Selenium metabolism and platelet glutathione peroxidase activity in healthy Finnish men: effects of selenium yeast, selenite, and selenate

Georg Alfihan, Antti Aro, Heikki Arvilommi, and Jussi K Huttunen

ABSTRACT The mean dietary selenium intake in Finland increased from 40 to 100 μg/d in 1987 because of the addition in 1985 of selenium to fertilizers. A selenium-supplementation study was performed in 1987 on the same men as were followed in a 1981 study that had a similar design (200 μg Se/d). Selenite and selenate, but not selenium yeast increased platelet glutathione peroxidase (GSHPx) activity by 30% compared with placebo, much less than the 70% found in the previous study. Selenium yeast and selenite increased plasma selenium after 11 wk from 1.39 μmol/L to peak values of 2.15 and 1.58 μmol/L, respectively. Only yeast selenium was incorporated into red cells. From a regression plot based on present and literature data, it was estimated that the plasma selenium concentration needed to achieve maximal platelet GSHP activity was 1.25–1.45 μmol/L. At the present selenium intake in Finland, 100 μg/d, GSHPx activity is saturated in plasma and red cells and almost saturated in platelets. Am J Clin Nutr 1991;53:120-5.

KEY WORDS Selenium, glutathione peroxidase, platelets, blood, urine, human supplementation

Introduction

The activity of the only known mammalian selenoenzyme, glutathione peroxidase (GSHPx), in various tissues is commonly used (1, 2) as a functional measure of the selenium status of animals. The enzyme activity reaches a plateau in different tissues at different intake levels of selenium (3). It has been suggested that a dietary intake of Se that maintains maximal GSHP activity may be used as a criterion for the human nutritional selenium requirement (4). When the intake exceeds 40 μg Se/d for a body weight of 60 kg, maximal activity of GSHPx in human plasma is seen (5). The corresponding selenium concentration of whole blood is 1.13 μmol/L (5). At higher intakes of selenium (estimated to be 60–80 μg/d), GSHPx reaches maximal activity in whole blood and red cells. The corresponding selenium concentrations are 1.26 (6) and 1.77 μmol/L (7), respectively.

GSHPx activity in platelets reflects intermediate-term selenium status, ie, the intake during the preceding 1–2 wk (8). The daily intake required for maximal activity of the enzyme in the platelets is not known. On the basis of results of a bioavailability trial conducted in Finland during 1981–1982 (basal intake 55 μg/d), we estimated that platelet GSHPx reaches maximum activity at a plasma selenium concentration of 1.26–1.70 μmol/L. In 1985, 6 y after that trial, the selenium intake of the entire Finnish population was raised to 100 μg/d (9) by the nationwide addition of selenium to artificial fertilizers (10). The higher basal intake gave us an opportunity to study in the same subjects whether a basal intake of 100 μg Se/d (corresponding to a plasma selenium concentration of 1.39 μmol/L) is enough to induce maximal platelet GSHP activity. The study design also allowed us to compare the long-term effects of selenite and selenate supplementation under controlled circumstances.

Subjects and methods

Subjects

The subjects were 45 middle-aged blood donors, residents of the previously low-selenium area of Jyväskylä, who had participated in the previous selenium bioavailability study (8). Their mean age was 51 ± 7 (± SD). They were allocated to the same supplement group as before except that the subjects who had previously received selenium-enriched wheat were now supplemented with selenite. Thus, 10 subjects received selenium-enriched yeast, 10 received selenite, 10 received selenate, and 15 received placebo. On the basis of self-reported history (chronic diseases) and clinical chemistry tests (excessive alcohol consumption and liver and kidney function) carried out in connection with the baseline examination, all subjects were healthy.

Study design

The subjects in each of the three selenium-receiving groups were supplemented daily with one tablet containing 200 μg Se either as selenium-rich yeast, sodium selenite, or sodium selenate (Leiras Pharmaceuticals, Ltd, Turku, Finland) for 16 wk. A 1-wk pre- and a 10-wk postsupplementation period were included in the study design. The study was carried out in 1987–88, ie, 2 y after the addition of selenium to fertilizers began (10). Fifteen of the original 20 control subjects served as controls and received...
TABLE 1
Baseline values*

<table>
<thead>
<tr>
<th>Form of selenium</th>
<th>Selenium-rich yeast (n = 10)</th>
<th>Selenite (n = 10)</th>
<th>Selenate (n = 10)</th>
<th>Placebo (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>52 ± 7</td>
<td>51 ± 7</td>
<td>51 ± 7</td>
<td>50 ± 7</td>
</tr>
<tr>
<td>Plasma Se (μmol/L)</td>
<td>1.40 ± 0.12</td>
<td>1.38 ± 0.10</td>
<td>1.34 ± 0.05</td>
<td>1.44 ± 0.16</td>
</tr>
<tr>
<td>Red cell Se (μmol/L)</td>
<td>2.24 ± 0.45</td>
<td>2.41 ± 0.36</td>
<td>2.30 ± 0.27</td>
<td>2.26 ± 0.24</td>
</tr>
<tr>
<td>Platelet GSHPx† (U/g protein)</td>
<td>173 ± 35</td>
<td>168 ± 38</td>
<td>166 ± 25</td>
<td>185 ± 28</td>
</tr>
<tr>
<td>Plasma GSHPx (U/L)</td>
<td>366 ± 40</td>
<td>386 ± 49</td>
<td>381 ± 47</td>
<td>358 ± 46</td>
</tr>
<tr>
<td>Red cell GSHPx (U/g Hb)</td>
<td>9.2 ± 0.9</td>
<td>10.2 ± 1.4</td>
<td>9.4 ± 1.0</td>
<td>9.4 ± 1.3</td>
</tr>
<tr>
<td>Urinary Se‡ excretion (μmol/d)</td>
<td>0.72 ± 0.08</td>
<td>0.65 ± 0.10</td>
<td>0.81 ± 0.25</td>
<td>0.98 ± 0.49</td>
</tr>
<tr>
<td>Plasma α-tocopherol (μmol/L)</td>
<td>25 ± 4</td>
<td>23 ± 5</td>
<td>25 ± 4</td>
<td>25 ± 2</td>
</tr>
</tbody>
</table>

* ± SD.
† Glutathione peroxidase.
‡ n = 5.

a placebo tablet daily. Chemical analysis of selenite and selenate tablets was carried out to confirm their chemical composition. The placebo tablets contributed < 1 μg Se/d. The majority of the total selenium in the enriched yeast consisted of selenomethionine (11). Selenoglutathione, selenodiglutathione, and selenocysteine were identified among the remaining selenium compounds (11). The subjects were unaware of the type of supplement received and they were encouraged to continue on their usual diets. Informed consent was obtained from all participants. The study protocol was approved by the Ethical Committee of The National Public Health Institute, Helsinki.

**Sampling**

Venous fasting blood samples were taken between 0800 and 1000 into 3.8% trisodium citrate (0.1 vol) or heparin-containing vacuum tubes. Blood samples were taken 1 wk before supplementation; at baseline; during supplementation weeks 4, 7, 11, and 16; and after supplementation during weeks 20 and 26. Platelets were separated from citrated plasma and sonicated as described by Levander et al (3). Heparin plasma and packed red blood cells were separated by centrifugation at 1000 × g and stored at −70 °C. The frozen (−70 °C) platelet sonicates, plasma, and red cells were sent to Helsinki by air once per week.

Twenty-four-hour urine samples from five randomly chosen subjects in each group (n = 20) were taken at baseline, during supplementation weeks 11 and 16, and 4 and 10 wk after the end of supplementation. Urine was collected into acid-washed plastic containers without preservative, the volume was measured, and 10-mL samples were stored at −70 °C.

**Analytical methods**

Plasma selenium was determined by electrothermal atomic absorption spectrometry with nickel as the matrix modifier (12). This method is not applicable to samples with a high iron or phosphate concentration. Therefore, all other samples were determined by acid-digestion fluorimetry (13).

Platelet, plasma, and red blood cell GSHPx activity was determined with a method described by Levander et al (3), modified for an automated reaction rate analyzer (LKB 2086, LKB Wallac, Bromma, Sweden). Red blood cells were prediluted with water and frozen again at −70 °C. Before the assay the hemolysates were allowed to react with Drabkins solution. The activity is expressed as units (U)/g protein for platelets, U/L for plasma, and U/g hemoglobin (Hb) for red cells. One unit is defined as nanomoles of NADPH oxidized per minute.

The precision (±SD) of selenium determinations between series of the reference serum, Seronorm 105 (Nycomed Co, Oslo), was 1.09 ± 0.03 μmol/L (8 series, 24 observations, recommended value 1.14 ± 0.07 μmol/L) (14), and the precision of the reference urine, Seronorm 108, was 0.61 ± 0.01 μmol/L (8 series, 32 observations, recommended value 0.62 ± 0.04 μmol/L) (14). The precision between series for platelet GSHPx activity of a pooled platelet sonicate sample was 81.2 ± 5.4 U/g protein (8 series, 48 observations).

Plasma α-tocopherol was determined by reverse-phase HPLC (15). All samples were analyzed in duplicate.

**Statistical methods**

Statistical analyses were performed by use of SAS/Stat (16). Analysis of variance (ANOVA) was used to determine the statistical significance of differences between the groups, and Duncan’s multiple-range test and Student’s t test were used for the significance between means. Spearman correlations of individual data and linear-regression analysis were also performed.

**Results**

The baseline plasma and red blood cell selenium concentrations; the urinary excretion of selenium; the platelet, plasma, and red blood cell GSHPx activities; and plasma α-tocopherol concentration were similar in the four treatment groups (Table 1). No statistically significant correlations were observed between any of these variables in the entire study population at baseline (data not shown).

Supplementation with Se-rich yeast increased the plasma selenium concentration from 1.40 ± 0.12 μmol/L to a plateau at
FIG 1. Effect of Se-rich yeast, selenite, and selenate supplementation on plasma selenium concentration. Each point represents the mean ± SEM of 10-15 subjects. Different letters at indicated weeks denote significantly different means at P ≤ 0.05.

2.12 ± 0.26 μmol/L after 11 wk (Fig 1). Four weeks after the end of supplementation, the plasma selenium concentration decreased to 1.78 ± 0.18 μmol/L, but even after 10 wk it was still 14% higher than the placebo level (P < 0.01).

Of the two inorganic supplements, only selenite increased plasma selenium significantly above placebo (P < 0.01). The plasma selenium concentration in the selenite group reached a peak after 7 wk of supplementation, at the level of 1.58 ± 0.16 μmol/L. After the end of supplementation it returned rapidly to baseline values.

Only Se-rich yeast affected the red blood cell selenium concentration (Fig 2). The concentration increased almost linearly from the baseline concentration of 2.36 ± 0.31 μmol/L to 4.06 ± 0.56 μmol/L at the end of the supplementation period and continued to rise up to the peak concentration of 4.45 ± 0.45 μmol/L 4 wk after cessation of supplementation. The concentration observed 10 wk after the discontinuation of supplementation was still almost twice the baseline concentration.

Selenite and selenate supplementation raised the platelet GSHPx activity significantly. The maximum level occurred after 4 wk, and this level was maintained throughout the remainder of the supplementation period (Fig 3). The percent increases of the activity, as compared with the placebo group, were ~30% for both selenite and selenate. The activity returned rapidly back to baseline values after cessation of supplementation in both groups. Se-rich yeast did not influence the platelet GSHPx activity. None of the supplements had any effect on plasma or red blood cell GSHPx activity (data not shown).

The mean urinary excretion of selenium was 0.79 ± 0.29 μmol/d (n = 20) before the supplementation (Fig 4). The greatest increase in urinary selenium excretion during supplementation was observed in subjects who received selenate [0.81 ± 0.25, 2.43 ± 0.79, and 3.22 ± 1.81 μmol/d at baseline and at 11 and 16 wk of supplementation, respectively (P < 0.05)]. The rise in urinary selenium excretion, compared with baseline values, during the first 11 wk in the yeast (+56%, P < 0.01) and selenite (+85%, p < 0.001) groups was smaller and did not further increase between weeks 11 and 16. The mean urinary selenium excretion returned to baseline levels in 4 wk after the end of supplementation in all three groups.

According to balance studies conducted in areas of low (17, 18) and moderate (19, 20) selenium intake, 24-h urinary selenium excretion accounts for ~50% of the dietary intake. On the basis of these data, the mean basal intake at baseline in 18 of the 20 subjects (excluding two samples exceeding +2 SD) was ~110 μg/d (mean urinary selenium output 0.72 μmol/d, or 57 μg/d, range 0.50-1.22 μmol/d, or 40-97 μg/d). The intake of the placebo group (n = 5) calculated on the basis of all samples
Despite its major effects on the concentration of plasma and red blood cell selenium, in a similar experiment in healthy Belgians, 

\[
\text{Se} \text{ cis-plast APR cm } 0.82 \text{ /ol/d, or 65 /ol/d, range 0.50-1.43 /ol/d, or 40-113 /ol/d).}
\]

**Discussion**

The nationwide enrichment of fertilizers with selenium in Finland enabled us to repeat, in the same volunteers, our earlier study (8) on the effects of selenium supplements on selenium status in subjects whose basal intake was almost twice as high as it had been in 1981-1982. The main finding of our study is that 200 g of selenite or selenate still raised the platelet GSHPx activity, but the increase was substantially less than in the previous experiment (~30% vs ~70%, as compared with placebo). This observation indicates that the daily intake of 100-110 g Se is almost sufficient to induce maximal activity of platelet GSHPx.

An estimate of the amount of dietary Se needed for maximal activity of platelet GSHPx is obtained by plotting the percent increments in enzyme activity in various supplementation studies conducted among healthy adults (8, 21-25) and in the present study against the respective basal plasma selenium concentrations (Fig 5). The linear-regression plot suggests that maximal activity is achieved when the plasma selenium concentration is ~1.25-1.45 /mol/L. This estimate is in good agreement with the present data; a small but significant rise in the enzyme activity was observed in selenite- and selenate-supplemented subjects, with a mean basal plasma selenium concentration of 1.39 /mol/L (corresponding to a dietary intake of ~100 /g/d).

The relationship between platelet GSHPx activity and the daily intake is dependent on the nature of the Se supplement. Thus, yeast Se did not materially influence the activity of the enzyme despite its major effects on the concentration of plasma and red blood cell selenium. In a similar experiment in healthy Belgians with a mean basal plasma selenium concentration of 1.11 /mol/L, 100 g Se-enriched yeast for 60 d raised platelet GSHPx activity only slightly, but only after 45 d on supplementation (26). The slow response suggests that selenomethionine is first deposited into tissue pools before being converted into the precursor available to GSHPx (27). Preferential incorporation of selenomethionine into proteins other than GSHPx at an adequate selenium status is also supported by animal experiments (28, 29). Such a selective distribution would also explain the small or nonexistent response in platelet GSHPx in the yeast-supplemented subjects in the present study. Another reason for the small response could lie in the composition of selenium-enriched yeast. The non-selenomethionine fraction may not be as readily absorbed or available for GSHPx synthesis as selenomethionine (no animal or human data are available), thus decreasing somewhat the effective dose of selenium compared with the pure inorganic forms.

Selenium supplementation did not influence plasma GSHPx activity in our previous study (basal intake 55 /g/d and plasma concentration 0.88 /mol/L) (8) nor in the present study (the respective values were 100 /g/d and 1.39 /mol/L). Lack of effect is consistent with other studies with no response (21, 24) or a small increase (26) in plasma GSHPx activity in subjects with a basal plasma selenium concentration ~0.88 /mol/L. In fact, Yang et al (5) suggested that plasma GSHPx is maximally active when the daily intake of selenium exceeds 40 /g/d in Chinese people with a mean body weight of 60 kg.

Selenium supplementation did not influence the red cell GSHPx activity in the present study. A significant association between red cell (or whole blood) selenium concentration and red cell GSHPx activity has been reported only in the low-selenium areas of New Zealand (6, 7, 30). The New Zealand data suggest that the activity of erythrocyte GSHPx is maximal when the red blood cell selenium concentration exceeds 1.77 /mol/L, corresponding to an estimated selenium intake of 60-80 /g/d. Our results agree with these data and indicate that the basal red cell selenium concentration (2.40 /mol/L) in Finland is almost adequate selenium status is also supported by animal experiments (28, 29). Such a selective distribution would also explain the small or nonexistent response in platelet GSHPx in the yeast-supplemented subjects in the present study. Another reason for the small response could lie in the composition of selenium-enriched yeast. The non-selenomethionine fraction may not be as readily absorbed or available for GSHPx synthesis as selenomethionine (no animal or human data are available), thus decreasing somewhat the effective dose of selenium compared with the pure inorganic forms.

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**FIG 5.** Relationship between basal plasma Se concentration and percent increase of platelet GSHPx activity compared with baseline or placebo during selenium supplementation. Symbols stand for type of selenium (200-256 /g/d) supplement: Se-rich yeast, □; Se-rich wheat, ●; selenate ■, and selenite □. Numbers stand for reference: 1, present study; 2, reference 21; 3, reference 8; 4, reference 24; 5, reference 25; 6, reference 23; 7, reference 22.
1987–1988 was sufficiently high to induce maximal GSH-Px activity in erythrocytes.

Only yeast selenium (mostly selenomethionine) was incorporated into red blood cells. This pattern was also seen in our previous study in which Se-rich yeast and selenium-rich wheat but not selenate raised erythrocyte selenium (8). In vivo tracer experiments (31) showed that selenomethionine is predominantly incorporated into hemoglobin, and selenite, into selenocysteine of erythrocyte GSH-Px. Hence, lack of effect of selenite and selenate on the erythrocyte selenium concentration is probably explained by the fact that the amount of GSH-Px protein was already maximal at baseline.

Daily supplementation with 200 μg Se-rich yeast resulted in a plasma selenium peak of 2.15 μmol/L after 11 wk. This finding is in excellent agreement with a study from New Zealand in which volunteers were supplemented for 8–13 wk with 200 μg Se/d as selenium-rich wheat (23) or selenium-rich yeast (22). The net increases in plasma selenium concentration in the present study, in the previous study (8), and in the two studies from New Zealand (23, 22) were 0.72, 1.26, 1.39, and 1.74 μmol/L, respectively. In each case the peak concentration was approximately the same. A slightly higher dose (256 μg/d) as selenium-rich yeast used in a Danish study resulted in a peak at 2.53 μmol/L after 15 wk of supplementation, the net increase in plasma selenium being 1.46 μmol/L (21).

In the present experiment selenite supplementation raised plasma selenium concentration by 14% (P < 0.01), whereas selenate had no effect. The effect of selenite is supported by Mutanen et al (32), who reported that supplementing healthy women in Helsinki (after the selenium fertilization took effect) by 150 μg selenite/d raised the plasma selenium concentration <10%. In our previous study selenate (200 μg/d) increased plasma Se concentration to the baseline concentration (~1.39 μmol/L) of the present study. Taken together, these results suggest that supplementation by selenate results in an equilibrium at ~1.39 μmol/L, a conclusion that is consistent with a study from New Zealand (22). The difference between the two selenium species is probably explained by more efficient intestinal absorption and more rapid urinary excretion of selenate (33, 34), also observed here.

The difference in urinary excretion of the three forms of selenium were compared in terms of percent of dose excreted during weeks 11 and 16. For selenite and Se-rich yeast the values were 34–48% and 40–45%, respectively, and for selenate, 64–95%. These values agree with data (33, 35) from New Zealand residents (selenite 2 1-3 1%, wheat 54%, and selenate 62-8 1%) and females (selenite 2 1-3 1%, wheat 54%, and selenate 62-8 1%) and for selenate, 64–78% and 98–99%, respectively. These values agree with data (33, 35) from New Zealand (22). The difference between the two selenium species was already maximal at baseline.

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References


