning a pregnancy, were not taking a selenium supplement of >25 µg/d, and had not been diagnosed with liver disease. Eighty-eight subjects were enrolled after informed consent was obtained. The Vanderbilt Institutional Review Board approved the protocol. Subjects were compensated for participation.

**Selenium Supplements.** Selenite was purchased from Kelatron Corp. (Ogden, UT) as sodium selenite. Dr. V. Badmaev (Sabinsa Corp., Piscataway, NJ) provided selenomethionine and Paul A. Willis (Cypress Systems, Inc., Fresno, CA) provided SelenoExcel High-Selenium Yeast. This yeast preparation contained ~1,200 µg selenium/g and was estimated, based on proprietary analyses, to have ~75% of its organic selenium in the form of selenomethionine.3

Capsules ordered as placebos or to contain 200, 400, and 600 µg selenium of each form were made by Integrative Therapeutics, Inc. (Wilsonville, OR). All capsules contained Fast-Flo Edible Lactose (Foremost Farms, Baraboo, WI) as the filler. Capsules were assayed for selenium in our laboratory and the results are mean ± SD (n = 3). Selenite capsules contained 202 ± 30, 380 ± 56, and 601 ± 49 µg selenium; selenomethionine capsules contained 158 ± 17, 338 ± 34, and 507 ± 25 µg selenium; and yeast capsules contained 226 ± 16, 439 ± 27, and 703 ± 48 µg selenium. Placebos contained only the filler and their selenium content was not detectable (<0.05 µg).

**Protocol.** The study was a randomized, placebo-controlled intervention. After enrollment, subjects were admitted as inpatients to the General Clinical Research Center at Vanderbilt University Medical Center.

Research nurses recorded height, weight, and medical histories of each participant. Subjects stayed overnight at the General Clinical Research Center. After an overnight fast, 20 mL blood was taken by venipuncture and processed. Before discharge, subjects were given the study capsules and instructed to take one capsule each morning with food beginning that morning.

Participants returned for outpatient visits after weeks 4, 8, 12, and 16 for fasting blood sampling and to exchange their pill bottle for a new one. Compliance was measured by counting capsules remaining in the bottle and by questioning the participants. At each outpatient visit, subjects completed a brief questionnaire about how they were tolerating the study capsules and, in particular, whether they had noticed any hair loss or changes in nail condition, menstrual cycle, appetite, or gastrointestinal function.

The study coordinator phoned participants midway between appointments to encourage compliance and to remind subjects to fast overnight before their appointments. Subjects completed a 24-hour urine collection just before their final blood sampling in the General Clinical Research Center.

**Sample Collection.** At each clinic visit, blood was sampled by venipuncture after an overnight fast. Fifteen milliliters were collected in tubes containing EDTA as an anticoagulant. Plasma was separated and stored at ~70°C. An additional 5 mL blood was collected into a tube without EDTA. Serum was separated for albumin determination.

Participants voided their first morning urine and then collected all urine for the following 24 hours. At the end of the collection period, the total volume of urine was recorded for each individual. Each urine sample was mixed and aliquots were stored at ~70°C.

**Assays and Calculations.** Albumin concentration was determined in serum at the Vanderbilt Clinic laboratory using the bromcresol green method (12). Glutathione peroxidase activity was determined in plasma using the assay we have employed in other human studies (13). The substrate was 0.25 mmol/L H₂O₂.

Plasma and urine selenium concentrations were measured using a fluorometric assay (14, 15). Our standard human plasma sample (16) was included in every assay to assess reproducibility. Based on 23 assays, this standard plasma had a selenium concentration of 121 ± 5 µg/L. The 24-hour urine selenium excretion was calculated by adjusting the urine selenium concentration to reflect the total urine volume.

Selenoprotein P was measured using a sandwich ELISA with two monoclonal antibodies, N22 and N11, which were provided by Dr. Takeshi Naruse (Kaketsuken, Kumamoto, Japan). Each antibody recognizes a specific binding site in the NH₂-terminal region of selenoprotein P. The capture antibody, N22, was incubated on 96-well microtiter plates overnight at 4°C. The plates were washed with a solution containing Block Ace (Dainippon Pharmaceuticals, Osaka, Japan) to block remaining binding sites. Coated plates were stored at ~20°C until needed. Plasma samples were prepared in two dilutions and each dilution was measured in duplicate. Our human standard plasma was included on each plate. The diluted plasma was combined with the biotinylated antibody, N11. Standard curves that ranged from 0.15 to 5 ng/mL were constructed using purified human selenoprotein P, which was also a gift of Dr. Takeshi Naruse (Kaketsuken). The sample or standard solution was then added to the N22-coated wells of the ELISA plate and incubated at 37°C for 1 hour. Each well was washed and incubated for an additional hour with streptavidin-horseradish peroxidase at 37°C. Again, the plate was rinsed, and 3,3′,5,5′-tetramethylbenzidine base, a substrate of horseradish peroxidase, was added to the plate. The plate was incubated in the dark for 30 minutes. H₂SO₄ (0.5 mol/L) was added to each well to stop the reaction. Absorbance at 450 nm was measured in each well and the selenoprotein P concentration of the plasma (µg/mL) was calculated for each sample using the standard curve. Based on 56 readings, our human standard plasma was measured to have a mean selenoprotein P concentration of 5.5 ± 0.8 mg/L. These assay results of the standard plasma show the reproducibility of the selenium and selenoprotein P assays.

“Other” selenium is all the plasma selenium not in the selenoproteins. “Other” selenium was determined by calculation. We calculated the selenium content of each selenoprotein by expressing plasma selenoprotein P and glutathione peroxidase concentrations as a fraction of their respective averages in selenium-replete individuals: 5.5 µg/L for selenoprotein P (derived from analysis of the human standard plasma) and 159 units/L for glutathione peroxidase (the initial value in this study of selenium-replete subjects). This fraction was then multiplied by the respective concentrations of selenium previously determined to be present within each selenoprotein in selenium-replete subjects (64 µg/L for selenoprotein P and 17 µg/L for plasma glutathione peroxidase; refs. 16, 17). We calculated “other” selenium by subtracting the selenium content of the two selenoproteins from the total selenium.

3 P.A. Willis, personal communication.
present in the plasma (18): “Other” selenium (µg/L) = plasma selenium (µg/L) – [glutathione peroxidase (units/L/159 units/L)] × 17 µg/L – [selenoprotein P (mg/L/5.5 mg/L) × 64 µg/L].

Creatinine was measured in the urine by an established method (19) adapted to a 96-well plate. The amount of creatinine excreted in 24 hours was determined by multiplying creatinine concentration by total urine volume.

**Compliance and Withdrawals.** Eighty-two participants completed the study. Two participants withdrew from the study for reasons unrelated to the capsules and four due to the odor of the capsules or minor gastrointestinal complaints. One subject’s data were excluded due to noncompliance, bringing the final number of subjects analyzed to 81. Their compliance was estimated to be 98.2% with a range of 80.4% to 100%.

**Sample Size and Statistical Analysis.** Based on our previous work,4 we estimated that to detect a difference in the mean plasma selenium levels among groups with different forms of selenium supplementation a sample size of 10 in each group would have 92% power to detect a difference in means of 41 µg/L (the difference between the 200 µg supplement group mean of 170 µg/L and placebo group mean of 129 µg/L) assuming a common SD of 28 using a two-group t test with a 0.05 two-sided significance level. Subjects were randomized within blocks of 22, with 4 placebo and 2 in each of the 9 supplement groups. Therefore, the initial estimate was for 110 subjects total. Due to practical and financial constraints, a decision was made to end the study after the 88th patient (the fourth block of 22). This decision was made while the data were blinded and before any analyses.

A Pearson χ² test or Fisher’s exact test was used to assess categorical comparisons between groups. Differences between group means for continuous measurements were tested by the Student’s t test or the Mann-Whitney U test. Comparisons among the four treatment groups were assessed with a one-way ANOVA or a Kruskal-Wallis test. The Student’s Newman-Keuls and Tukey tests were used for multiple comparison procedures. Before-after comparisons were analyzed by the paired t test or the Wilcoxon signed-rank test. A general linear model repeated-measures ANOVA was employed to assess changes from baseline between groups. Correlations were tested with both the Spearman rank-correlation test and the Pearson correlation. Linear regression models were used to produce best-fit lines and equations for relationship between the dose and selenium levels. Ps < 0.05 were considered statistically significant and all tests were two-tailed. Continuous variables are mean ± SD unless otherwise indicated.

**Results**

Participant characteristics are displayed in Table 1. Seventy-three percent of the subjects were female. Males and females were not significantly different in age, body mass index, or albumin concentration.

**Plasma Selenium Biomarkers before Supplementation.** The initial plasma selenium biomarkers are presented in Table 2. The mean plasma selenium concentration was similar to the mean U.S. serum selenium value of 125 µg/L determined in the Third National Health and Nutrition Examination Survey (20). That value is considered to indicate a selenium-replete status. Therefore, the values determined for the two selenoproteins can be predicted to be optimal (or saturated) values. Of the selenium in plasma, 64% was calculated to be in selenoproteins; therefore, 36% was “other” selenium, presumably mostly selenomethionine in albumin and other plasma proteins (9, 21).

Comparison of the biomarkers with one another (Table 3) revealed that neither selenoprotein correlated with plasma selenium nor did they correlate with each other. Therefore, although selenium biomarkers correlate with one another in selenium-deficient individuals (8, 13, 16), they did not in these selenium-replete individuals.

**Plasma Selenium Biomarkers during Supplementation.** Plasma selenium concentration increased markedly with selenomethionine and yeast supplementation but much less with selenite supplementation (Fig. 1A). The largest increase occurred in the first 4 weeks of supplementation. By week 16, the plasma selenium level seemed to have been approaching a plateau. Supplementation with 158, 338, and 507 µg selenium as selenomethionine caused plasma selenium concentration to increase significantly (P < 0.05) above the placebo value by 71%, 108%, and 178%, respectively. Supplementation with 226, 439, and 703 µg selenium as yeast caused plasma selenium concentration to increase significantly (P < 0.05) by 46%, 108%.

### Table 1. Characteristics of subjects

<table>
<thead>
<tr>
<th></th>
<th>Women</th>
<th>Men</th>
<th>P</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. subjects in study</td>
<td>59</td>
<td>22</td>
<td>81</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>35.8 ± 10.0 (59)</td>
<td>37.1 ± 10.6 (22)</td>
<td>0.63</td>
<td>36.2 ± 10.1</td>
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<td>Weight (kg)</td>
<td>74.7 ± 18.8 (38)</td>
<td>82.6 ± 11.7 (21)</td>
<td>0.01</td>
<td>76.8 ± 17.5</td>
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<tr>
<td>Body mass index</td>
<td>27.6 ± 6.5 (58)</td>
<td>26.2 ± 3.5 (21)</td>
<td>0.76</td>
<td>27.2 ± 5.9</td>
</tr>
<tr>
<td>Albumin (g/L plasma)</td>
<td>39 ± 4 (48)</td>
<td>40 ± 4 (19)</td>
<td>0.49</td>
<td>39 ± 4</td>
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</table>

**NOTE:** Data are mean ± SD (n). Values for women and men were compared by the Mann-Whitney test.

### Table 2. Initial plasma selenium biomarkers

<table>
<thead>
<tr>
<th>Selenium (µg/L)</th>
<th>Women</th>
<th>Men</th>
<th>P</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selenoprotein P (mg/L)</td>
<td>5.2 ± 1.0 (59)</td>
<td>5.7 ± 0.7 (22)</td>
<td>0.02</td>
<td>5.3 ± 0.9</td>
</tr>
<tr>
<td>Glutathione peroxidase</td>
<td>154 ± 28 (49)</td>
<td>176 ± 41 (13)</td>
<td>0.13</td>
<td>159 ± 32</td>
</tr>
<tr>
<td>“Other” selenium</td>
<td>45 ± 16 (49)</td>
<td>39 ± 12 (13)</td>
<td>0.22</td>
<td>44 ± 15</td>
</tr>
</tbody>
</table>

**NOTE:** Data are mean ± SD (n). Values for men and women were compared by the Mann-Whitney test.

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4 R.F. Burk et al., unpublished results.
and 182%, respectively. In contrast, the two lower doses of selenium as selenite (202 and 380 μg) did not raise plasma selenium significantly. The highest dose (601 μg) raised plasma selenium after 16 weeks only by 26% (P < 0.05). The plasma selenium concentration of the placebo group did not change over the 16 weeks of supplementation (P = 0.63, repeated-measures ANOVA).

The other two plasma selenium biomarkers, glutathione peroxidase and selenoprotein P, showed no tendency to increase during the study in any of the treatment groups (Fig. 1B and C). This lack of response to the selenium supplements confirms that the study subjects were selenium replete before supplementation was begun. Because selenoprotein concentration was not changed by the supplements, the increase observed in total plasma selenium concentration was caused by enlargement of the nonspecific selenium pool designated as "other" selenium.

To allow comparison of the forms of selenium administered, the plasma selenium concentration achieved after 16 weeks of supplementation was compared with selenium dose adjusted for body weight (Fig. 2). Each form of selenium raised the plasma selenium with a different efficiency. Slopes of the best-fit lines were selenomethionine > yeast > selenite. Setting the selenomethionine slope at 100%, the yeast slope was 73% and the selenite slope was 11%. This gives an indication of the relative increases in plasma selenium concentration that were achieved with each form of the element.

### Urinary Selenium Excretion after 16 Weeks of Supplementation

Excretion of selenium in the urine by the placebo group was 55 ± 22 μg/24 hours (n = 13). Selenium is also lost from the body in the stool and in sloughed skin cells. Thus, the average daily selenium loss, and therefore intake (because this group was under steady-state conditions), was above the recommended dietary allowance of 55 μg. This is another confirmation of the selenium-replete status of the subjects.

Urinary selenium excretion after 16 weeks of supplementation is shown in Fig. 3. When corrected for placebo excretion level and expressed as percentage of administered selenium, the percentage of the dose excreted did not vary significantly between the doses of a given form of the element (data not shown). However, it differed (P < 0.05) between the forms selenomethionine (60 ± 26%; n = 26) and selenite (41 ± 15%; n = 20). Percent urinary excretion of selenium administered as yeast was 52 ± 23% (n = 18), which was not significantly different from percent excretion of either of the other forms of selenium. Thus, a greater fraction of the dose of selenium administered was excreted in the urine when the form was selenomethionine than when the form was selenite.

The slope of each best-fit line in Fig. 3 is another indicator of the fraction of administered selenium that was excreted in urine. Setting the line-of-best-fit slope of the selenomethionine group at 100%, the slope of the yeast group was 77% and that of the selenite group was 60%. The urinary excretion results

### Table 3. Correlations among initial plasma selenium biomarkers

<table>
<thead>
<tr>
<th>Plasma biomarkers</th>
<th>All subjects</th>
<th>Subjects in lowest tertile of plasma selenium*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>R²</td>
</tr>
<tr>
<td>Selenium vs selenoprotein P</td>
<td>81</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Selenium vs glutathione peroxidase</td>
<td>62</td>
<td>0.03</td>
</tr>
<tr>
<td>Selenoprotein P vs glutathione peroxidase</td>
<td>62</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

**NOTE:** Correlations and P values are based on the Pearson correlation.

*Lowest tertile of baseline plasma selenium was defined as ≤116.24 μg/L.

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**Figure 1.** Plasma selenium biomarkers in subjects supplemented daily with selenium in three forms. -X-, placebo; -○-, - △-, and -□-, selenium doses as selenite (202, 380, and 601 μg/d, respectively); -○-, - △-, and -□-, selenium doses as selenomethionine (158, 338, and 507 μg/d, respectively); -○-, - △-, and -□-, selenium doses as yeast (226, 439, and 703 μg/d, respectively). A. Selenium concentrations (n = 6-8 with placebo n = 15). B. Plasma glutathione peroxidase activities (n = 4-7 with placebo n = 12). C. Plasma selenoprotein P concentrations (n = 6-8 with placebo n = 15). Points, mean; bars, 1 SD.
are compatible with supplemental selenium being absorbed to a greater extent in the form of selenomethionine than in the forms of yeast and selenite.

Discussion

Plasma Biomarkers before Selenium Supplementation. Like other healthy North Americans, the subjects were selenium replete when they entered this study. This conclusion is based on the initial plasma selenium concentration being above the level at which plasma selenoproteins are optimized (7, 8), the mean daily urinary selenium excretion by the placebo group indicating an intake greater than the recommended dietary allowance, and, most importantly, the lack of effect of selenium supplementation on the plasma selenoproteins (Fig. 1B and C). This last observation implies that the selenium administered as supplements was not needed to support the synthesis of these selenoproteins. Thus, the initial selenium biomarker values can be considered to indicate a selenium-replete state.

Epidemiology studies often rank subjects according to their plasma selenium concentrations (e.g., in tertiles). The incidence of pathologic conditions or responses to chemoprevention in each tertile is then determined. Table 3 shows that plasma selenium did not correlate with either selenoprotein in these selenium-replete subjects. Even when biomarkers of the lowest tertile of plasma selenium concentrations were compared with one another, there were still no correlations. This indicates that forming groups of selenium-replete subjects according to plasma selenium concentration will not constitute groups that differ in selenium nutritional status at least as indicated by plasma selenoprotein P or glutathione peroxidase. Thus, factors that lead to an increase in “other” selenium in plasma, such as consumption of plant foods rich in selenomethionine, need to be considered as the cause of differences in plasma selenium and as potential influences on any clinical events found to correlate with it.

Plasma Biomarkers and Forms of Selenium Administered. Selenium concentration was the only plasma selenium biomarker that was affected by supplementation (Fig. 1). Because supplementation did not cause an increase in selenoprotein P or glutathione peroxidase, the category of “other” selenium accounted for the increase. Figure 4 depicts some established relationships of ingested selenium forms with plasma forms of the element. “Other” plasma selenium comprises selenomethionine in albumin and other proteins and small-molecule forms, such as the selenosugars synthesized in the liver and bound for the urine. Small-molecule forms account for <3% of plasma selenium (22), indicating that the “other” selenium is mostly selenomethionine.

Forms, such as selenite and selenocysteine (in selenoproteins), enter directly into the selenium pool (Fig. 4). They cannot be converted to selenomethionine and therefore cannot enter the methionine pool and contribute to the “other” selenium. This accounts for the relative inability of selenite supplements to raise plasma selenium in this study (Fig. 1A).

Bioavailability of Selenium in the Supplements. An earlier study carried out by our group in selenium-deficient Chinese subjects indicated that selenium given in the form of selenomethionine had almost twice the bioavailability of
selenium given as selenite based on the biomarkers selenoprotein P and glutathione peroxidase (8). Those biomarkers cannot be used to estimate bioavailability in the present study because they were optimized when the study started and therefore did not respond to selenium supplementation. Neither can plasma selenium concentration be used here to estimate bioavailability because the forms administered had different characteristics of incorporation into plasma. This leaves only urinary excretion as an estimate of bioavailability (or absorption) of selenium in this study.

If the assumption is made that a steady state in the metabolism of selenium and selenomethionine existed after the 16 weeks of supplementation, urinary excretion of the element should have been proportional to absorbed selenium. Urinary excretion will actually be less than the amount of selenium absorbed because some endogenous selenium is lost in sloughed skin cells and in feces. More selenium was excreted in the urine when it was administered as selenomethionine than when it was administered as selenite. Therefore, we conclude that selenium was better absorbed, and consequently more bioavailable, when administered as selenomethionine than when given as selenite. This leads to the conclusion that selenium in the form of selenomethionine has greater bioavailability than selenium in the form of selenite. Selenium was excreted in the urine by subjects given selenomethionine (60%) than by subjects given selenite (41%), and similar results on urinary selenium excretion were obtained in the study carried out in China. When averaged, these findings suggest that absorption of selenite selenium is approximately two-thirds of the absorption of selenomethionine selenium. Animal studies have generally shown similar bioavailabilities of these two forms of selenium (23), but stable isotope studies in human beings have indicated that only ~50% of selenite selenium is absorbed (24) and >90% of selenomethionine selenium is absorbed (25). Thus, it seems reasonable to assign relative selenium bioavailabilities of 1.0 for selenomethionine and 0.64 (average of estimated relative absorption in this study and bioavailability in the China study) for selenite. This difference in bioavailabilities has implications for formulation of selenium supplements.

Selenite selenium is much less effective than selenomethionine selenium in raising plasma selenium concentration in selenium-replete individuals (Fig. 2). Part of this difference can be attributed to less efficient absorption, but the slope of plasma selenium concentration versus dose of supplementation for selenite is only 11% that of selenomethionine (Fig. 2). Thus, selenite acts as predicted by Fig. 4 in not being available to the methionine pool. This limits its plasma incorporation to the selenoproteins and the tiny small-molecule fraction.

The yeast preparation used contained an estimated 75% of its selenium as selenomethionine. Urinary excretion of selenium by this group was intermediate between the selenomethionine and the selenite groups (Fig. 3), suggesting that absorption of selenium in the form of yeast is greater than selenite selenium but less than selenomethionine selenium. Yeast was also intermediate between the other two forms in raising plasma selenium concentration (Fig. 2). The slope of selenium concentration versus dose of selenium as yeast was 73% that of selenomethionine. This value closely approximates the fraction of selenium estimated by the manufacturer to be selenomethionine in yeast. Both these findings are compatible with the minor forms of selenium in the yeast, having little effect on plasma selenium and perhaps not even being absorbed. Thus, additional work on yeast is needed to determine the absorption and metabolism of its different chemical forms of selenium.

Implications for Safe Upper Levels of Selenium Intake. Some of the subjects in this study ingested >800 µg selenium/d for 16 weeks. This is considerably more than the Institute of Medicine’s tolerable upper level of 400 µg/d (4). No signs of selenium toxicity (hair loss and nail changes) were observed, in agreement with Chinese observations in subjects with intakes of ~800 µg (26). Moreover, plasma selenium concentrations (Fig. 1A) were lower than levels determined in healthy farmers in Enshi County, an area in China with high selenium in the soil where selenium toxicity is not recognized now but was reported in the past (27).

Conclusions
This study of selenium-replete subjects compared the effects of supplementing three forms of selenium in moderate to high doses on selenium biomarkers. Plasma selenium concentration was responsive to the supplements in proportion to their

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5Y. Xia et al., unpublished observations.
selenomethionine content. We propose that the increment in plasma selenium concentration during supplementation can be used to estimate compliance and ensure safety of selenomethionine supplements given in chemoprevention studies.

Bioavailability of selenium, as estimated by urinary excretion after 16 weeks of supplementation, was greatest for selenomethionine, less for yeast, and least for selenite. Yeast contains selenium mostly as selenomethionine but has a significant amount of selenium in other forms. The results of this study are compatible with sharply differing fates of different yeast selenium forms.

Some of the subjects in this study had total daily intakes of over 800 μg selenium for 16 weeks. No signs of selenium toxicity were detected in them. Thus, we conclude that total intakes of selenium in the range of 800 μg/d can be used safely in studies of limited duration if the subjects are monitored closely for signs of selenium toxicity.

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