PCR, but also cytogenetic diagnosis of fetal chromosomal aneuploidies by FISH.

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6. van Wijk IJ, van Vugt JMG, Ko¨nst AAM, Mulders MAM, Florijn WJ, Oudejans RRHM. Determination of Se-P. After plasma albumin-globulin separation, the selenium content of the albumin fraction was measured and the selenium content of plasma Se-P was deduced from the total plasma selenium minus the sum of selenium coming from plasma albumin and GSHPx.

7. In a previous study, we showed that plasma selenium was significantly decreased in a group of institutionalized elderly women compared with a free-living group and a group of young adult women living in the same area (12). To investigate the etiology of the differences in selenium status among these groups, we studied plasma selenium distribution among the three protein compartments.

The three groups of nine women have been described elsewhere (12). Blood samples were collected in the fasting state.

Affinity chromatography on a tandem column system of heparin-Sepharose CL-6B (Pharmacia) and blue Sepharose CL-6B (Pharmacia) was performed for the separation of GSHPx, Se-P, and albumin in human plasma according to reported methods (2, 8). To establish a correspondence between the activity and the selenium content of plasma GSHPx, GSHPx activity and selenium were measured in all unretained fractions because the unretained fraction from heparin-Sepharose and reactive blue columns correspond to the GSHPx fraction (7).

Albumin and globulins were separated from one another by precipitation with ammonium sulfate at half-saturation at neutral pH. Equal volumes of plasma and saturated ammonium sulfate solution were mixed and left for 30 min in an ice bath. After refrigerated centrifugation at 2600g for 15 min, the supernatant was removed. To the precipitate, we added 1 mL of deionized water and 1 mL of saturated ammonium sulfate solution. The precipitate was suspended by shaking, and the tube was placed in an ice bath for 30 min and then centrifuged. The supernatant was removed, combined with the first supernatant, and designated the albumin fraction. The final precipitate was considered the globulin fraction.

Plasma albumin and albumin in the supernatant were measured by immunoprecipitation (BNA-Behring). For the plasma samples, globulin fractions, and albumin fractions, selenium was determined by gas chromatography–mass spectrometry (13). For plasma and column fractions, GSHPx was assayed using a modified Günszter method (14).

All values were calculated as the mean ± SD. Significance was set as P < 0.05.

Affinity chromatography was performed on two human plasma samples for estimating the percentage of selenium associated with GSHPx in plasma. In the sum of unretained fractions, GSHPx activities were 274 and 296 U/L, respectively, for the two samples, and selenium concentrations were 12.7 and 15.4 µg/L, respectively. This value was further used to convert the measured GSHPx activity to the amount of selenium GSHPx of the other human samples. GSHPx activity recovery was estimated to be satisfactory because in the initial plasma samples, the GSHPx activities were 290 and 305 U/L, respectively.

Albumin recovery was estimated by the albumin measurement in plasma and in the albumin fraction; the recovery for 23 samples was 92.7% ± 16.7%. Selenium

Distribution of Selenium in Plasma of French Women: Relation to Age and Selenium Status, Véronique Ducros,†,‡ Monique Ferry,† Patrice Faure,† Nicole Belin,‡ Jean-Charles Renversez,‡ Daniel Ruffieux,† and Alain Favier‡ (1 Laboratoire de Biochimie C and 3 Laboratoire de Biochimie A, Hôpital Michallon, BP 217, 38043 Grenoble Cedex 09, France; 2 Service de Gériatrie, Centre Hospitalier, 26953 Valence Cedex 9, France; * author for correspondence: fax 33-4-76-76-5821, e-mail Veronique.Ducros@ujf-grenoble.fr)

Human plasma selenium is associated with three proteins: selenoprotein P (Se-P), glutathione peroxidase (isoform 3 of GSHPx), and albumin (1, 2). Most of the methods used to study the distribution of plasma selenium among these proteins are based on gel filtration (3–6) or on affinity chromatography (2, 7–9). Gel filtration is less sensitive, resolves less well, and has given higher results than affinity chromatography for GSHPx (7). Affinity chromatography is time-consuming and unsuitable for routine analyses. The only direct method to determine plasma Se-P is a RIA (10, 11). We developed an indirect determination of Se-P. After plasma albumin-globulin separation, the selenium content of the albumin fraction was measured and the selenium content of plasma Se-P was determined from the total plasma selenium minus the sum of selenium coming from plasma albumin and GSHPx.

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recovery was measured by comparing the sum of the three selenoprotein pools to the total plasma selenium; the recovery was 97.0% ± 8.4%. Our indirect determination of Se-P was compared with the direct measurement of Se-P by RIA. For 10 serum samples, the mean (SD) selenium values were 14.9 (14.8) and 17.3 (13.8) μg/L for direct and indirect determinations of Se-P, respectively (P > 0.05, Friedman test). The results for Se-P determined by RIA expressed in units/mL were transformed to μg/L of selenium, using the value 1 unit = 64 μg/L of plasma selenium (11).

Selenium associated with GSHPx was decreased in the two elderly groups compared with the younger group, and selenium associated with albumin was decreased in the institutionalized elderly. Selenium associated with the Se-P pool was significantly different (P = 0.02) between the two elderly groups.

Plasma selenium distribution among the three selenoproteins in healthy people has been reported a few times showing slight differences in the percentages of distribution when an affinity chromatography technique was used (2, 7–9). The percentage of total plasma selenium varied from 52% to 70% for Se-P selenium, from 15% to 39% for GSHPx selenium, and from 9% to 17% for “albumin” selenium compartments. Affinity chromatography can give only an estimate of plasma selenium present in selenoproteins. Specific methods such as immunofluorescence are more accurate to determine the percentage of selenium associated with proteins. Using immuno-precipitation, other authors found reference values of 12 – 16% for GSHPx (15, 16) and 36 – 44% for Se-P (10, 11). These percentages corresponded to GSHPx selenium concentration of ~15.2 μg/L and a Se-P selenium concentration of 32 μg/L, for a total plasma selenium concentration of 87 μg/L (10, 16). Total plasma selenium of 87 μg/L could be considered a normal plasma selenium reference for Europeans. Therefore, our selenium content determinations of the three selenoproteins appear to be good indirect estimates. Moreover, albumin-globulin separation is not very cumbersome, is not expensive in terms of reagents, and can be performed easily for routine analysis.

GSHPx should not be considered a good indicator of selenium status in the elderly, but Se-P may be. GSHPx selenium was decreased in the elderly, whereas Se-P selenium was not (one-way ANOVA among the three groups was not significantly different). However, Se-P selenium was significantly decreased in the group of institutionalized elderly compared with the group of free-living elderly (Student t-test, P = 0.02), suggesting that Se-P could be a better index of selenium deficiency than GSHPx in the elderly. The correlation coefficient was higher between total plasma selenium and Se-P selenium (r = 0.70) than between total plasma selenium and GSHPx selenium (r = 0.42). Previous direct determinations of Se-P among subjects of European countries have also demonstrated a similar correlation coefficient (r = 0.68) between serum Se-P and selenium (17). Moreover, another group of researchers (18) found a higher correlation between a selenium fraction (probably Se-P) and selenium than between extracellular GSHPx and selenium. The correlation coefficient of 0.42 between plasma selenium and GSHPx selenium is similar to those reported between serum selenium and extracellular GSHPx activity (0.49 – 0.58) (17). Previous direct determinations of Se-P have also demonstrated that the Se-P concentration is not related to age (11, 17).

The selenium associated with albumin was significantly lower in the institutionalized elderly compared with the younger group. Selenium associated with albumin is considered a good reflection of selenium intake. Because dietary selenium intakes were almost identical in the three groups (Table 1), the selenomethionine intake should be nearly the same for the three groups. Thus, the amount of selenium associated with albumin is dependent only on the plasma concentration of albumin. Plasma albumin was significantly lower in the institutionalized group than in the two other groups. This non-selenoprotein compartment cannot be indicative of the presence of selenium deficiency. This decrease is more related to the decrease of total plasma albumin observed in the group of institutionalized elderly.

Published studies are not in agreement concerning a progressive decline of selenium status with age, but several studies have reported a decrease of selenium status with institutionalization (19 – 22). Plasma selenium concentrations are related in part to dietary protein intake (19, 20), but the dietary protein and selenium intakes were quite similar for the two groups of elderly subjects in our previous study (12). In addition to negative effects on food intake, hospitalization and nutrient-medication interactions could represent additional factors in poor selenium status (12). The decrease of plasma GSHPx in the elderly is not clear-cut, but several lines of evidence suggest a decrease of GSHPx in old age (21, 23, 24).

We conclude that the method of albumin-globulin separation can be used to measure the proportion of plasma selenium located in the three selenoprotein compartments.

<table>
<thead>
<tr>
<th>Table 1. Plasma albumin, selenium status, and selenium content of the plasma selenoproteins of the three groups of women. *</th>
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<tbody>
<tr>
<td><strong>Selenium in GSHPx, μg/L</strong></td>
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<tr>
<td><strong>Selenium in Se-P, μg/L</strong></td>
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<tr>
<td><strong>Selenium in albumin, μg/L</strong></td>
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<tr>
<td><strong>Albumin, g/L</strong></td>
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<tr>
<td><strong>Selenium, μg/L</strong></td>
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<tr>
<td><strong>GSHPx, U/L</strong></td>
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<tr>
<td><strong>Dietary selenium intake, μg/day</strong></td>
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<tr>
<td><strong>Young adults</strong></td>
</tr>
<tr>
<td>(n = 9)</td>
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<tr>
<td>19.6 ± 3.2</td>
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<tr>
<td>27.8 ± 11.8</td>
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<tr>
<td>36.9 ± 7.1</td>
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<tr>
<td>44 ± 6</td>
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<tr>
<td>84.3 ± 14.1</td>
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<tr>
<td>391 ± 64</td>
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<td>35.1 ± 6.6</td>
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</table>

* Results given as mean ± SD.

* * P < 0.05 vs mean for young adults.

* c P = 0.02 (Student t-test) vs mean for free-living elderly.

* Results from previous study (12).

* d P < 0.05 vs mean for free-living elderly.
Indirect determination of Se-P should be considered a good estimate of Se-P. Moreover, Se-P, which is not related to age, seems to be a better index of selenium deficiency in the elderly than selenium associated with GSHPx.

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References