Serum Selenium Is Associated with Plasma Homocysteine Concentrations in Elderly Humans

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ABSTRACT Low selenium levels in humans have been associated with several pathologies; however, an earlier animal investigation found a direct association between Se intake and total plasma homocysteine (tHcy) concentrations. To date, the importance of serum selenium levels in association with tHcy in humans has not been determined. We evaluated the cross-sectional association of blood selenium concentrations with plasma tHcy and other determinants of this cardiovascular disease risk factor. We estimated protein intake and measured the blood status of selenium, tHcy, and several other related factors in serum such as folate, vitamin B-12, and creatinine. Serum selenium was inversely associated with tHcy, explaining 5.8% of tHcy variance with respect to 2.2% accounted for by serum folate. Furthermore, there was a 63% decreased risk of higher tHcy concentrations (>14 μmol/L) for subjects with serum selenium in the highest tertile (P = 0.013). We also found an inverse association of protein intake with tHcy in men (β = -0.144; P = 0.036), which disappeared after controlling for serum Se concentrations (β = -0.055; P = 0.003). In conclusion, selenium should be considered as a potential factor to lower tHcy. In addition, the described association between protein intake and homocysteine levels could be mediated by this trace element. J. Nutr. 134: 1736–1740, 2004.

KEY WORDS: • homocysteine • selenium • folate • cardiovascular disease • trace elements

Homocysteine (tHcy) is a metabolic product of methyl-group donation by the amino acid methionine; it is emerging as a risk factor for cardiovascular disease (1), Alzheimer’s disease (2), and neural tube defects (3).

Among the factors known to influence homocysteine metabolism are genetic and physiologic characteristics, and several nutrients. B vitamins such as folate, B-6, and riboflavin are important cofactors for several enzymes involved in homocysteine metabolism (4,5), and cobalamin (B-12) is the final methyl-group donor in the remethylation pathway of homocysteine to methionine (4). In this connection, several trials assessed the effects of folate, vitamin B-6, and B-12 supplements on homocysteine (6,7). In those studies, although high-dose folate acid supplements reduced fasting levels of homocysteine, vitamin B-6 and B-12 supplements had a minimal effect in healthy populations. Protein intakes were also reported to be inversely correlated with tHcy, whereas vegetarian diets and coffee consumption seemed to increase tHcy by different mechanisms (5,8).

Although all of these established factors cannot explain total tHcy variation, there are few studies attempting to identify an association between tHcy and other modifiable factors that could diminish plasma homocysteine concentrations. Recently, Uthus et al. (9) observed that tHcy decreases in rats fed a low-selenium diet and increases with selenium supplementation. Also, these authors recently reported an interactive effect of dietary selenium and folate (10) in such a way that in selenium-deprived rats, some of the effects of folate deficiency seem to be ameliorated, probably by shunting the buildup of tHcy to glutathione. In addition, Se has also been implicated in the etiology of cardiovascular disease and other pathologies, although its exact mechanism of action is not yet fully understood (11).

Following our team’s research line in nutritional factors associated with homocysteine concentrations (12), this paper reports research on the cross-sectional association among selenium, folate, and homocysteine as part of an ongoing prospective study about diet, blood antioxidant status, biomarkers of oxidative damage, and disease in a selected group of elderly people.

SUBJECTS AND METHODS

Study subjects. The study sample comprised a cohort of 202 (85 men, 117 women) institutionalized elderly population recruited from 14 nursing homes of Asturias (Northern Spain).

A medical history of each subject was obtained before enrollment in the study. Those subjects with a history of cancer or cardiovascular disease were not included because these were end-points for our
prospective study. For this analysis, we excluded patients taking antiepileptic drugs or thyroid hormones because these could interfere with plasma tHcy levels (13), and those consuming vitamin or mineral supplements on a regular basis. Although antihypertensive medication was reported to affect tHcy (5), people taking these drugs were not excluded because we found no differences in tHcy levels between those who were taking this medication and those who were not (data not shown).

All participants were mentally and physically able to participate in the study and all of them gave their informed consent. Ethics approval was obtained by the Committee on Ethical Research of the Oviedo University Hospital.

**Anthropometric and dietary intake assessment.** Quetelet’s BMI was determined as weight (kg) divided by height squared (m^2). Weight was assessed using a 500 g-precision scale (SECA Hamburg), and height was registered using a stadiometer exact to 1 mm (Año-Sayol) with subjects barefoot and in light clothes.

Dietary intake was assessed by means of an FFQ specifically designed for each of the 14 institutions, once they had kindly provided us with the menus of the previous year. Trained dietitians asked about cooking practices, number and amount of ingredients used in each recipe, as well as questions concerning menu preparation (e.g., type of oil used, type of milk). The FFQ contained all of the individual foods (not food groups) that were present in the menus of each institution. During an interview, subjects were asked item-by-item whether they usually ate each food and, if so, how much they used to eat. For this purpose, 3 different serving sizes of each cooked food were presented in pictures to the participants so that they could choose from up to 7 serving sizes (from “less than the small one” to “more than the large one”). For some of the foods consumed, amounts were recorded in household units, by volume, or by measuring with a ruler. All subjects were asked whether they had access to foods other than those being offered by the institution (through their relatives, for example). Food intake was analyzed for energy and macro- and micronutrient content by using the nutrient Food Composition Tables developed by the CSIC (14).

**Blood measurements.** Blood samples were drawn by venipuncture after a 12-h fast and collected in separate tubes for serum and plasma. Samples were kept on ice and centrifuged (1000 g) after a 12-h fast and collected in separate tubes for serum and plasma. Samples were kept on ice and centrifuged (1000 X g, 15 min) within 2–4 h after the collection. Plasma and serum aliquots were maintained at −70°C until analyses were performed.

Total plasma homocysteine was determined using reverse-phase HPLC with fluorometric detection (15). To ensure the stability of the analytical procedure, cysteine and cysteineglycine (2 aminothiols) were measured together with homocysteine in the same sample and we determined that their concentrations (absolute and relative to homocysteine) were within the normal range. Serum folate and vitamin B-12 were analyzed on the Access automated immunoassay analyzer from Beckman using a competitive-binding immunoenzymatic assay. Serum creatinine was determined by standard methods. Selenium was analyzed by graphite-furnace atomic absorption spectrometry in serum (16).

### Statistical analyses.

All analyses were performed with SPSS 11.0 for Windows. General characteristics of the sample are expressed as means ± SD and range. Goodness of fit to normal distribution was investigated with the Kolmogorov-Smirnov test. Because folate and vitamin B-12 did not satisfy the normality criterion, a logarithmic transformation was performed. Logistic and linear regression analyses were adjusted for age, gender, and creatinine levels. We introduced creatinine as a covariate because the kidney plays a major role in plasma homocysteine homeostasis, and serum creatinine levels affect tHcy concentration (17). Significant differences in mean tHcy by selenium and folate levels were tested by using generalized linear models with tHcy as the dependent variable and age, gender, and serum creatinine as covariates. Adjusted tHcy means were derived from these same models. Statistical parameters presented are β (standardized regression coefficient) and R² (coefficient of multiple determination).

Study participants were classified into tertiles of distribution of serum folate, selenium, and plasma homocysteine, to perform the logistic regression and calculate the odds ratio (OR) of being in the highest tertile of tHcy across tertiles of folate and selenium. Differences were considered significant at $P \leq 0.05$ level.

### RESULTS

Women were slightly older than men (76.4 ± 5.9 vs. 73.6 ± 7.3 y). Although energy intake, serum folate, and creatinine concentrations were higher in men, no significant differences were observed for tHcy, vitamin B-12, or selenium between sexes (Table 1). The significance of serum folate, vitamin B-12, and selenium as predictors of homocysteine levels is presented in Table 2. Folate and selenium had an inverse association with tHcy ($β = −0.150$ and $−0.242$, respectively). Serum folate explained a smaller proportion of plasma tHcy variation than selenium (2.2% explained by serum folate vs. 5.8% explained by serum selenium).

Protein intake had an inverse association with tHcy in men that persisted after controlling for the effect of serum folate ($β = −0.185, P = 0.008$). Nevertheless, when serum selenium was included in the same regression model, protein intake was no longer associated with tHcy, whereas serum selenium had a negative association (Table 3).

There was a 63% (OR = 0.37, $P = 0.013$) decreased risk of high homocysteine concentration for those individuals with serum selenium $>1190.5$ nmol/L and 62% (OR = 0.38, $P = 0.015$) for subjects with serum folate $>11.9$ nmol/L (Table 4). The highest levels of tHcy (15.7 μmol/L) corresponded to those subjects in the lowest tertiles of both selenium (<987.9 nmol/L) and folate (<8.6 nmol/L) (Fig. 1).

### TABLE 1

Characteristics of the study population by gender

<table>
<thead>
<tr>
<th></th>
<th>Men (n = 85)</th>
<th>Women (n = 117)</th>
<th>Range (P5–P95)1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>73.6 ± 7.32</td>
<td>76.4 ± 5.9**</td>
<td>62.9–85.5</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.3 ± 3.9</td>
<td>28.3 ± 5.4</td>
<td>20.2–36.6</td>
</tr>
<tr>
<td>Serum folate, nmol/L</td>
<td>12.1 ± 8.4</td>
<td>13.2 ± 8.4</td>
<td>5.9–36.2</td>
</tr>
<tr>
<td>Serum vitamin B-12, pmol/L</td>
<td>297.4 ± 164.5</td>
<td>290.3 ± 148.2</td>
<td>148.8–537.3</td>
</tr>
<tr>
<td>Serum selenium, nmol/L</td>
<td>1085.5 ± 223.4</td>
<td>1122.5 ± 204.8</td>
<td>810.0–1466.1</td>
</tr>
<tr>
<td>Serum creatinine, μmol/L</td>
<td>143.5 ± 28.2</td>
<td>129.2 ± 28.5**</td>
<td>95.0–182.4</td>
</tr>
<tr>
<td>Plasma homocysteine, μmol/L</td>
<td>13.0 ± 6.0</td>
<td>12.6 ± 4.3</td>
<td>6.0–22.7</td>
</tr>
<tr>
<td>Energy intake, MJ/d</td>
<td>8.9 ± 2.0</td>
<td>7.9 ± 1.6*</td>
<td>5.4–11.7</td>
</tr>
<tr>
<td>Protein intake, g/d</td>
<td>85.5 ± 21.0</td>
<td>79.0 ± 19.5*</td>
<td>53.1–111.3</td>
</tr>
</tbody>
</table>

1 P₅, 5th percentile; P₉₅, 95th percentile.
2 Values are means ± SD. Asterisks indicate different from men, * $P \leq 0.05$, ** $P \leq 0.01$. 

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**Fig. 1.**
Multivariate regression analysis of serum folate, selenium and vitamin B-12 on plasma homocysteine in elderly humans

<table>
<thead>
<tr>
<th>Regression coefficients¹</th>
<th>β</th>
<th>R²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum folate,² nmol/L</td>
<td>-0.150³</td>
<td>0.022</td>
<td>0.022</td>
</tr>
<tr>
<td>Serum Se, nmol/L</td>
<td>-0.242</td>
<td>0.058</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum vitamin B-12,² pmol/L</td>
<td>-0.082</td>
<td>0.006</td>
<td>0.214</td>
</tr>
</tbody>
</table>

¹ β-coefficient: standardized regression coefficient; R²: coefficient of multiple determination.
² Variables were logarithmically transformed.
³ Values adjusted for age, gender, and serum creatinine.

DISCUSSION

The major finding of the present study was the detection of an independent inverse association between tHcy and serum selenium concentrations in an elderly human population. Although interest in Se was due initially to its potential toxicity (18), greater importance is now given to its nutritional aspects because epidemiologic studies have associated low Se status with a higher incidence of cancer, cardiovascular disease, and other pathologies (19,20). Mortality has also been observed to be lower in areas with high concentrations of selenium in soils, as long as these are below toxic levels (21).

In a study performed in patients with peripheral vascular disease, optimal concentrations of antioxidant vitamins and selenium were suggested to decrease the risk of cardiovascular disease protecting not only against peroxidation of lipids, but also hyperhomocysteinemia (22). As far as we know, this is the only other study in humans with no supplementation on the association of serum Se and plasma tHcy.

Although most of the studies in human populations showed the adverse effect of low selenium levels, in an animal study, selenium deficiency was found to decrease total homocysteine levels (9). Animal studies on Se and tHcy used principally selenite; however, this form does not occur in foods (23). Because we found that the effects of Se and tHcy were contrary to those reported in rats (9), we hypothesized that Se in foods would have different effects in humans. In addition, the animals of the previous study were fed a selenium-deficient diet, whereas our study sample had adequate selenium intake levels.

On the basis of that animal study on Se and tHcy, Venn et al. (24) determined the effect of selenium supplements on plasma tHcy concentrations in humans, finding that selenium did not influence plasma tHcy. Hence, our results do not agree with theirs; however, it must be considered that they performed a supplementation trial to study the effect of Se intake, whereas we focused on serum selenium as an index of selenium status. Consequently, our results are not comparable to theirs because we observed an inverse linear trend across quintiles of serum selenium for tHcy concentrations (β = −0.954; P < 0.001), whereas the lack of an effect of selenium supplements on

Effect of adjustment for serum selenium or serum folate on the association between protein intake and homocysteine levels in elderly subjects

<table>
<thead>
<tr>
<th>Covariates¹</th>
<th>β</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model A²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein intake, g/d</td>
<td>-0.144</td>
<td>0.036</td>
</tr>
<tr>
<td>Model B³</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein intake, g/d</td>
<td>-0.185</td>
<td>0.008</td>
</tr>
<tr>
<td>Serum folate, nmol/L</td>
<td>-0.191</td>
<td>0.005</td>
</tr>
<tr>
<td>Model C⁴</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein intake, g/d</td>
<td>-0.055</td>
<td>0.243</td>
</tr>
<tr>
<td>Serum selenium, nmol/L</td>
<td>-0.219</td>
<td>0.003</td>
</tr>
</tbody>
</table>

¹ β-coefficient: standardized regression coefficient.
² Protein intake, age, gender, and serum creatinine.
³ Protein intake, folate, age, gender, and serum creatinine.
⁴ Protein intake, serum selenium, age, gender, and serum creatinine.

Logistic regression analysis between tertiles of serum folate and selenium and the risk of being in the highest tertile of plasma homocysteine (tHcy) in elderly humans

<table>
<thead>
<tr>
<th>Serum folate, nmol/L</th>
<th>n</th>
<th>tHcy¹</th>
<th>OR (95% CI)¹,²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;8.6</td>
<td>67</td>
<td>14.5</td>
<td>5.13</td>
<td>—</td>
</tr>
<tr>
<td>8.6–11.9</td>
<td>68</td>
<td>11.9</td>
<td>4.7</td>
<td>0.38</td>
</tr>
<tr>
<td>&gt;11.9</td>
<td>67</td>
<td>11.8</td>
<td>5.1</td>
<td>0.38</td>
</tr>
<tr>
<td>&lt;987.9</td>
<td>67</td>
<td>14.3</td>
<td>5.8</td>
<td>—</td>
</tr>
<tr>
<td>987.9–1190.5</td>
<td>69</td>
<td>12.8</td>
<td>4.8</td>
<td>0.71</td>
</tr>
<tr>
<td>&gt;1190.5</td>
<td>66</td>
<td>11.1</td>
<td>3.9</td>
<td>0.37</td>
</tr>
</tbody>
</table>

¹ Values were adjusted for age, gender, and serum creatinine.
² Highest tertile of tHcy > 14 μmol/L.
³ Values are means ± SD.

FIGURE 1 Mean homocysteine (tHcy) by tertiles of serum folate and selenium. ¹Selenium (nmol/L): tertile 1 (n = 67): <987.8; tertile 2 (n = 69): 987.8–1190.4; tertile 3 (n = 68): >1190.4. ²Folate (nmol/L): tertile 1 (n = 67): <8.6; tertile 2 (n = 68): 8.6–11.9; tertile 3 (n = 67): >11.9. Asterisks indicate different from the low-folate/low-selenium group. *P ≤ 0.05, **P ≤ 0.01.
plasma tHcy in humans may be attributable to a threshold effect, as the authors suggest (24).

There are considerable variations in subjects’ selenium concentrations in different countries around the world and, within countries, between areas. The mean serum selenium values in our study are similar to those described for the Spanish people (25), but are lower than those reported for other populations (26). This is not surprising because selenium present in serum is directly dependent on the quantity and the way in which selenium is ingested in the daily diet, and selenium content of foods varies depending on the Se content of the soil (27).

To date, several studies have described folate as the most powerful nutritional factor for reducing plasma tHcy (5,28); according to our results, however, serum selenium appears to have a greater effect on regulating homocysteine concentrations than folate. In addition, serum levels of folate and selenium were not correlated (data not shown). In our study, subjects with high selenium had a moderately low tHcy whereas the opposite was not true because the high-folate/low-selenium group had higher tHcy within the lowest tertile, whereas the opposite was not true. This conversion is directly dependent on the quantity and the way in which selenium present in serum (25), but are lower than those reported for other populations (26). This is not surprising because selenium present in serum and intake levels of folate (25) have been studied previously in an apparently healthy human population (30,31), whereas the effect of serum selenium on tHcy has not been studied previously in an apparently healthy human population.

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The third important finding in our study was the negative association of protein intake with tHcy levels in men, which was also noted in other studies (5,8). This association does not appear in women because of the narrow range of protein intake in this group. As shown in Table 3, this effect disappears after adjusting for plasma selenium levels. Other authors suggested that this association could be mediated by vitamin B-12, whose intake is associated with proteins (8). However, in light of our results, we propose selenium as the factor that is mediating the association of protein intake and tHcy because protein-rich foods are the most important products for providing this element in the daily diet (32,33).

The association of Se status with the risk of heart disease was studied mainly with a focus on its role in antioxidant defenses (34); nevertheless, our data suggest another possible mechanism for Se, which is the cooperation in the defense against high tHcy levels. When interpreting these results, we must consider that any cross-sectional study design precludes any causal inferences. Hence, further observational and experimental studies are warranted before firm conclusions can be made regarding the importance of the influence of selenium on plasma homocysteine levels to assess the possible benefits and harm of Se supplementation.

ACKNOWLEDGMENT

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LITERATURE CITED


