Effect of Intermittent Supplementation with Selenate on Selenium Status of Rats Fed Selenium-Deficient Diet

Satoru SUGIHARA1, Kenji FUKUNAGA1, Toshimasa NISHIYAMA2 and Munehiro YOSHIDA1,3,*

1Laboratory of Food and Nutritional Sciences, Department of Biotechnology, Faculty of Engineering, and 3High Technology Research Center, Kansai University, Yamate 3–3–15, Suita, Osaka 564–8680, Japan 2Department of Public Health, Kansai Medical University, Fumizono 10–15, Moriyachi, Osaka 570–8506, Japan

Summary To examine the selenium (Se) status of rats intermittently supplemented with Se, we measured tissue Se contents and glutathione peroxidase (GPx) activities in rats fed a Se-deficient diet intermittently supplemented with selenate. In experiment 1, four groups of male 4-wk-old Wistar rats were fed a Torula yeast-based Se-deficient diet (Se content, <0.01 μg/g) for 28 d. During the experimental period, the diet of each group was supplemented with sodium selenate (0.17 μg Se/g) for 0, 1, 2 or 7 d/wk. The tissue Se contents and GPx activities both increased gradually with an increase in frequency of the selenate supplementation, and significant linear regressions were observed between the frequency and these Se indices. In particular, the correlation coefficient in the liver and plasma indices was nearly equal to a value of 1.0. In experiment 2, three groups of rats were fed the Se-deficient basal diet for 28 d. Among these, one group was daily supplemented with sodium selenate to the Se-deficient diet at a level of 0.17 μg Se/g, and another group was intermittently supplemented with the selenate at a level of 1.19 μg Se/g for 1 d/wk. The tissue Se contents and GPx activities both were increased by the selenate supplementation and no significant difference was observed between daily and weekly supplementation in the Se indices except in erythrocyte Se. These results indicate that Se status in the growth period is dependent on total Se intake in this period and that weekly intermittent supplementation with Se can maintain adequate Se status.

Key Words selenium, selenium status, selenate, intermittent supplementation, glutathione peroxidase

Selenium (Se) is an essential trace element in human nutrition and plays several important roles in the form of selenoenzymes including glutathione peroxidase (GPx), the family of deiodinases or thioredoxin reductase (1). Dietary Reference Intakes for the Japanese in 2005 presented values of 30 and 25 μg/d as the Recommended Dietary Allowances (RDA) of Se for adult men and women, respectively (2). Several reports estimated that the average Se intake in the Japanese population was about 100 μg/d/capita (3–6). This estimated value is obviously higher than the RDA. However, daily Se intake in individuals may vary since foods with high Se content are limited to particular food groups such as fish and imported wheat in Japan (6). In fact, analysis of Se in Japanese duplicate portion studies showed that Se contents in total diets varied from less than the RDA to more than 100 μg (7). In general, an individual Japanese person eats various types of food during a week. Accordingly, daily Se intake of Japanese probably varies intra-individually from less than RDA to more than 100 μg; we may take both a low Se meals and a high Se meals during a week; an adequate Se intake is considered to be maintained not at daily level but at weekly one in most of the Japanese.

In many animal experiments on Se nutrition, dietary Se content is kept constant during the entire experimental period and there has been little information on the effect of intermittent supplementation with Se on Se status. In the present study, we measured tissue Se contents and GPx activities in rats fed a diet intermittently supplemented with a high level of Se and rats fed a diet daily supplemented with an adequate level of Se and compared the Se status of the both groups whose weekly Se intakes were similar to each other.

Materials and Methods

Animal feeding. The experimental protocol was reviewed and approved by the Animal Ethics Committee of Kansai Medical University and followed the Guide for the Care and Use of Experimental Animals of the Prime Minister's Office of Japan. Four-week-old male Wistar rats, weighing 40 to 50 g each, were housed in stainless steel wire mesh cages in a room with a controlled 12 h light (8:00 to 20:00) and dark cycle at a temperature of 22 to 24 ºC and a humidity of 60%. The
rats were given deionized water ad libitum and pair-fed each experimental diet during the entire experimental period.

Because bioavailability of dietary Se is dependent on chemical species of Se (8), evaluation of intermittent supplementation with Se may be affected by Se species supplemented. The AIN-93G diet, which is known to be the most standard experimental diet for rats, uses sodium selenate as a Se source (9). Thus, in the present study, we selected sodium selenate as a Se source in evaluation of intermittent supplementation with Se.

In experiment 1, we examined the association of Se status with frequency (d/wk) of supplementation with Se at an adequate level. Twenty-four rats were randomly divided into 4 groups (groups 1-A to D). Three groups (groups 1-A, B and C) were fed a Torula yeast-based Se-deficient diet for 28 d. Among these groups, groups 1-B and 1-C were intermittently supplemented with sodium selenate (0.17 μg Se/g) to the basal diet for 1 and 2 d/wk, respectively. Since fluorometric analysis showed that Se content in the AIN-93G diet prepared in our laboratory was 0.17±0.005 μg/g, we considered that this value corresponds an adequate Se level in rats and set the supplementary Se level at 0.17 μg/g. The supplementation in group 1-B was performed on days 1, 8, 15 and 22, and that in group 1-C was performed on days 1, 5, 8, 12, 15, 19, 22 and 26 after the start of the feeding. Group 1-D was fed the selenate-supplemented basal diet for 28 d. Composition of the basal Se-deficient diet was as follows (%): Torula yeast (KR yeast® kindly supplied by Kojin Co., Tokyo, crude protein and Se contents were 51.2% and 0.011 μg/g, respectively), 35.2; sucrose, 51.8; soybean oil, 8.0; AIN93G salt mixture (except for sodium selenate), 3.5; AIN93G vitamin mixture, 1.0; choline bitartrate, 0.2; dl-methionine, 0.3. Analysis showed the basal diet to contain less than 0.01 μg/g of Se.

In experiment 2, we examined the Se status in rats receiving a high level of Se once a week compared with that in rats receiving the adequate level (0.17 μg/g) of Se every day. To equalize Se intake per week, the high level set at 7 times (1.19 μg/g) the adequate level. Eighteen rats were randomly divided into 3 groups (groups 2-A to C) and fed the Se-deficient basal diet for 28 d. Among these groups, group 2-B was daily supplemented with sodium selenate to the Se-deficient diet at a level of 0.17 μg Se/g, and group 2-C was intermittently supplemented with the selenate at a level of 1.19 μg Se/g on 1 d/wk. The supplementation with selenate in the group 2-C was performed on days 1, 8, 15 and 22 after the start of feeding.

After feeding for 28 d, in both experiments 1 and 2, the rats were anesthetized by diethyl ether, the blood was collected into a heparinized tube by heart puncture and the liver was excised, washed, blotted and weighed.

Assays. The heparinized blood was centrifuged to separate erythrocytes. The obtained erythrocytes were washed with saline and burst in 9 volumes of a hypotonic buffer (5 mM sodium phosphate buffer, pH 7.0). The liver pieces, about 1 g each, were homogenized with 9 volumes of saline in a Teflon-glass homogenizer.

GPx was assayed by a modified method of Paglia and Valentine (10) with 0.29 mM tert-butyl hydroperoxide as the peroxide substrate (11). Units of the enzyme activity were defined as μmol NADPH oxidized per min. Se was analyzed by high performance liquid chromatography with a fluorometric detector (12). Protein was measured by the method of Lowry et al. (13) with bovine serum albumin as a standard.

Statistics. Experimental data were assessed by an analysis of variance (ANOVA) followed by Scheffe’s multiple comparison test, using a personal computer (eMac, Apple Computer, Cupertino, CA) with operating system Mac OS 9.2 and statistical program package StatView-J version 5.0 (Abacus Concepts, Berkeley, CA).

Results and Discussion

In experiment 1, no significant differences were observed in the body weight or animal growth irrespective of the dietary Se supplementation during the entire feeding period of 28 d. At the end of the experimental period, the means±SE (n=6) of body weight (g) were as follows: group 1-A, 224±4; group 1-B, 221±6; group 1-C, 230±7; group 1-D, 232±4. Similarly, the effect of Se supplementation was least on the liver weight (data not shown).

<table>
<thead>
<tr>
<th>Group</th>
<th>Frequency of supplementation (d/wk)</th>
<th>Liver Se content (ng/g)</th>
<th>Erythrocytes Se content (ng/ml packed cell)</th>
<th>Plasma Se (ng/ml)</th>
<th>GPx activity 1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Liver (unit/g protein)</td>
</tr>
<tr>
<td>1-A</td>
<td>0</td>
<td>23±2a</td>
<td>33±4a</td>
<td>8±1a</td>
<td>24±2a</td>
</tr>
<tr>
<td>1-B</td>
<td>1</td>
<td>40±2a</td>
<td>35±3a</td>
<td>15±2a</td>
<td>29±2a</td>
</tr>
<tr>
<td>1-C</td>
<td>2</td>
<td>81±5b</td>
<td>31±7a</td>
<td>15±1a</td>
<td>57±12a</td>
</tr>
<tr>
<td>1-D</td>
<td>7</td>
<td>237±11c</td>
<td>108±17c</td>
<td>140±12b</td>
<td>329±21b</td>
</tr>
</tbody>
</table>

1Frequency of supplementation with sodium selenate to a basal Se-deficient diet (Se content was less than 0.01 μg/g) at a level of 0.17 μg Se/g.

2Values are means±SE (n=6). Means in the same column not sharing a common superscript differ significantly (p<0.05).
Table 2. Regression analysis between frequency of Se supplementation (X)\(^1\) and tissue Se contents or GPx activities (Y)\(^2\) in experiment 1.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Regression equation</th>
<th>Correlation coefficient (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Se content</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>( Y = 16.6 + 31.4X )</td>
<td>0.985</td>
</tr>
<tr>
<td>Plasma</td>
<td>( Y = -5.68 + 20.1X )</td>
<td>0.947</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>( Y = 22.9 + 11.6X )</td>
<td>0.798</td>
</tr>
<tr>
<td>GPx activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>( Y = -6.86 + 46.5X )</td>
<td>0.962</td>
</tr>
<tr>
<td>Plasma</td>
<td>( Y = 1.12 + 0.619X )</td>
<td>0.945</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>( Y = 2.73 + 0.567X )</td>
<td>0.888</td>
</tr>
</tbody>
</table>

\(^1\) X: frequency of Se supplementation (d/wk).
\(^2\) Y: tissue Se contents or GPx activities (units are the same as described in Table 1).

Table 3. Effect of daily or weekly supplementation with selenate on tissue Se contents and GPx activities in rats fed a Se-deficient diet.

<table>
<thead>
<tr>
<th>Group</th>
<th>Supplement with Se</th>
<th>Se content(^3)</th>
<th>GPx activity(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Liver (ng/g)</td>
<td>Erythrocytes (ng/mL packed cell)</td>
</tr>
<tr>
<td>2-A</td>
<td>None</td>
<td>13±1(^a)</td>
<td>15±2(^a)</td>
</tr>
<tr>
<td>2-B</td>
<td>Daily (^1)</td>
<td>302±18(^b)</td>
<td>97±12(^c)</td>
</tr>
<tr>
<td>2-C</td>
<td>Weekly (^2)</td>
<td>316±17(^b)</td>
<td>72±4(^b)</td>
</tr>
</tbody>
</table>

\(^1\) Daily supplementation with sodium selenate to the basal diet (Se content was less than 0.01 μg/g) at a level of 0.17 μg Se/g was performed.

\(^2\) Supplementation with sodium selenate to the basal diet at a level of 1.19 μg Se/g was performed once a week.

\(^3\) Values are means±SE (n=6). Means in the same column not sharing a common superscript differ significantly (p<0.05).

Table 1 shows the response to daily or intermittent supplementation with selenate at a level of 0.17 μg Se/g in Se contents and GPx activities of the liver, plasma and erythrocytes. The Se contents and the GPx activities both increased gradually with an increase in frequency of the selenate supplementation. However, the response somewhat varied by indices monitored. The intermittent supplementation did not elevate erythrocyte Se.

Table 2 summarizes the results of regression analysis between the frequency of selenate supplementation and tissue Se contents or GPx activities. Significant linear regressions were observed between the frequency and these Se indices. In particular, the correlation coefficient in the liver and plasma indices was nearly equal to a value of 1.0. This indicates that tissue Se contents or GPx during the experimental period were dependent on the total Se intake in this period.

In experiment 2, no significant differences were observed in animal growth or liver weight irrespective of the dietary Se supplementation similarly to those in experiment 1. At the end of the experimental period, the means±SE (n=6) of body weight (g) were as follows: group 2-A, 212±5; group 2-B, 212±4; group 2-C, 208±6. No adverse effect was observed on dissection findings in any rats including those which received the high level (1.19 μg/g) of Se once a week.

Table 3 shows the effect of daily supplementation with 0.17 μg Se/g of selenate or of once a week supplementation with 1.17 μg Se/g of selenate on the tissue Se contents and GPx activities. The Se contents and the GPx activities were both increased by the selenate supplementation. Although no significant difference was observed between the daily and once a week supplementation in the tissue GPx activities, a slight difference in the Se contents was observed between the rats with the daily supplementation and those with the once a week supplementation. The once a week supplementation caused lower erythrocyte Se contents than the daily supplementation. This lower response to the intermittent supplementation with selenate in erythrocytes Se was also observed in experiment 1. The reason for these phenomena is unclear. There is one possibility that Se directly uptaken by erythrocytes and not associated with erythrocyte GPx decreased rapidly because the collection of liver and blood from the animals was performed 2 or 6 d after the last administration of selenate.

Since analysis of Se in Japanese duplicate portion studies showed that daily Se intake varied from less than the RDA to more than 100 μg (7), it is considered that Japanese people other than those having an extremely unbalanced diet take both a low Se daily diet and a high Se daily diet during a week. In other words,
Japanese people maintain an adequacy in Se status based on the Se intake per week. We attempted to evaluate Se status of rats supplemented with Se intermittently. The present experimental result that Se status was dependent on total Se intake over the entire experimental period indicates that the once a week intermittent intake of a high Se diet can maintain adequate Se status.

There are two types of strategies to overcome a deficiency in trace elements; increase of the daily intake from diet and intermittent administration at a high dose. As concerns the former, trials employing addition of iodine to table salt (14) or addition of iron to a seasoning (15) have been performed with a view to increasing daily intake of these trace elements. The latter has been also performed to confront deficiencies of iodine (16) or iron and zinc (17) in developing countries. In general, the former is adopted to prevent a trace element deficiency and the latter is adopted to cure it. However, as regards Se, it was reported that both daily consumption of a selenite-fortified table salt and weekly administration of a selenite-tablet were available to prevent Keshan disease (18). In the present study, no difference was observed between daily and weekly supplementation with selenium in Se status of rats monitored by tissue Se contents and GPx activities. This indicates that intermittent supplementation with a high level of Se can be available for not only correction but also prevention of Se deficiency.

As described in the former section, bioavailability of dietary Se is dependent on the chemical species and effect of the intermittent supplementation on Se status may vary with the Se species. Therefore, a further investigation using various Se sources other than selenite is necessary for the evaluation of intermittent supplementation with Se. In addition, although no adverse effect was observed in rats receiving 1.19 µg Se/g of selenite in the present study, it is necessary to assess the toxicological effect of intermittent supplementation with a high Se dose for a long period.

REFERENCES