Effects of organic and inorganic selenium supplementation on selenoenzyme activity in blood lymphocytes, granulocytes, platelets and erythrocytes

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ABSTRACT

The blood selenium (Se) concentration in the U.K. population has declined by approx. 50% between 1974 and 1991, reflecting a large decrease in dietary Se supply, with intakes only half the reference nutrient intake of 1 μg/kg body weight. Tissue levels of Se are readily influenced by dietary intake. Therefore selenoprotein activity may be sub-optimal due to low Se status, and thus compromise normal cell function. To examine the effects of changing Se intake on selenoproteins, we have determined the relative effectiveness of organic selenomethionine and inorganic sodium selenite (50 μg of Se daily for 28 days) in modulating glutathione peroxidase activities in blood cells from 45 healthy men and women, from a U.K. population. Transient and acute changes in lymphocyte, granulocyte and platelet phospholipid-hydroperoxide glutathione peroxidase (GP<sub>4</sub>) activity occurred by day 7 or 14 of sodium selenite treatment and by day 7 in lymphocytes from selenomethionine-treated subjects compared with controls taking a placebo. In contrast, GP<sub>4</sub> activity in granulocytes and platelets in the selenomethionine group increased gradually over the 28 days. Cytosolic glutathione peroxidase (GP<sub>1</sub>) activity in these blood cells from both treatment groups increased gradually over the 28 days. For each cellular selenoenzyme activity a significant inter-individual difference (P < 0.001) in the extent of the response to Se supplementation was observed, but this was not related to blood Se concentrations either before or after treatments. Significant inverse correlations were evident between baseline enzyme activities and percentage change in activity after 28 days of supplementation [e.g. lymphocyte GP<sub>4</sub>, r = -0.695 (P < 0.001)], indicating that pre-treatment activity may be sub-optimal as a result of poor Se status. The different and contrasting effects that Se supplementation had on blood selenoenzyme activities may be indicative of a difference in metabolic need for Se regulated at the level of Se-dependent cell function.

INTRODUCTION

Selenium (Se) is an essential nutrient associated with the function of major metabolic pathways in the cell, where it is incorporated as selenocysteine at the active site of a wide range of proteins. Under physiological conditions the Se in selenocysteine is almost fully ionized, and consequently it is an extremely efficient biological catalyst [1]. It has been suggested that up to 100 selenoproteins may exist in mammalian systems [2], and

Key words: glutathione peroxidase, selenium, selenoprotein, supplementation.
Abbreviations: GP×1, cytosolic glutathione peroxidase; GP×3, extracellular glutathione peroxidase; GP×4, phospholipid-hydroperoxide glutathione peroxidase; Se-l-Met, l-selenomethionine.
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up to 30 have been identified by $^{75}$Se labelling in vivo [3]. To date, 15 selenoproteins have been purified or cloned, allowing further characterization of their biological function [4]. These include four glutathione peroxidase enzymes, which represent a major class of functionally important selenoproteins. The Se peroxidases are genetically, structurally and kinetically different, and yet have both common and individual functions [1]. Classical glutathione peroxidase (GP × 1), so called because it was the first to be discovered, is present in the cell cytosol, where it functions as an antioxidant by directly reducing \( \text{H}_2\text{O}_2 \) and lipid hydroperoxides [5]. It may also act as a storage vehicle for Se [2,6], as it contains four selenocysteine residues in a tetrameric structure. A membrane-associated phospholipid-hydroperoxide glutathione peroxidase (GP × 4) has been identified which is directly responsible for the reductive destruction of lipid hydroperoxides [7]. The enzyme is a monomer and its activity is preserved in preference to GP × 1 when dietary Se supply is low [8]. Extracellular glutathione peroxidase (GP × 3) is another selenoprotein with antioxidant potential, but there is some debate as to its function in plasma because of low plasma concentrations of glutathione (GSH). However, other thiols, such as thioredoxin, can act as electron donor and support an antioxidant role for GP × 3 in plasma [9]. Thioredoxin is a protein disulphide important to antioxidant defences and the regulation of cell growth. Three thioredoxin reductases have been identified as selenocysteine-containing enzymes which catalyse the NADPH-dependent reduction of thioredoxin and therefore play a regulatory role in its metabolic activity [10,11].

Although the mechanisms involved have yet to be fully elucidated, it is well established that Se is important for a healthy immune response [12], which may be mediated in part by leucocyte selenoenzyme activity. It has been shown that dietary supplementation of Se-replete humans with 200 \( \mu \)g of sodium selenite Se enhances T-lymphocyte immune responses [13].

An adequate dietary supply of Se is essential for selenoenzyme activity. Consequently, the decreased intake of Se by the U.K. population is of concern, since it may result in sub-optimal selenoenzyme activity, which may have deleterious long-term health implications. We have therefore investigated the effects of different forms of Se supply on selenoenzyme activity in plasma, erythrocytes, platelets, lymphocytes and granulocytes isolated from healthy adults.

**MATERIALS AND METHODS**

A total of 45 healthy non-smoking subjects aged 25–50 years participated in the study. None of the subjects had any history of cardiovascular, endocrine or gastrointestinal disorders, and none were receiving medication or taking any nutritional supplements. Fifteen subjects were randomly allocated to each of three treatment groups receiving sodium selenite, selenomethionine or placebo. There were seven females and eight males in each group. Prior to treatment, subjects were screened for evaluation of Se, vitamin E and vitamin C status. Thereafter, for 28 days each subject took one capsule/day of 50 \( \mu \)g of sodium selenite (\( \text{Na}_2\text{SeO}_3 \)), 50 \( \mu \)g of l-selenomethionine (Se-L-Met) or visually identical placebo (soya bean oil with no Se). The capsules were taken with breakfast every day. Capsules were manufactured by RP Scherer (Swindon, Wilts., U.K.) to our specification, and the Se content was confirmed by analysis in our laboratory.

Blood (30 ml) was withdrawn from the antecubital vein of each subject into heparinized evacuated tubes. Samples were obtained on day 0 immediately before taking the first supplement, and then 2, 7, 14 and 28 days later. All samples were obtained after an overnight fast.

After the centrifugation of blood (100 \( \times \)g, 20 °C, 17 min), the platelet-rich plasma was centrifuged further (900 \( \times \)g, 20 °C, 12 min). The supernatant was removed and the remaining platelet pellet was resuspended into cold Hanks/Hepes buffer to wash, then centrifuged (900 \( \times \)g, 4 °C, 12 min), and the clean platelet pellet was frozen slowly to –70 °C.

The remaining platelet-free blood cells were reconstituted to the original volume using Hanks/Hepes buffer salt solution (pH 7.3) (Sigma) and then layered over a discontinuous gradient formed by first layering 1 ml of Histopaque (density 1.077 g/ml; Sigma) over 3 ml of Mono-poly resolving medium Ficoll-Hyapaque (density 1.114 g/ml; ICN). This was centrifuged (900 \( \times \)g, 20 °C, 30 min) to obtain lymphocyte and granulocyte fractions. Cells were harvested, washed with Hanks/Hepes buffer, and the pellets frozen slowly to –70 °C.

A further sample of whole blood was centrifuged (1500 \( \times \)g, 4 °C, 15 min) for isolation of plasma and erythrocytes. The latter were washed three times with iso-osmotic PBS, pH 7.4, and finally resuspended to the original volume.

Plasma and erythrocyte Se concentrations were measured fluorimetrically, following acid digestion and coupling of Se to diaminophthalene, using the method of Olsen et al. [14] as modified by Arthur [15].

Plasma, erythrocyte, lymphocyte, granulocyte and platelet GP × 1 activity was determined using hydrogen peroxide as substrate by a method similar to that of Paglia and Valentine [16]. The reaction pH was adjusted to 7.6 in the presence of 5 mM GSH, and following addition of 2.2 mM \( \text{H}_2\text{O}_2 \) a linear reaction rate was used to calculate activity. One unit of glutathione peroxidase activity is defined as that which oxidizes 1 mol of NADPH/min in the assay system. GP × 4 activity in blood cells was measured by the method of Weitzel et al. [17] using phosphatidylcholine hydroperoxide as substrate and 0.1 % peroxide-free Triton to solubilize cell membranes.
One unit of GP × 4 activity is defined as that which oxidizes 1 μmol of NADPH/min.

Platelet protein concentration was determined by the Biuret method, and lymphocyte and granulocyte protein concentrations were assayed by the bicinchoninic acid (BCA) method. All analyses were performed in duplicate.

Statistical analysis was undertaken in collaboration with Biomathematics and Statistics (Rowett Research Institute). Mean values for the three groups were compared by analysis of variance. An effect was tested for significance by comparing it with its standard error using a t-test. Data for males and females did not differ, and their results have therefore been amalgamated.

The study was approved by the Joint Ethical Committee of the Grampian Region and the University of Aberdeen, and informed consent was obtained from the subjects.

RESULTS

Initial plasma Se levels were lower than in many other European countries [18] (Table 1). The response to supplementation with Na₂SeO₃ or Se-L-Met varied considerably between individuals. The plasma Se concentration increased over 28 days in 93% of Se-treated subjects, but did not change in the placebo group (Table 1). Despite the short supplementation period, the erythrocyte Se concentration increased in 60% and 65% of subjects on Se-L-Met and Na₂SeO₃ supplements respectively (P < 0.05), with no change in the placebo group. The extent of the increases in plasma and erythrocyte Se concentrations varied considerably (Table 1), and could not be attributed to variations in baseline Se concentrations.

Plasma and erythrocyte glutathione peroxidase activities increased in 70% of subjects on Na₂SeO₃, and in only 50% of those on the Se-L-Met supplement (Table 1). As with blood Se concentrations, the increase in selenoenzyme activities in response to both Se treatments varied significantly (P < 0.01) between individuals. Consequently, the difference between mean baseline and day 28 activity did not reach statistical significance.

A more striking effect of Se supplementation was evident from measurement of GP × 1 and GP × 4 activity in isolated platelets, lymphocytes and granulocytes. In untreated controls an intra-individual variation in glutathione peroxidase activity was observed, and was most pronounced in granulocytes compared with platelets and lymphocytes, with mean coefficients of variation for GP × 1 of 28 ± 18%, 36 ± 14%, and 34 ± 19% for platelets, granulocytes and lymphocytes respectively, and for GP × 4 of 23 ± 15%, 58 ± 18% and 42 ± 22% for platelets, granulocytes and lymphocytes respectively. Despite the natural variation in glutathione peroxidase activities, the effects of the Na₂SeO₃ and Se-L-Met treatments on glutathione peroxidase activities in blood cells differed. However, the responses of individuals within groups were also very different.

Transient and acute changes in lymphocyte, granulocyte and platelet GP × 4 activities occurred by day 7 or 14 of Na₂SeO₃ treatment, and this was mirrored in lymphocytes from Se-L-Met-treated subjects compared with controls, whereas GP × 4 activity in granulocytes and platelets from the Se-L-Met group increased gradually over the 28 days (Figure 1). The activity of GP × 1 in lymphocytes, granulocytes and platelets from both treatment groups appeared less variable across the groups, increasing gradually over the 28 days (Figure 2). Although individual effects are evident, differences between mean values for the different treatment groups were not statistically significant because of the large variation in

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo</th>
<th>Na₂SeO₃</th>
<th>Se-L-Met</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocyte Se (μg/g of Hb)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>0.419 ± 0.04 (0.153–0.695)</td>
<td>0.341 ± 0.07 (0.119–0.389)</td>
<td>0.371 ± 0.05 (0.246–0.572)</td>
</tr>
<tr>
<td>Day 28</td>
<td>0.401 ± 0.07 (0.237–0.491)</td>
<td>0.375 ± 0.12* (0.247–0.537)</td>
<td>0.417 ± 0.07* (0.284–0.628)</td>
</tr>
<tr>
<td>Plasma Se (μg/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>80 ± 13 (58–101)</td>
<td>81 ± 9 (58–95)</td>
<td>84 ± 14 (66–105)</td>
</tr>
<tr>
<td>Day 28</td>
<td>82 ± 11 (60–103)</td>
<td>93 ± 14** (84–125)</td>
<td>103 ± 17*** (88–136)</td>
</tr>
<tr>
<td>Plasma GP × 3 activity (units/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>499 ± 80 (426–711)</td>
<td>539 ± 150 (378–740)</td>
<td>607 ± 40 (579–981)</td>
</tr>
<tr>
<td>Day 28</td>
<td>599 ± 80 (406–740)</td>
<td>630 ± 150 (370–932)</td>
<td>708 ± 90 (611–852)</td>
</tr>
<tr>
<td>Erythrocyte GP × 1 activity (units/g of Hb)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>62 ± 16 (42–81)</td>
<td>58 ± 10 (43–75)</td>
<td>59 ± 19 (32–99)</td>
</tr>
<tr>
<td>Day 28</td>
<td>59 ± 19 (48–81)</td>
<td>65 ± 10 (42–83)</td>
<td>68 ± 15 (40–99)</td>
</tr>
</tbody>
</table>

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inter-individual responses. Since baseline levels of activity differed between groups, data have been evaluated both as absolute levels of activity and as the percentage change in activity by day 28. An important observation, therefore, is the significance of the difference ($P < 0.001$) in the extent of the response to Se supplementation between individuals. The differences could not be attributed to blood Se concentration, but were related to baseline selenoenzyme activities. For both $\text{GP}_{\times 1}$ and $\text{GP}_{\times 4}$ there were significant inverse correlations between baseline activity and the extent of the increase in activity following supplementation (Table 2). The lower the baseline selenoenzyme activity, the greater the increase in response to Se supplementation, an effect shown for $\text{GP}_{\times 1}$ activity in lymphocytes and platelets in Figure 3.

**DISCUSSION**

The health implications of the decline in Se status in the U.K. over the past two decades have not been systematically investigated. Dietary intake of Se in the U.K. is now reported to be only half the U.K. Reference Nutrient Intake (RNI) of $1 \mu g$ of Se/kg body weight [19–21]. The present study highlights the relatively poor Se status of a U.K. population, with plasma Se levels lower than in many other European countries [18], and levels in erythrocytes half the value previously reported for U.K. populations [22,23]. Se supplementation of our volunteers with $50 \mu g$ of Se/day as $\text{Na}_2\text{SeO}_3$ or $\text{Se-L-Met}$ increased the activities of Se-dependent peroxidases in plasma and blood cells, indicating that functional Se status may be improved by nutritionally achievable levels of Se. However, the data in Table 2 demonstrate a large variation in individual responses to treatment which...
Table 2  Simple regression analysis showing correlation coefficients between baseline selenoenzyme activity and percentage change in activity from baseline after 28 days of Se supplementation
Ranges of values are also given for the extent of the percentage change in activity.

<table>
<thead>
<tr>
<th>Blood cell</th>
<th>Correlation</th>
<th>Na₂SeO₃</th>
<th>Se-L-Met</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>% Change</td>
<td>r</td>
</tr>
<tr>
<td>Platelet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GP × 1</td>
<td>-0.574</td>
<td>&lt; 0.01</td>
<td>9–179</td>
</tr>
<tr>
<td>GP × 4</td>
<td>-0.572</td>
<td>&lt; 0.01</td>
<td>20–142</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GP × 1</td>
<td>-0.642</td>
<td>&lt; 0.001</td>
<td>20–364</td>
</tr>
<tr>
<td>GP × 4</td>
<td>-0.354</td>
<td>&lt; 0.05</td>
<td>33–349</td>
</tr>
<tr>
<td>Granulocyte</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GP × 1</td>
<td>-0.355</td>
<td>&lt; 0.05</td>
<td>38–354</td>
</tr>
<tr>
<td>GP × 4</td>
<td>-0.499</td>
<td>&lt; 0.02</td>
<td>13–110</td>
</tr>
</tbody>
</table>

Figure 3  Relationship between percentage change in GP × 1 activity after Se supplementation and baseline activity in lymphocytes and platelets

reflected a subsequent incorporation of Se into selenoproteins in the organs of the body. This phenomenon could be investigated by measuring the turnover of stable isotopes of Se in specific proteins. Mechanisms for selenocysteine incorporation into functional selenoproteins involve an inorganic precursor, probably seleno-phosphate [2]. The fact that most dietary Se occurs in an organic form, as either selenocysteine or selenomethionine, emphasizes that the in vivo conversion into an inorganic precursor may potentially be an important regulator of Se bioavailability. This regulatory control may confer protection against excessive incorporation of Se into selenoproteins during protein synthesis, and may be of particular importance in preventing toxicity from excessive intakes. The transient acute peaks in GP × 4 activity in blood cells were not evident for blood cell GP × 1 with either Se treatment, which may reflect the predominance of GP × 1 activity over that of GP × 4 in blood cells, which produce H₂O₂, the preferred substrate for GP × 1.

The significant inverse correlation between pretreatment selenoenzyme activity and the extent of the increase in activity following 28 days on a Se supplement suggests that activity may be sub-optimal as a result of the habitual pre-treatment Se status (Table 2, Figure 3). However, it does not explain why some subjects with relatively low initial selenoenzyme activity did not show an increase in enzyme activity, despite increases in plasma Se concentration. This was particularly pronounced in response to Se-L-Met supplementation, where increases in plasma and erythrocyte glutathione peroxidase activities occurred in only half the subjects. In animal studies where rats were supplemented with selenomethionine and fed a diet low in methionine, a large proportion of the selenomethionine was incorporated non-specifically into tissue proteins, thereby com-
implications of a lowered Se intake in the U.K. Se is an important for a healthy immune response [28]. The different and contrasting effects that Se supplementation had on white blood cell and platelet glutathione peroxidase activities may be indicative of a difference in metabolic requirements for Se that is regulated at the level of Se-dependent cell function, such as the endogenous peroxide concentration. Transient sharp increases in the activity of GP x 4, an extremely stable enzyme which is preferentially spared in Se-depleted animal models, may indicate that it is a marker of acute increases in selenium requirement in humans. GP x 4 reacts with phospholipid hydroperoxides as well as small soluble hydroperoxides [25], and is also capable of metabolizing cholesterol and cholesteryl ester hydroperoxides in oxidized low-density lipoprotein. Consequently, it is essential to the destruction of fatty acid hydroperoxides, which, if not reduced to hydroxy fatty acids, will lead to uncontrolled radical chain reactions that are deleterious to the integrity of membranes. However, lipid peroxidation is only inhibited by GP x 4 if membranes contain sufficient vitamin E, consistent with the proposed synergism between the two antioxidant activities [26]. In animal models, the amount of GP x 4 mRNA present in tissues does not exactly reflect the distribution of enzyme activity [8]. The mechanism for the activation/inactivation of the enzyme is unknown, but the evidence of high activity in membranes of differentiating spermatogenic cells suggests a possible relationship between cell differentiation and peroxide levels [27]. Subjects who do not respond to Se supplementation with increased GP x 4 activity may have sufficient basal levels of expression, an inability to express the protein or an impaired ability to activate the protein.

Although the mechanisms involved have yet to be fully elucidated, it is well established that dietary Se is important for a healthy immune response [28]. The effects of Se deficiency can include decreased T-cell counts, and impaired lymphocyte proliferation and responsiveness [29]. A sub-clinical requirement for Se by lymphocytes and granulocytes will clearly not be as great when the immune system is not overly stressed. However, the principle cell types that mediate the eradication of pathogenic agents and tumour cells in the circulation are the T-lymphocytes, the synthesis and activation of which may be Se-dependent [29].

Many new functional roles for Se are being identified that still have to be elucidated. This should not, however, detract from further investigation into the long-term implications of a lowered Se intake in the U.K. Se is an integral component of at least three major metabolic systems essential for normal cell metabolism [4]. It is likely, therefore, that changes to selenoprotein function caused by changes in Se intake will have the potential to cause sub-optimal cell function, which may increase the risk of disease.

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