Dietary and Biochemical Selenium Status of Urban 6- to 24-Month-Old South Island New Zealand Children and their Postpartum Mothers

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ABSTRACT A community-based, cross-sectional survey was conducted in the South Island of New Zealand to assess the dietary and biochemical selenium status of children (n = 136) and their mothers (n = 302), and to assess factors influencing selenium status. Serum and plasma samples from children and their mothers were analyzed for selenium using graphite furnace atomic absorption spectrometry. Dietary selenium intakes were analyzed from 3-d weighed diet records, and food sources of selenium were quantified. Mean dietary selenium intakes in infants (6–11.9 mo), toddlers (12–24 mo), and mothers were below recommended levels. Toddlers had higher selenium intakes than infants (13.7 ± 8.4 and 7.9 ± 6.2 μg/d, respectively, P = 0.0001) and the selenium density of their diets was also higher [3.2 ± 1.7 and 2.4 ± 1.7 μg/(MJ·d), respectively, P = 0.003]. Household smoking was associated with lower serum selenium concentrations in infants and toddlers (P = 0.02). South Island women who were currently pregnant had lower plasma selenium concentrations (0.74 ± 0.15 μmol/L) than nonpregnant lactating and nonpregnant nonlactating women (0.94 ± 0.16 and 0.93 ± 0.16 μmol/L, respectively, P = 0.0001). Clearly, pregnant women, infants, and toddlers are at risk of suboptimal selenium status, and further research is warranted to assess potential effects in these groups. The finding of an association between household smoking and lower selenium concentrations in children should be investigated further. Dietary interventions are recommended to improve dietary selenium intakes in South Island children and their mothers. J. Nutr. 134: 3290–3295, 2004.

KEY WORDS: • selenium status • dietary selenium • children • infants • mothers • New Zealand

Selenium, a trace element with antioxidant properties, has an essential role in the enzyme glutathione peroxidase (GPx) and other important selenoproteins (1). Historically, New Zealand residents have had low dietary intakes of selenium due to low soil levels; consequently, blood selenium and GPx concentrations have been suboptimal (2). Recent reports suggest that blood selenium concentrations in New Zealand residents have increased due to factors including wheat importation (3), but are still below the level required for maximal GPx activity (4–6).

Pregnant women may be at risk of low selenium concentrations due to increased selenium demands for the growing fetus, and maternal selenium status influences blood selenium concentrations of the newborn through both breast milk and fetal transfer (7–9). In addition, lactating women were shown to have lower blood selenium concentrations than nonlactating, postpartum women (8,10), reflecting both loss of selenium to breast milk, and the increased demands of lactation.

During infancy, rapid growth and development lead to an increased requirement for selenium. Possible implications of low selenium status in infants include bronchopulmonary dysplasia and chronic lung disease in preterm infants (11,12). Low selenium levels were also implicated in an increased risk of certain childhood diseases including asthma (13,14), an illness with high incidence in New Zealand (15).

Data on dietary selenium intakes in relation to biochemical selenium status in both children and their mothers are limited, and food sources of selenium, particularly for infants and young children, are unknown. Such information is essential to formulate effective dietary strategies to ensure optimal selenium status of New Zealand mothers and their young children. Therefore, the aims of this study were to assess biochemical selenium status and dietary selenium intakes in infants (6–11.9 mo), toddlers (children 12–24 mo), and their mothers in the South Island of New Zealand. In addition, dietary sources of selenium and factors influencing serum selenium levels in children and their mothers were investigated.

SUBJECTS AND METHODS

A community-based, cross-sectional survey of 6- to 24-mo-old South Island children and their mothers was conducted in Christchurch, Dunedin, and Invercargill between May 1998 and March 1999 (16). Dietary data, a demographic questionnaire, and
blood samples for biochemical assessment were collected during 2 home visits for each participant. Maternal plasma and infant serum samples were collected for iron, zinc, folate, and selenium analysis. Only the results of selenium analysis are presented here (children, n = 136; mothers, n = 302). The Ethics Committee of the University of Otago, Dunedin, New Zealand granted ethical approval for the survey, and written informed consent was obtained from each primary caregiver participating in the survey.

Children and their primary caregivers were randomly selected to participate in the survey using multi-stage sampling, as described in detail elsewhere (16). Inclusion criteria were that children were aged between 6 to 24 mo, and were apparently healthy. Of the 532 eligible families identified, 323 (61%) agreed to participate in the survey. Children aged between 6 and 11.9 mo were classified as infants, whereas children aged between 12 and 24 mo were classified as toddlers.

When feasible, a venipuncture blood sample was collected from each child (nonfasting) during a home visit (n = 261; 81.4%). A venipuncture sample was taken from each mother (fasting) during a 2nd home visit (n = 310). Blood for serum and plasma samples was collected into a trace-element free vacutainer (Becton Dickinson) then separated (10–20 min at 16500 × g). From the 261 blood samples collected from children, 136 (52%) were available for selenium analysis 3 y after collection, and 302 (97%) blood samples from mothers were available. Because selenium was the third priority nutrient for blood sample analysis, sufficient sample was not available for all children. Of the 302 mothers, 30 (10%) were pregnant at the time of the study.

Serum and plasma selenium concentrations were determined by graphite furnace atomic absorption spectrophotometry, using a modified version of the method of Jacobson and Lockitch (17). Multiple aliquots of a control pooled plasma sample were analyzed during each batch of analyses to ensure analytical accuracy and precision. In addition, an external control, Utah Reference Plasma (Batch No. 66816, Lot No. 4103), was analyzed with each batch. Analyzed values were within the expected range given by the manufacturer (i.e., 1.20–2.00 μmol/L, certified mean concentration: 1.60 μmol/L), with means of 1.57 ± 0.13 μmol/L (n = 14, CV 8.3%), and 1.62 ± 0.16 μmol/L (n = 21, CV 10%) for the serum and plasma analyses, respectively.

Three-day weighed diet records were collected for each child and mother using dietary scales accurate to within ± 1 g (Model Salter Electronic, Salter Housewares). Records were taken on randomly selected nonconsecutive days, with 2 weekdays and 1 weekend day included. Average daily nutrient intakes and major food sources of energy and selenium were calculated using the New Zealand Food Composition Database (NZFCD) (18). The selenium concentrations of infant formulas were taken from the manufacturers’ labels when available (18 and 13 μg/L for Nurture Premium and Heinz infant formula, respectively, Heinz Watti’s). The analyzed selenium values reported in a Christchurch-based study were used for other brands of infant formula in the present study (Similac, Ross Products, 4.6 μg/L, Karicare, Nutricia, 4.6 μg/L, Enfamil, Mead Johnson, 3.9 μg/L, SMA, Wyeth, 5.2 μg/L, and Isomil, Ross Products, 8.2 μg/L) (19). For the remaining brands of formula consumed, a selenium concentration of 5.3 μg/L was assumed on the basis of the average concentration of the formulas above. Selenium intakes from complementary foods for breast-fed infants and toddlers were assessed, but because breast milk consumption was not quantified and therefore total intakes were not available, these intakes (n = 75) were excluded from statistical analysis. In total, 230 and 302 diet records were available for non-breast-fed children and mothers, respectively. Mean dietary selenium intakes for infants, toddlers, and mothers were compared with current U.S. and Canadian Estimated Average Requirements (EAR) and Adequate Intakes (AI) for infants (20), because these recommendations are the most current and are similar to those proposed for new Australian and New Zealand Nutrient Reference Values for selenium (21).

All statistical analyses were conducted using the survey commands of Stata version 5.0 to account for the complex survey design. Results are expressed as means ± SD. The contribution of food groups to selenium intakes was assessed for infants, toddlers, and mothers, and presented as the percentage of total selenium from each selected food group. Differences in mean dietary intakes and selenium concentrations in both children and mothers were assessed using ANOVA. Distribution of data was checked using histograms and normal probability plots, and the log was taken to normalize data if necessary. Multiple-linear regression was carried out to investigate the effect of independent variables on selenium concentrations in children and their mothers, after adjusting for all other variables in the model. Variables were then excluded in a stepwise sequence if they did not significantly improve the model fit (P > 0.10), and did not provide significant adjustment (P < 0.20) for the effects of remaining variables in the model. Independent variables assessed in the maternal analysis included log dietary selenium (μg/d), age (y), household income (low, medium, high), maternal university education (tertiary education: Yes/No), current pregnancy, and smoking in the household (smoker in the household: Yes/No). Independent variables assessed for serum selenium in children included age (mo), smoking in the household, maternal university education, household income, ethnicity (New Zealand European or Other), and prematurity. Variables were included in the final multivariate model when P < 0.20. Associations were considered significant when P < 0.05.

**RESULTS**

The characteristics of the study population were described in detail previously (16). Characteristics differed slightly for the children whose serum was available for selenium analysis (n = 136), compared with the original survey population (n = 323) and the population for whom serum selenium was not available (n = 187). A greater number of children for whom selenium status was available were Caucasian (90 vs. 84%), respectively, and there were more families with high household incomes.

Infants tended to have a higher mean serum selenium concentration than toddlers (Table 1). Serum selenium concentrations did not differ between breast-fed and nonbreast-fed children. The daily selenium intake by toddlers was higher than that of infants, as were dietary selenium density and protein and energy intakes. Comparison with U.S. and Canadian Dietary Reference Intakes (20) showed that the selenium intake of infants was well below the AI of 15–20 μg/d and that of toddlers below the EAR of 17 μg/d. Infants and toddlers who were breast-fed had a selenium intake from complementary foods of 4.4 and 10.5 μg/d, respectively, but because breast milk intake was not determined, total selenium intake was not available for these children.

Pregnant women had significantly lower plasma selenium concentrations than either nonpregnant lactating (NPL) or nonpregnant, nonlactating (NPNL) women (Table 2). Significantly higher intakes of energy, protein and selenium were reported in NPL women compared with the other 2 groups, and the selenium density of diets was higher for NPL and NPNL women than for pregnant women. Dietary selenium intakes in mothers in all 3 groups were lower than the U.S. and Canadian EARs of 45, 49 and 59 μg/d for women, pregnant women, and lactating women, respectively.

The major food sources of dietary selenium for children and mothers were bakery products and cereals (Table 3). Meat and fish/seafood combined comprised a major source of selenium in mothers (32%), but not in infants and toddlers. Fast food was also a good source of selenium for mothers and toddlers. The main sources of selenium for nonbreast-fed infants were bakery products and cereals, and infant foods (including infant formula). Comparatively, nonbreast-fed toddlers were receiving the majority of dietary selenium from bakery products and
cereals, and dairy and egg products. Infant foods (including formula) were not a major source of selenium for toddlers.

Multivariate analysis (Table 4) showed that household smoking was significantly associated with lower serum selenium concentrations in infants and toddlers (P = 0.012). Serum selenium in children from nonsmoking households was 0.71 ± 0.24 μmol/L (n = 106) and from smoking households 0.57 ± 0.17 μmol/L (n = 29) (P = 0.004). Maternal university education was also associated with higher serum selenium concentrations in the children (P = 0.032). Household income and the child’s age were not associated with serum selenium concentrations. Maternal selenium status and dietary selenium intake in children were not significantly associated with serum selenium concentrations; therefore, this was not included in the multivariate analysis. For mothers, smoking in the household was associated with decreased maternal plasma selenium concentrations, but this association was not significant after adjusting for other factors (maternal university education, age, current pregnancy, income, selenium intake). Current pregnancy was significantly associated with lower plasma selenium concentrations (P = 0.001), and maternal age tended to be positively associated with plasma selenium (P = 0.055).

**DISCUSSION**

This cross-sectional survey provides unique data on blood selenium concentrations, and information on selenium intakes and food sources of selenium for infants, toddlers, and their mothers. The results show that dietary selenium intakes and blood selenium concentrations in these groups in the South Island of New Zealand are at the lower end of international levels.

Although there are no established dietary or plasma selenium cutoff levels for selenium deficiency or inadequacy, our infants and toddlers are at the lower end of the range of reported values for both serum selenium concentrations and dietary selenium intakes. Serum concentrations in our infants and toddlers (0.69 and 0.61 μmol/L, respectively) were at the lower end of the reference ranges calculated in a group of German infants of 0.11–1.47 μmol/L for 4- to 12-mo-old infants and 0.43–1.63 μmol/L for 1- to 5-y-old children (22), and were also among the lowest reported from other countries, including Austria (0.61 μmol/L) (23), Canada (1.6 μmol/L) (17) and Finland in 1990 (1.25 μmol/L) (24).

These selenium concentrations reflected the low dietary intakes of these children. The intake for our infants (7.9 μg/d) was slightly higher than that reported in 7-mo-old formula-fed Finnish infants (6.4 μg/d) (24), whereas intakes of our toddlers (13.7 μg/d) was lower than that of Finnish children aged 13 mo (16.4 μg/d). Intakes in U.S. infants were considerably higher, with reported mean intakes at 11 mo of 18 μg/d, and 46 μg/d at 2 y of age (25). The selenium intake for our infants was below the U.S. and Canadian AI of 15–20 μg/d, and that for toddlers was below the U.S. and Canadian EAR of 17 μg/d (20).

Clearly, response rate bias may be an issue in this study, because less than half of the original blood samples collected from children were available for analysis, and the overall response rate for the study was 61%. Comparison of the children for whom serum selenium analysis was available with the original survey population showed that a higher percentage of children were Caucasian (90 vs. 84%, respectively), and there were more families with high household incomes. Similarly, comparison of the original survey population with the

**TABLE 1**

<table>
<thead>
<tr>
<th>Serum Se, μmol/L</th>
<th>Infants (6–11.9 mo)</th>
<th>Toddlers (12–24 mo)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast-fed</td>
<td>22</td>
<td>0.69 ± 0.27</td>
<td>7</td>
</tr>
<tr>
<td>Nonbreast-fed</td>
<td>16</td>
<td>0.70 ± 0.34</td>
<td>91</td>
</tr>
<tr>
<td>Se intake, μg/d</td>
<td>42</td>
<td>7.9 ± 6.2</td>
<td>188</td>
</tr>
<tr>
<td>Se density, μg/(MJ·d)</td>
<td>42</td>
<td>2.4 ± 1.7</td>
<td>188</td>
</tr>
<tr>
<td>Energy intake, kJ/d</td>
<td>42</td>
<td>3227 ± 521</td>
<td>188</td>
</tr>
<tr>
<td>Protein intake, g/d</td>
<td>42</td>
<td>26 ± 7</td>
<td>188</td>
</tr>
</tbody>
</table>

1 Dietary selenium intakes exclude infants and toddlers currently breast-fed.
2 Values are means ± SD.

**TABLE 2**

<table>
<thead>
<tr>
<th>Plasma Se, μmol/L</th>
<th>PNNL</th>
<th>P+2</th>
<th>NPL</th>
<th>All women</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>198</td>
<td>30</td>
<td>74</td>
<td>302</td>
<td></td>
</tr>
<tr>
<td>Plasma Se, μmol/L</td>
<td>0.93 ± 0.16</td>
<td>0.74 ± 0.15</td>
<td>0.94 ± 0.16</td>
<td>0.91 ± 0.18</td>
<td>0.055</td>
</tr>
<tr>
<td>Dietary Se intake, μg/d</td>
<td>36 ± 22</td>
<td>33 ± 16</td>
<td>46 ± 32</td>
<td>38 ± 25</td>
<td>0.004</td>
</tr>
<tr>
<td>Se density, μg/(MJ·d)</td>
<td>5.0 ± 2.8</td>
<td>4.3 ± 2.8</td>
<td>5.5 ± 3.3</td>
<td>5.0 ± 2.8</td>
<td>0.235</td>
</tr>
<tr>
<td>Energy intake, kJ/d</td>
<td>7181 ± 1930</td>
<td>7583 ± 2074</td>
<td>8682 ± 2219</td>
<td>7590 ± 2110</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Protein intake, g/d</td>
<td>70 ± 21</td>
<td>69 ± 22</td>
<td>83 ± 22</td>
<td>73 ± 22</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

1 Values are means ± SD.
2 P+, pregnant (n = 24) and pregnant and lactating (n = 6).
reflected the lower selenium density of their diets compared with that in diets of nonpregnant women, influenced in particular, by a lower contribution of selenium from meat and fish/seafood. Another influence on selenium status might be the physiologic demands of pregnancy or hemodilution, resulting in lower plasma selenium concentrations. In the present study, concentrations were not adjusted for hemodilution; however, selenium utilization and demands of the fetus were shown to result in lower plasma selenium concentrations in pregnant women, even after adjustment for hemodilution (29,30). The effect of low selenium status during pregnancy is not clear; however, it would certainly increase the risk of suboptimal selenium status in the growing fetus.

The higher dietary intakes of selenium, protein, and energy of lactating mothers than of pregnant and nonpregnant women were also reported in lactating mothers in the U.S. (10), and are likely due to the high energy demands of lactation. However, the higher selenium intakes were not reflected in plasma selenium concentrations, which were similar to those of NPNL women, presumably because the extra selenium consumed was required for breast milk. Indeed, lower plasma selenium concentrations in lactating women compared with nonlactating women were reported previously, and were attributed to selenium loss in breast milk (8).

Multiple regression analysis did not show a significant relation between dietary selenium intakes and plasma selenium concentrations of mothers or children. This lack of association might result from a number of factors, including inaccurate estimates of selenium intakes of individual subjects because of inadequacy of the NZFCD (18) or because of an insufficient length of diet recording for assessment of selenium intake. The NZFCD does not account for geographical variations in the selenium content of certain foods, such as bread, which is higher in areas of New Zealand in which Australian wheat is imported than in the South Island where locally grown wheat is used (3). Thus, intakes in our South Island subjects may be overestimated. An alternative reason for the lack of association might be the narrow range of selenium intakes and serum/plasma selenium concentrations in a low-selenium population such that there may not have been sufficient variability in these small groups of subjects. However, taken as a group, the dietary data obtained still provide valuable information on the contribution of selenium from different food sources as well as average intakes for each group; at this level, low dietary intakes were reflected in lower selenium status.

### TABLE 3

<table>
<thead>
<tr>
<th>Food group</th>
<th>Infants</th>
<th>Toddlers</th>
<th>NPNL</th>
<th>P+1</th>
<th>NPL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bakery products/cereals</td>
<td>30</td>
<td>29</td>
<td>27</td>
<td>31</td>
<td>27</td>
</tr>
<tr>
<td>Meat</td>
<td>11</td>
<td>11</td>
<td>22</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Fish/seafood</td>
<td>2</td>
<td>3</td>
<td>10</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>Dairy/egg</td>
<td>12</td>
<td>28</td>
<td>11</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>Fast food</td>
<td>3</td>
<td>12</td>
<td>16</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>Fruit and vegetables</td>
<td>11</td>
<td>6</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Infant foods/formula</td>
<td>27</td>
<td>4</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Miscellaneous foods2</td>
<td>3</td>
<td>7</td>
<td>9</td>
<td>11</td>
<td>9</td>
</tr>
</tbody>
</table>

1 P+: pregnant (n = 24) and pregnant and lactating (n = 6).
2 Miscellaneous foods include fats and oils, nuts, beverages, recipes, soups, sugar, and confectionary, and other.

### TABLE 4

**Multivariate analysis of factors influencing serum selenium levels in children (r² = 0.12), and plasma selenium levels in mothers (r² = 0.15)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Children (n = 135) β Est.1 (95% CI)</th>
<th>P-value</th>
<th>Mothers (n = 250) β Est. (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>1.52 – 03 (-6.33 – 03, 7.60 – 03)</td>
<td>0.991</td>
<td>5.06 – 03 (-1.27 – 06, 0.01)</td>
<td>0.055</td>
</tr>
<tr>
<td>Household smoking</td>
<td>-0.12 (-0.22, -0.03)</td>
<td>0.012</td>
<td>-0.05 (-0.12, 0.01)</td>
<td>0.099</td>
</tr>
<tr>
<td>Maternal university</td>
<td>0.02 (8.87 – 03, 0.20)</td>
<td>0.032</td>
<td>0.03 (-0.02, 0.08)</td>
<td>0.236</td>
</tr>
<tr>
<td>Premature</td>
<td>0.08 (-0.03, 0.18)</td>
<td>0.157</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Log Se intake, µg/d</td>
<td>—</td>
<td>0.08 (-8.87 – 03, 0.16)</td>
<td>0.076</td>
<td></td>
</tr>
<tr>
<td>Pregnant</td>
<td>—</td>
<td>-0.15 (-0.22, -0.07)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Household income2</td>
<td>≥$50,001</td>
<td>-0.07 (-0.22, 0.08)</td>
<td>0.382</td>
<td></td>
</tr>
<tr>
<td>$20,001–50,000</td>
<td>-0.08 (-0.21, 0.05)</td>
<td>0.225</td>
<td>-0.42 (-0.12, 0.03)</td>
<td>0.278</td>
</tr>
</tbody>
</table>

1 β Est., β estimate.
2 Reference group: $1–20,000.
Because both consumption and selenium concentration of breast milk were not determined, selenium intake from breast milk and total intakes for these children could not be estimated; thus, they were excluded from the dietary analysis. Breast-fed infants were shown to have higher serum levels of selenium compared with formula-fed infants in several studies (12,19,31,32). In the present study, serum concentrations of infants and toddlers who were breast-fed and those who were not did not differ. Serum concentrations are no doubt influenced by the effect of low maternal selenium status on breast milk concentrations as well as the selenium content of complementary foods.

Smoking in the household was significantly associated with lower serum selenium concentrations in infants and toddlers. The effect of household smoking was reported in a recent study of children aged 4–18 y living in England (33), in which plasma selenium in children was significantly lower if either or both parents smoked. Although the authors could not rule out the effect of passive smoking, it was noted that other lifestyle factors associated with socioeconomic status may have affected selenium levels. Adjusting for lifestyle factors (household income and maternal university education) in the present study did not remove the association of household smoking and serum selenium concentrations. It is possible that young children are more susceptible to the effects of passive smoking, and further investigation is required to confirm this finding because the public health implications may be significant. Data from NHANES III suggested an increased risk of asthma in children with both low selenium levels and exposure to cigarette smoke (14).

Maternal smoking was associated with lower plasma selenium concentrations in mothers, although not significantly. Lloyd et al. (34) also reported lower plasma selenium levels in English residents who smoked than in nonsmokers, and Patterson et al. (35) found lower plasma selenium concentrations and GPx activities in New Zealand smokers than nonsmokers, with even lower levels in women who were heavy smokers. Reduced selenium concentrations may be due in part to the decreased energy and selenium intakes in smokers or to an increased demand for selenium as a result of cigarette smoking.

The monitoring of blood selenium concentrations of both mothers and young children is essential to determine whether they are at risk of inadequate selenium status. This study shows that the selenium status of South Island infants and toddlers and their mothers is suboptimal. The mean serum selenium levels are among the lowest reported internationally, and the majority of this population was not meeting the EARs or AIs for selenium intakes. The implications of suboptimal selenium status in these children are unclear, but associations with diseases including cancer and asthma were reported (14,15,36,37). Further research on the requirements of selenium for this age group is warranted. Dietary strategies to improve selenium status in both infants and toddlers are recommended and include encouraging mothers who formula feed their infants and toddlers to choose brands that contain adequate dietary selenium. Regulations may be required to ensure that all infant formulas contain selenium at a level similar to that of the breast milk of selenium-sufficient mothers. Selenium-rich weaning foods, including fish, meat, and unrefined cereals should be recommended. Pregnant women may be at increased risk of low selenium status, putting the developing fetus at risk, and an increase in the consumption of foods high in selenium is recommended. The association between household smoking and lower serum selenium levels has potential health implications and requires further investigation.

**LITERATURE CITED**


