Selenium intake and metabolic balance of 10 men from a low selenium area of China

Xianmao Luo, MD, Huijuan Wei, MD, Chunlin Yang, Jiu Xing, Changhong Qiao, BS, Yimin Feng, Jun Liu, Zing Liu, BS, Qin Wu, Yongxing Liu, Barbara J Stoecker, PhD, Julian E Spalholz, PhD, and Shiang P Yang, PhD

ABSTRACT Selenium intake and urinary and fecal Se excretion of 10 healthy men from a low Se area in China were determined for three consecutive days, in summer, fall, and winter of 1983, and the spring of 1984 while self-selected diets were being consumed. Mean daily Se intake was 8.8 μg/day with a range of 2.3-35.5 μg/day, and was far below the recommended range of safe and adequate Se intake of 50-200 μg Se/day (National Academy of Sciences/National Research Council). Mean urinary and fecal Se outputs were 3.7 and 3.4 μg Se/day, respectively. Mean Se balance during this time was +1.8 μg Se/day. Apparent absorption of Se approximated 57%. The low Se intake in this area is a cause for concern since the residents of Molimo may be at risk for Se deficiency diseases.

KEY WORDS Selenium balance, selenium intake, selenium status in China, human selenium nutrition

Introduction

Selenium (Se) has been known to be essential element for animals since the 1950's (1). During the past few years there has been increasing evidence of the nutritional essentiality of Se in humans. Perhaps the most important evidence was the isolation of the Se-containing enzyme glutathione peroxidase (GSH-Px) from human erythrocytes (2), furthermore, beneficial responses to Se supplementation have been observed in the setting of total parenteral nutrition (3), and in certain people living in low Se areas of the People's Republic of China (4, 5).

Present epidemiological studies suggest that low Se status might be related to an increased incidence of cancer (6) and cardiovascular diseases (7). In a review by Griffin (8), the association between Se nutrition and carcinogenesis in animals was emphasized, and epidemiological studies showing a correlation between low environmental selenium and human cancer were discussed. The practical value of Se for prevention of human cancer remains in doubt (8) and is currently a subject of research in China.

In 1980, the US National Research Council (9) established a safe and adequate range of daily Se intake of 50-200 μg Se for adults and children over 7 years of age. However, there have been relatively few investigations of the minimal Se requirement for humans, and information on the amount of Se actually consumed by varied population groups is still limited (10-14). Se intakes estimated on the basis of food purchased rather than food eaten probably indicate a higher intake of Se than would be expected in the general population (14).

1 From the Cancer Institute, Chinese Academy of Medical Sciences, Beijing, PRC (XL, HW, JX, CQ, YF, JL, ZL), and the Edemic Disease Institute, Weichang, PRC (CY, QW, YL), and Department of Food and Nutrition, Texas Tech University, Lubbock, TX (BJS, JSP).

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3 Address reprint requests to: Dr Barbara Stoecker, Department of Food and Nutrition, Texas Tech University, Lubbock, TX 79409.

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While Keshan disease is the best known Se deficiency disease of China, Kaschin-Beck disease, a lesser-known disease affecting mainly the joints of both animals and humans, has been recognized in China and is also preventable with dietary Se supplements (15). The site of the experiments reported here, Molimo, is a low Se area where these two diseases are prevalent. The present study was designed to estimate the daily Se intake and the metabolic balance of residents eating self-selected diets in Molimo. It was thought that these balance studies would provide a realistic measure of the actual Se consumption of people living in a Keshan and Kaschin-Beck disease area.

Methods

After an explanation of the experimental protocol to the people of Molimo, ten apparently healthy male adults were selected from volunteers for the study. Subjects gave informed consent and were compensated (3 renminbi per day). Study procedures were approved by the Texas Tech University Committee for Protection of Human Subjects and by the Department of Science and Education, Ministry of Health, People’s Republic of China. Table 1 describes the age, weight, and smoking status of the subjects. Duplicate meals, feces and 24-h urine samples were collected on each of three consecutive days in June, September, and December of 1983 and in March of 1984 (Table 2).

All subjects ate self-selected diets. No fish was eaten. Liver and kidney, which are good sources of Se, are not usually consumed by these subjects, and were not consumed during the study. Subjects were instructed to collect an exact duplicate of everything consumed each day and project staff assisted them at each meal. A three-day record with weights or volumes of food items eaten was additionally maintained for each person. Weighed aliquots of 1/9 of each food item were composited, in the village, and because the diets were very simple. GSH-Px-like activity was measured in the presence of human hemoglobin. GSH-Px activity of homogenized, and refrigerated on the same day of collection. This sampling technique was used because of limited storage, handling, and transportation facilities in the village, and because the diets were very simple.

Urine and feces were measured or weighed in the morning after each 24-h period. As a preservative, a 2% solution of sodium benzoate was added to the samples after collection (1 ml for 10 g diet or feces or for 100 ml of urine). Samples were collected in acid-washed polyethylene bottles. All samples were subsequently refrigerated and transported to Beijing where they were frozen until analysis. Feces were dried at 60°C for 72 h, ground in a coffee mill, and then kept in a desiccator. Fasting blood samples were drawn via vena mediana cubiti early in the morning. About 6 ml blood was treated with 0.1 ml of 10.0% potassium oxalate and centrifuged, and plasma was stored at -20°C. The RBC were maintained in 2 ml ACD solution (Acid-Citrate-Dextrose, Citric acid 0.540 g, tri sodium citrate 1.485 g, dextrose 1.687 g and water to 67.5 ml) at 4°C until analysis for GSH-Px activity (usually finished within 72 h).

Selenium levels were measured from digestates of the diet, urine, and feces by a modification (16) of the 2,3-diaminonaphthalene fluorometric method of Watkinson (17). Reagent purification included removal of residual Se from H2SO4 using 48% HBr, redistillation of hexane, and removal of fluorescent substances from 2,3-diaminonaphthalene with hexane. A standard reference material (kale) from the International Atomic Energy Agency (a generous gift of Dr. Bowen, Reading University, UK.) was used. Recoveries of added Se were grain and diet, 96-102%; blood, 93%; urine, 103%; feces, 96%; and kale, 109%.

GSH-Px activities were assayed by a modification of the coupled method of Paigi and Valentine (18) using hydrogen peroxide as substrate. One unit of enzyme activity was defined as 1 micromole NADPH oxidized/min and the results were expressed as units/ml plasma or units/g Hb for RBC. GSH-Px activity using erythrocyte lysates as a measure of the Se status of humans may be hampered by human hemoglobin (19). Much of the Se in human erythrocytes (85-90%) is reported to be sequestered by human hemoglobin (19). In our assay procedure the GSH-Px activity of human hemoglobin contributed negligible amounts of GSH-Px-like activity when measured in the presence of sodium azide, using H2O2 as the substrate and monitoring oxidation of NADPH at 340 nm as described by Paglia and Valentine (18). In a pilot study in China, GSH-Px activity in human plasma or erythrocyte lysate was not found to be significantly different using either H2O2 or cumene hydroperoxide as substrate. The data were subjected to one-way analysis of variance and compared by Duncan's multiple range test (19).

Results

The distribution of the Se content of the 113 composite diets is shown in Figure 1. Se levels in all diets were far below the recom...
Selenium intake and balance in China

Median: 6.0 µg/day
Mean: 8.8 µg/day

10
20
30
40
50
Number of diets

Selenium intake (µg/day)

FIG 1. The percentage distribution of Se content of 113 diet composites from Molino, China.

The recommended range of safe and adequate intake of Se was 50-200 µg Se/day (9). The mean daily intake of Se was 8.8 µg with a range of 2.3-35.5 µg. Since the distribution was upwardly skewed because of a few relatively high Se levels, the median of 6.0 µg Se/day might be more representative than the mean daily Se intake. To our knowledge, the daily Se intake of subjects of Molino is one of the lowest reported to date in the world (Table 3). The low daily Se intake resulted in the low Se status of the subjects (Table 4). Plasma and RBC GSH-Px activities were less than 60% of the levels seen in Beijing residents. Plasma and RBC Se concentrations were also extremely low compared to residents of Beijing and the USA.

As shown in Table 5, a seasonal variation of Se intake was observed. The Se intake in summer was significantly higher than in the other three seasons (p < 0.05). In the spring, the Se intake was less than in either the fall

<table>
<thead>
<tr>
<th>Country and area</th>
<th>Se intake</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canada</td>
<td>168†</td>
<td>21</td>
</tr>
<tr>
<td>US, California</td>
<td>90-168*</td>
<td>22</td>
</tr>
<tr>
<td>Japan</td>
<td>100*</td>
<td>23</td>
</tr>
<tr>
<td>US, North Carolina</td>
<td>93†</td>
<td>24</td>
</tr>
<tr>
<td>US, Maryland</td>
<td>81§</td>
<td>11</td>
</tr>
<tr>
<td>US, Washington, DC</td>
<td>71§</td>
<td>25</td>
</tr>
<tr>
<td>United Kingdom, Britain</td>
<td>60*</td>
<td>26</td>
</tr>
<tr>
<td>New Zealand</td>
<td>56*</td>
<td>27</td>
</tr>
<tr>
<td>Finland</td>
<td>30*</td>
<td>28</td>
</tr>
<tr>
<td>New Zealand</td>
<td>24§</td>
<td>10</td>
</tr>
<tr>
<td>China, Keshan disease area</td>
<td>11†</td>
<td>29</td>
</tr>
<tr>
<td>China, Molino</td>
<td>8.8§</td>
<td>present study</td>
</tr>
</tbody>
</table>

| Calculation of Se content in food based on consumption information. |
| Chemical analysis of diet composites prepared on the basis of food consumption information or idealized diets. |
| Chemical analysis of diet composites collected from subjects. |
TABLE 4
Selenium status of residents in different locations

<table>
<thead>
<tr>
<th>Location</th>
<th>Plasma Se (nmol/L)</th>
<th>GSH-Px (units/mL)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ng/g Hb</td>
<td>ng/g Hb</td>
<td>ng/g Hb</td>
</tr>
<tr>
<td>Molimo†</td>
<td>30</td>
<td>23.9 ± 1.1</td>
<td>100.9 ± 2.9</td>
</tr>
<tr>
<td>Beijing</td>
<td>20</td>
<td>93.4 ± 5.5</td>
<td>262.8 ± 24.9</td>
</tr>
<tr>
<td>USA</td>
<td>50</td>
<td>80.0 ± 10.0</td>
<td>740.0 ± 40.0</td>
</tr>
<tr>
<td>USA</td>
<td>174</td>
<td>97.0 ± 2.3</td>
<td>810.0 ± 31.8</td>
</tr>
</tbody>
</table>

* Mean ± SEM.
† Mean of analyses of blood samples collected from 10 subjects in Oct, Nov, and Dec of 1983.

or winter (p < 0.05). Se intake in the fall did not differ from that of the winter. The Se concentration of urine in fall was less than that in summer (p < 0.05) but was not significantly different from urine samples taken in the winter or spring.

Se in the feces of these subjects declined from 4.5 and 4.3 μg Se/day in summer and fall to 2.7 and 2.1 μg Se/day in winter and spring (p < 0.05). The mean balance of Se varied from +1.5 to +4.4 μg Se/day in summer, fall and winter, but decreased to −1.1 μg Se/day in spring. The means of apparent Se absorption in the four seasons did not vary significantly (p > 0.05). On the average, our subjects excreted 52% of their total body Se losses in urine and 48% in the feces with a Se balance of +1.8 μg/day. The mean apparent absorption of Se during the four seasons was 57.2%.

Table 6 shows the daily intake of some macronutrients. These macronutrient intakes did not vary significantly in summer, fall, and winter. Significant decreases (p < 0.05) during spring were observed in energy and protein intake (vs fall) and carbohydrate intake (vs fall and winter). Correlation between Se intake and the intakes of energy, protein, carbohydrate, and fat were positive but were not statistically significant (0.1 > p > 0.05).

Discussion

Diet composites actually prepared and consumed by the 10 subjects in Molimo, China, were analyzed in this study. The data, therefore, are believed to represent true Se intakes of the people of this area.

Data from New Zealand indicated that the minimum dietary Se requirement for maintenance of human health is probably not more than 20 μg/day (10). In China, Keshan disease is absent in areas where the dietary Se intake is at least 30 μg Se/day (5). On the average, self-selected diets in this study provided only 8.8 μg Se/day (median 6.0 μg) for men. Moreover, of these diets, 88 or 97% had Se levels less than 20 or 30 μg Se/day, respectively. It is likely that the residents of Molimo are at risk of Keshan and

TABLE 5
Selenium balance in 10 men during the four seasons**†

<table>
<thead>
<tr>
<th>Season</th>
<th>n</th>
<th>Intake</th>
<th>Urine</th>
<th>Feces</th>
<th>Balance</th>
<th>Apparent absorption %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Summer</td>
<td>10</td>
<td>13.3 ± 1.0a</td>
<td>4.3 ± 0.3a</td>
<td>4.5 ± 0.6a</td>
<td>+4.4 ± 1.5a</td>
<td>63.1 ± 6.5a</td>
</tr>
<tr>
<td>Fall</td>
<td>10</td>
<td>9.2 ± 0.3a</td>
<td>2.7 ± 0.3a</td>
<td>4.3 ± 0.6a</td>
<td>+2.2 ± 0.7a</td>
<td>52.4 ± 6.8a</td>
</tr>
<tr>
<td>Winter</td>
<td>10</td>
<td>8.1 ± 1.4a</td>
<td>3.9 ± 0.6a</td>
<td>2.7 ± 0.3a</td>
<td>+1.5 ± 1.7a</td>
<td>57.7 ± 6.8a</td>
</tr>
<tr>
<td>Spring</td>
<td>10</td>
<td>4.7 ± 0.4a</td>
<td>3.8 ± 0.4a</td>
<td>2.1 ± 0.3a</td>
<td>−1.1 ± 0.6a</td>
<td>53.4 ± 6.8a</td>
</tr>
<tr>
<td>Annual mean</td>
<td></td>
<td>8.8 ± 0.7</td>
<td>3.7 ± 0.2</td>
<td>3.4 ± 0.3</td>
<td>+1.8 ± 0.8</td>
<td>57.2 ± 3.3</td>
</tr>
</tbody>
</table>

* Mean ± SEM.
† Means in a column not sharing a common superscript are significantly different (p < 0.05).
‡ Apparent absorption, % = Se intake-fecal Se/Se intake × 100.
Kaschin-Beck disease as a consequence of Se deficiency, and the low Se intake in this area is a cause for concern.

Located in a mountainous northern district of Hebei province, Molimo is an isolated area where locally grown food constitutes almost the entire diet. The amount of Se in the soil is so low that the major grains and beans contain only about 0.005 ppm of Se (except soybean which contains about 0.012 ppm of Se). The average Se level in vegetables is less than 0.003 ppm on a wet weight basis. Low Se in most foods is the reason that the Se levels are so low in the composite diets of our subjects, and in the people of Molimo in general.

Several factors may be involved in the seasonal variation of Se intake: (a) During summer, and especially in the fall, heavy field work compelled our subjects to eat 3 meals/day. In the winter and spring, only 2 meals a day were eaten. (b) In summer and fall, diets were usually supplemented with purchased wheat flour products from Henan Province whose Se level is about 0.1 ppm or 20 times that found in local staple foods. (c) During the spring, pickled Chinese cabbage was the main vegetable eaten, and it contained less Se than did fresh vegetables.

Selenium and macronutrient intake appeared to decline progressively during fall and winter and dropped to the lowest values in the spring. In this area, Keshan disease occurs mainly during the winter and early spring. The possible role of seasonal variation in Se and macronutrient intake may contribute to the onset of Keshan disease and should be further investigated.

The high individual variance in daily Se intake (from 2.3 to 35.5 μg) probably could be attributed to day-to-day variability in the consumption of relatively few products that are good sources of selenium. In the case of our subjects, as mentioned above, imported wheat flour products from the market might contribute to infrequent high Se intakes.

In our subjects, the mean daily fecal and urinary Se losses were only 7.1 μg. These values are lower than those reported for six young American men consuming formula diets who needed 54 μg Se/day to replace fecal and urinary losses (32), or for men consuming self-selected diets whose Se requirement for balance was calculated at 80 μg/day (14). Mean body weight of our subjects was 60 kg compared to the mean 75-76 kg reported by Levander and coworkers for male subjects (14, 32). Selenium intake needed to maintain balance has been suggested to be related to lean body mass. Our results also differ from those of four young New Zealand women who needed about 24 μg Se/day to maintain a positive Se balance (10). It is possible that people from extremely low-Se areas, like Molimo, maintain their Se balance but at a much lower Se level with some compensative mechanism conserving their body Se reserves. These data do not mean that the Se intake is enough to support optimal physiological function. In another study, daily supplements of 150 μg Se as Na₂SeO₃ for 30 days did not raise GSH-Px levels of RBC or plasma to those levels seen in Beijing residents (33).

Despite considerable seasonal variation in Se intake, urinary Se remained more or less constant, except in the fall when a significant drop in urinary Se was observed compared to that of summer. The decrease of fecal Se excretion in winter and spring reflected the
decline of Se intake more markedly than did urinary Se loss. Levander and Morris (14) reported no seasonal variation in Se intake, but it is probable that the Chinese subjects had more seasonal variation in energy expenditure and food availability than did the North American subjects.

Our subjects excreted 52% of their total Se losses in the urine. This agrees closely with the 55% of total Se output via the urine calculated for four New Zealand women consuming diets which provided 24 µg Se/day (10). Percent of Se lost in the urine of our subjects was slightly lower than data obtained from two groups of American subjects (14, 32).

In spite of the low Se intakes, the apparent absorption of Se in our subjects (57 ± 3) remained similar to the 55 ± 5% apparent absorption calculated for four New Zealand women (10), the 62 ± 6% for six American young men (31) during Se depletion (32), and the 61 ± 2% and 68 ± 2% calculated for American men and women respectively, on self-selected diets (14). However, a linear regression of Se balance vs intake indicated that zero balance would be attained with 7.4 µg Se/day. This figure was much less than required for balance by either North American or New Zealand adults, even considering the slightly smaller body mass of Chinese subjects.

New Zealand workers had shown little loss of Se by routes other than urine and feces in their subjects consuming a diet with 24 µg of Se per day (10). Others have indicated that the sauna-induced sweat of North Americans contained low levels of Se (average of 1.3 µg/l) (34). Presumably, the net balance of our subjects should be less than +1.8 µg/day if dermal and respiratory losses of Se, particularly in summer and fall, were considered.

References
