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Glutathione peroxidase, selenoprotein P and selenium in serum of elderly subjects in relation to other biomarkers of nutritional status and food intake

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The relation of the intake of selenium and different food groups to serum levels of selenium, glutathione peroxidase and selenoprotein P*, and urinary selenium was studied in 50- to 69-year-old subjects (101 men, 105 women). Blood was sampled six times during the course of 1 year, and during the same time the subjects performed six 3-day weighed dietary records. The concentration of glutathione peroxidase in serum was higher among men (4.3 (0.74) mg/L) (mean (SD)) than among women (4.0 (0.76) mg/L,* $P < 0.03$*), whereas men had lower serum selenium (1.10 (0.17)* μ*mol/L) vs. 1.17 (0.19)* μ*mol/L,* $P < 0.01$*). The intake of selenium among men was calculated to be 36 (18–54)* μ*g/day and among women 29 (13–48)* μ*g/day (geometric means (90% central range)), but there was no significant gender difference when the selenium intake was corrected for energy intake. No difference between men and women was observed for serum selenoprotein P (1.47 (0.25) a.u. versus 1.47 (0.24) a.u. (mean(SD)) or urinary selenium excretion (0.31 (0.09)* μ*mol/d vs. 0.27 (0.08)* μ*mol/d). Serum selenium was significantly associated with selenoprotein P in both sexes but with glutathione peroxidase only in men. Serum selenium had a marked association with urinary selenium excretion. Selenium intake was significantly associated with serum selenium in men, and with selenoprotein P and urinary selenium in women. Among 11 major food groups, the intakes of fish and milk products were significantly associated with biomarkers of selenium status among women. Calculation of the association between biomarkers of selenium status and variables on intake and serum content of nutrients using multiple regression analysis resulted in most cases in low explanatory power. The variables most consistently related to more than one variable of selenium status were serum levels of retinol,* α*-tocopherol and eicosapentaenoic acid, and intake of protein. The study shows that individual biomarkers of selenium status are associated differently to variables on dietary intake and nutritional status.* (J. Nutr. Biochem. 8:508–517, 1997) © *Elsevier Science Inc. 1997*

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Introduction

The concentration of selenium in plasma or blood is often used as an index of selenium status in epidemiologic and clinical studies.[1–6] The plasma level responds more rapidly to changes in selenium balance than the blood level. The interpretation of such changes is complicated by the fact that the chemical composition of selenium compounds in plasma and erythrocytes is only partly known. It has been established for some time that extracellular glutathione

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peroxidase (eGSHPx) is a selenoprotein in plasma,[7] and recently another selenoprotein, selenoprotein P, has been found in human plasma, accounting for a large part of plasma selenium.[8] Radioimmunoassays have been developed to measure these proteins.[8–10]

The concentration of selenium and selenoproteins in tissues can be influenced by selenium intake,[11–13] but the relation of the intake of different foods to selenium and selenoprotein concentrations in humans is incompletely known. There is also a need to assess the influence of other life-style variables than dietary intake on biomarkers of selenium status. Moreover, because glutathione peroxidases and maybe other selenoproteins are believed to act as components of the oxidant defence, their interaction with other antioxidants needs further study. In the present study the mutual relationships of eGSHPx, selenoprotein P, and selenium in serum, and selenium in urine were investigated. In addition their associations to dietary intake, some life-style variables and serum antioxidant levels were studied.

Methods and materials

Study design

Nine hundred subjects (50 to 69 years old) living in Malmö were randomly selected from the population register and invited to participate in the study as described in detail elsewhere.[14–16] To screen the subjects for inclusion in the study, they completed a medical questionnaire and underwent a medical examination, including assays of clinical-chemical variables. Of the 900 subjects, 552 (61%) took part in this health survey.[15] Nineteen subjects were excluded for medical reasons or because of language problems, and from the remainder, 375 subjects were offered to participate in this dietary study. As evidenced by participation in the first blood sampling, 339 subjects actually started the study, and 206 subjects (101 men and 105 women) completed the study based on the participation in blood sampling and on the quality of dietary information provided. Of these subjects, 45 were smokers (31 men and 14 women).

Dietary assessment

During the course of approximately 1 year, the subjects performed six weighed 3-day dietary records, and six blood samples were taken during the same period. The sampling periods were evenly distributed during the year, and each 3-day record was performed within 1 week. For technical reasons, the first sampling preceded the first dietary record period by a mean of 38 days, and the last sampling was performed 26 days after the sixth dietary record period. For the four intermediary periods, the interval between the first day of record and the blood sampling was usually 1 day. The food data were coded by nutritionists and the nutrient intake was calculated using the Swedish Food Tables covering 34 nutrients for approximately 1,500 food items.[16,17] Besides, 24-hr urinary specimens from 66 subjects (34 men and 32 women) were obtained on 8 days during part of the experimental period.

Biochemical methods

Blood samples were obtained from an antecubital vein using vacuum tubes containing a serum separator. On the first and sixth occasions, the samples were drawn in the morning, while the subjects had been fasting for >8 hr, and on the other occasions the subjects were in the nonfasting state. After centrifugation of blood, serum was stored at −70°C until analysis. In the first serum sample, the concentrations of cholesterol, HDL-cholesterol, triglyceride, creatinine, albumin, cobalamin, folate, and alanine aminotransferase were measured.

Serum selenium, α-tocopherol, retinol, total carotenoids, and ascorbic acid were measured in six samples from 206 subjects, and eGSHPx and selenoprotein *P* were measured in six samples from 127 subjects. Urinary selenium was measured in eight daily samples from 66 subjects. Fatty acid composition of serum phosphatidylcholine was measured in the first sample from 206 subjects.

eGSHPx was assayed by a radioimmunoassay using a polyclonal antiserum as described elsewhere.[9] A calibration curve was constructed using different amounts of a normal human serum pool, and the within-assay and between-assay variation in the analysis of eGSHPx concentration were 7.0% and 7.4% (c.v.), respectively. Selenoprotein P was measured by a similar radioimmunoassay as described elsewhere.[10] Serum selenium was analyzed by atomic absorption spectrophotometry using Zeeman background correction.[18] Urinary selenium was measured using a fluorimetric method.[19] Retinol, α-tocopherol, carotenoids, ascorbic acid and fatty acid composition were measured as described previously,[20] and the data will be described in detail elsewhere.

Statistical calculations

Data on nutrient and food intake were calculated as the mean value of 18 days of weighed food records. These data were $\log_e$-transformed to improve normality, and transformed data were used unless indicated. Energy-adjusted intakes of selenium and major food groups were calculated as described by Stryker et al.[21] Data on ascorbic acid, creatinine, selenium, eGSHPx, and selenoprotein P in serum and urinary selenium were used without transformation, other data on serum concentrations were $\log_e$-transformed. Data on smoking habits were obtained from the questionnaire,[15] and in some calculations, the data was expressed as the amount of tobacco used (g/d) and in others smokers and nonsmokers were indicated by a categorical variable. Data on alcohol intake were obtained from the dietary records. Data on age, body mass index, tobacco, and alcohol habits were used without transformation. In comparison of selenium intake among men and women the data were backtransformed and expressed as the ratio of geometric means with its 95% confidence interval.[22] The associations of serum and urinary selenium and serum selenoprotein levels to other variables were assessed from linear correlation coefficients and from multiple linear regression analysis with an index of selenium status as the dependent variable and other variables as independent ones. Gender was indicated by a categorical variable (men = 0, women = 1).

Data on intake and serum levels were obtained six times during the study. Sampling No. 1 was performed in September–October, No. 2 in December to February, No. 3 in February to April, No. 4 in April to June, No. 5 in June to August, and No. 6 in September to October. Systematic seasonal variation in selenium intake or biomarkers of selenium status in serum at the six occasions was evaluated by Friedman's two-way analysis of variance (ANOVA).

Results

Selenium intake and selenium status

The dietary selenium intake was higher in men than in women and the difference was similar at all sampling times (*Table 1*). The geometric mean ratio (95% CI) men/women with respect to the mean intake at six recording periods was 1.26 (1.15, 1.38), but the energy-adjusted selenium intake was not significantly different in men and women (ratio:

Table 1 Selenium status in different seasons

	Sampling no.							
	1	2	3	4	5	6	Mean	*n*
Selenium intake (μg/d)								
Men	32	31	31	34	31	33	33	101
Women	26	25	24	26	27	23	26	105
Ratio	1.26	1.21	1.33	1.33	1.16	1.39	1.26	
Men-women (95% CI)	(1.09, 1.45)	(1.05, 1.39)	(1.16, 1.53)	(1.16, 1.52)	(1.00, 1.35)	(1.20, 1.60)	(1.15, 1.38)	
Serum selenium (μmol/l)								
Men	1.09	1.12	1.09	1.12	1.09	1.10	1.10	99–101
Women	1.16	1.17	1.17	1.18	1.18	1.15	1.17	102–105
Difference	−0.07	−0.05	−0.07	−0.07	−0.09	−0.06	−0.07	
Men-women (95% CI)	(−0.12, −0.01)	(−0.11, 0.01)	(−0.13, −0.02)	(−0.12, −0.01)	(−0.15, −0.03)	(−0.11, −0.03)	(−0.12, −0.02)	
eGSHPx (mg/l)								
Men	4.05	4.36	4.34	4.45	4.29	4.09	4.30	55–98
Women	3.99	4.01	3.96	4.03	3.86	3.80	4.00	71–99
Difference	0.06	0.35	0.38	0.42	0.26	0.12	0.30	
Men-women (95% CI)	(−0.17, 0.29)	(0.07, 0.63)	(0.06, 0.69)	(0.12, 0.72)	(−0.01, 0.52)	(−0.12, 0.36)	(0.04, 0.57)	
Selenoprotein P (a.u.)								
Men	1.48	1.51	1.48	1.47	1.47	1.46	1.47	55–82
Women	1.49	1.47	1.47	1.49	1.48	1.48	1.47	71–90
Difference	−0.01	0.04	0.00	−0.01	−0.01	−0.02	0.00	
Men-women (95% CI)	(−0.09, 0.07)	(−0.06, 0.14)	(−0.10, 0.10)	(−0.12, 0.09)	(−0.10, 0.08)	(−0.09, 0.06)	(−0.09, 0.09)	

Dietary selenium intake and serum selenium, glutathione peroxidase (eGSHPx), and selenoprotein P concentrations in 50- to 69-year-old subjects at six samplings during 1 year. Data on selenium intake are expressed as geometric means and the comparison between men and women is expressed as the ratio of geometric means with its 95% confidence interval. Data on serum levels are given as arithmetic means together with the gender difference (95% CI).

1.00 (0.92, 1.09)). Men had a significantly lower concentration of serum selenium, and the mean from six samplings for men was 6% lower than that of women, and a similar pattern was observed for all six occasions (*Table 1*).

In contrast, the mean concentration of eGSHPx in serum at six samplings was significantly higher in men than in women (7%), and in this case a similar pattern was observed at the four intermediary samplings, whereas the differences at the first and sixth samplings seemed smaller (*Table 2*). In contrast, there was no significant difference in the concentration of selenoprotein P in serum between men and women.

Friedman's two-way ANOVA indicated significant seasonal variation in glutathione peroxidase ($P = 0.004$) and serum selenium ($P = 0.003$) concentrations in the male group. Glutatione peroxidase concentrations were marginally lower at samplings 1 and 6, and serum selenium was highest at samplings 2 and 4, but the differences between samplings in this respect were small. There was no significant systematic seasonal variation in serum selenoprotein P or selenium intake in the whole group or the gender groups.

The mean 24-hr urinary selenium excretion measured on 8 days tended to be higher in men (0.31 (0.09) μmol/d) than in women (0.27 (0.08) μmol/d), but the difference (95% CI) 0.03 (−0.01, 0.08) μmol/d was not statistically significant. It could be calculated that the urinary excretion of selenium corresponded to 75% (32) (men) and 78% (31) (women) (mean(SD)) of the calculated dietary selenium intake.

Associations among biomarkers of selenium status

Serum selenium was correlated to eGSHPx in men but not in women (*Table 2*). Serum selenium was also significantly associated with serum selenoprotein P in both sexes (*Figure 1*). There were also strong positive correlations between serum selenium and urinary selenium excretion in both men and women. Selenoprotein P was significantly associated with urinary selenium excretion in men but not in women. eGSHPx was not significantly correlated to selenoprotein P or to urinary selenium.

Relation between the intake of selenium and other nutrients and biomarkers of selenium status

The mean selenium intake was associated with serum selenium in men, and the association tended to increase after correction for energy intake (*Table 2*). In contrast, selenium intake was associated with selenoprotein P only in women and to urinary selenium excretion again only in women after correction for energy intake. No significant correlations between eGSHPx and selenium intake were observed.

Besides the associations between selenium intake and selenium status there were only few significant correlations between nutrient intake and biomarkers of selenium status (*Table 2*). Several significant associations between protein intake and selenium status were observed, which can be explained by the preferential occurrence of selenium in protein-rich foods such as meat, milk, fish, and egg.

The association between selenium status and nutrient

Table 2 Biochemical markers of selenium status in relation to dietary intake

	Serum selenium (μmol/l)	eGSHPx (mg/l)	Selenoprotein P (a.u.)	Urinary selenium (μmol/d)
Men				
eGSHPx	0.31 ($P = 0.020$)			
Selenoprotein P	0.59 ($P < 0.001$)	0.15		
Urinary selenium	0.61 ($P < 0.001$)	−0.09	0.64 ($P = 0.019$)	
Energy	−0.08	0.07	−0.02	0.14
Fat (E%)	−0.06	0.14	−0.01	0.06
Protein (E%)	0.24 ($P = 0.018$)	0.01	0.16	0.08
Carbohydrate (E%)	−0.01	−0.02	0.02	−0.09
Alcohol (E%)	0.10	0.10	0.08	0.04
Selenium	0.23 ($P = 0.022$)	0.10	0.14	0.20
Selenium[a]	0.30 ($P = 0.003$)	0.08	0.20	0.17
Retinol equiv.	−0.01	0.08	0.15	−0.08
α-Tocopherol	0.02	0.17	0.10	0.14
Ascorbic acid	0.05	−0.16	0.07	−0.08
Women				
eGSHPx	0.02			
Selenoprotein P	0.35 ($P = 0.003$)	0.23 ($P = 0.055$)		
Urinary selenium	0.58 ($P = 0.001$)	0.15	0.22	
Energy	−0.07	0.05	−0.07	−0.27
Fat (E%)	−0.08	−0.09	−0.07	0.29
Protein (E%)	0.03	0.31 ($P = 0.009$)	0.34 ($P = 0.004$)	0.51 ($P = 0.004$)
Carbohydrate (E%)	−0.00	0.01	−0.11	0.18
Alcohol (E%)	0.10	0.10	0.08	0.04
Selenium	0.10	0.22 ($P = 0.07$)	0.30 ($P = 0.012$)	0.27
Selenium[a]	0.13	0.21 ($P = 0.008$)	0.34 ($P = 0.004$)	0.37 ($P = 0.04$)
Retinol equiv.	−0.05	0.28 ($P = 0.019$)	0.07	0.30
α-Tocopherol	0.08	−0.00	0.00	−0.29
Ascorbic acid	0.00	0.01	−0.08	0.05

Pearson correlation coefficients between different indices of selenium status and the intake of selenium and some other nutrients. Log_e-transformed data on nutrient intake (mean from 18 days) were used except for alcohol. Data on serum levels were means from six samples and urinary data were means from eight samples. The number of subjects (men/women) was 101/105 for serum selenium, 56/71 for eGSHPx and selenoprotein *P*, and 30/30 for urinary selenium. *P* values are given if ≤0.10.
[a], corrected for energy intake

intake was further studied by multiple regression analysis. Serum selenium, eGSHPx, selenoprotein P, or urinary selenium was set as the dependent variable, and the intake of selenium, energy, protein, total carbohydrates, alcohol, total fat, fibre, ascorbic acid, β-carotene, retinol, α-tocopherol, folate, and, moreover, gender as independent variables.

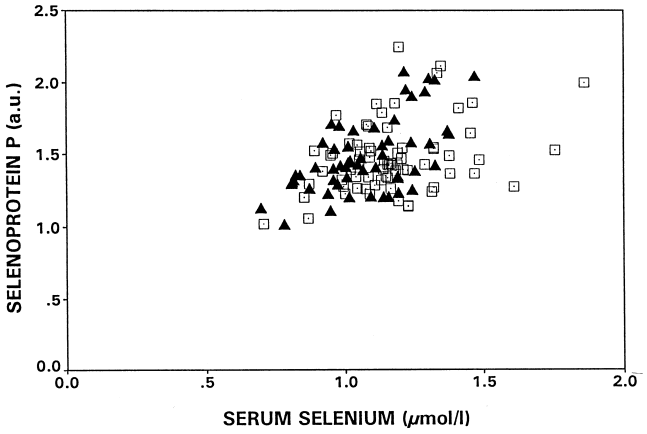

Figure 1 Relation between selenoprotein P and selenium in serum for 126 (55 men/71 women) 50–69-year-old residents of Malmö. The correlation coefficient for men was 0.59, $P < 0.001$ and for women 0.35, $P = 0.003$ (*triangles*, men; *squares*, women).

Only low explanatory power was observed when serum selenium in the total study group was the dependent variable, the adjusted R^2 being 0.07 when three variables (intakes of selenium ($P = 0.009(+)$), energy ($P = 0.0001(-)$) and α-tocopherol ($P = 0.08(+)$) were kept in the model. (The *P* value indicates the significance of the association, and the ensuing sign shows whether it was positive or negative). When the same calculation was made for the male group similar results were obtained, but in females no significant associations were observed.

When eGSHPx was the dependent variable, an adjusted R^2 of 0.09 was observed with only retinol intake as independent variable ($P = 0.0003(+)$). For the male group, no significant associations were found. In the female group, the adjusted R^2 was 0.15 with three variables remaining, energy ($P = 0.04(-)$), protein ($P = 0.05(+)$), and retinol ($P = 0.05(+)$).

When selenoprotein P was the dependent variable, a model with two independent variables was obtained, selenium intake ($P = 0.002(+)$) and fat intake ($P = 0.03(-)$) with an adjusted R^2 of 0.07. In the male group no such associations were found, and in the female group and adjusted R^2 of 0.07 was obtained with selenium intake as the only variable ($P = 0.013(+)$).

Finally, when urinary selenium was the dependent variable an adjusted R^2 of 0.40 was found with four intake

variables remaining (protein ($P = 0.022(+)$), fat ($P = 0.052(-)$), β-carotene ($P = 0.004(+)$), and fibre ($P = 0.016(-)$)). In the male group, an adjusted R^2 of 0.34 was obtained with six intake variables remaining in the model (energy ($P = 0.0009(+)$), carbohydrates ($P = 0.001(-)$), fibre ($P = 0.0013(+)$), retinol ($P = 0.02(-)$), and folate ($P = 0.09(-)$)). For the female group, the adjusted R^2 was 0.40 with four variables remaining (protein ($P = 0.02(+)$), fat ($P = 0.05(-)$), fibre ($P = 0.016(-)$), and β-carotene ($P = 0.004(+)$)).

Thus, also these calculations showed that selenium intake was associated to serum selenium and selenoprotein P but not to eGSHPx. The complex pattern of variables found to be related to urinary selenium may be attributable to the smaller number of subjects used or to strong intercorrelations between independent variables. There were no associations between selenium status or intake and smoking habits.

Relation between the intake of food groups and selenium status

For this calculation, the intake of 11 major food groups was corrected for energy intake, which, in most cases tended to give somewhat higher correlation coefficients between food intake and selenium status than without such correction. The most consistent positive associations to selenium status were observed for the women's intake of milk products ($r = 0.08$, $0.27/P = 0.02/$ and $0.45/P = 0.01/$ for serum selenium, selenoprotein P and urinary selenium, respectively) and fish ($r = 0.22/P = 0.023/$, $0.22/P = 0.06/$ and $0.40/P = 0.03/$ for serum selenium, selenoprotein P and urinary selenium, respectively). No significant correlations were observed between eGSHPx and the intake of food groups.

Multiple regression analysis was used to further study the association between serum selenium (dependent variable) and the intake of eleven food groups (independent variables in addition to energy intake and gender). In a model where fish intake ($P = 0.003(+)$) and energy intake ($P = 0.004(-)$) were the remaining independent variables, the adjusted R^2 was 0.06. For the male group, an adjusted R^2 of 0.11 was obtained with four variables, intake of potato ($P = 0.005(-)$), meat ($P = 0.02(+)$), fish ($P = 0.04(+)$), and rice plus pasta ($P = 0.05(+)$), and for the female group, the adjusted R^2 was 0.14 with three variables: fish intake ($P = 0.07(+)$), egg intake ($P = 0.09(-)$), and intake of rice plus pasta ($P = 0.0015(-)$). Also, when eGSHPx was the dependent variable, a low adjusted R^2 of 0.06 was observed with two independent variables remaining (intake of meat ($P = 0.04(+)$) and bread ($P = 0.05(+)$)). Separate calculations for the gender groups showed no significant associations.

With the selenoprotein P level as the dependent variable, a model with four independent variables emerged, intake of energy ($P = 0.04(-)$), fish ($P = 0.02(+)$), egg ($P = 0.02(+)$), and cereals plus grains ($P = 0.09(+)$) and an adjusted R^2 of 0.07. No associations were found in the male group, and for women three remaining variables were obtained, intake of fish ($P = 0.04(+)$), egg ($P = 0.01(+)$), and fats ($P = 0.01(-)$) with an adjusted R^2 of 0.15. When urinary selenium was the dependent variable an adjusted R^2 of 0.10 was found with two independent variables (intake of fish ($P = 0.02(+)$) and the gender variable ($P = 0.06(+)$). No consistent pattern was found for each gender group.

Thus, also in these calculations the intake of fish and to some extent those of egg and meat showed positive associations to selenium status. The reason why no relation was found for milk products as in the correlation analysis, is probably the presence of strong intercorrelations between independent variables.

Relation between concentrations of nutrients in serum and selenium status

Serum retinol was positively correlated to serum selenium, negatively to eGSHPx and also showed some association to selenoprotein P and urinary selenium excretion (*Table 3*). Also serum α-tocopherol was positively associated to serum selenium (*Figure 2*), selenoprotein P and urinary selenium. Significant associations between selenium status and the fatty acid composition of serum phosphatidylcholine were also observed, especially between selenium or selenoprotein P and individual or total n-3 fatty acids (*Table 3*). This can probably be explained by the association of these fatty acids with fish intake.

Relation of selenium status to general clinical-chemical variables

Because a major part of the eGSHPx in serum is produced in the kidney, the association of its concentration to serum creatinine was studied. Indeed, the inverse correlation observed between creatinine and eGSHPx was the most consistent association to clinical-chemical variables in both sexes (*Table 3*). Similar correlations were observed when the mean eGSHPx from six samplings, the mean of the first and sixth samplings, or data from the first sampling only was used. In most cases the correlation was not materially affected when the few subjects with supernormal values of serum creatinine were excluded from the calculation. Urinary selenium excretion showed a positive association to creatinine in both sexes, and selenoprotein P showed a positive association in men. As expected also serum retinol was correlated to serum creatinine. Among serum lipids, HDL cholesterol was associated with eGSHPx concentration in both sexes, but no mechanism for this association is known.

The associations between serum selenium and serum analytes (ascorbic acid, α-tocopherol, carotenoids, retinol, cobalamin, folate, creatinine, cholesterol, HDL-cholesterol, triglyceride, albumin, alanine aminotransferase) and gender were also studied using multiple regression analysis. In the whole study group, an adjusted R^2 of 0.16 was found with six variables in the model with P values <0.05 for cobalamin, α-tocopherol, and retinol, and <0.10 for albumin, ascorbic acid, and the gender variable (all associations positive). For the male group a significant association was observed only for serum α-tocopherol ($P = 0.01(+)$) and albumin ($P = 0.014(+)$) with an adjusted R^2 of 0.11. For women the adjusted R^2 was 0.25 with P values <0.05 for

Table 3 Relationships of biochemical markers of selenium status to plasma analysis and anthropometrical variables

	Serum selenium (μmol/l)	eGSHPx (mg/l)	Selenoprotein P (a.u.)	Urinary selenium (μmol/d)
Men				
α-Tocopherol	0.35 ($P < 0.001$)	0.29 ($P = 0.03$)	0.27 ($P = 0.047$)	0.44 ($P = 0.016$)
Retinol	0.28 ($P = 0.004$)	−0.31 ($P = 0.019$)	0.35 ($P = 0.009$)	0.28
Carotenoids	0.19 ($P = 0.06$)	0.09	0.30 ($P = 0.025$)	0.23
Ascorbic acid	0.16	0.06	0.29 ($P = 0.031$)	0.20
18:2	−0.13	0.16	−0.12	0.13
20:4	0.10	0.10	0.13	0.18
Total n-6	−0.14	0.19 ($P = 0.07$)	−0.08	0.15
20:5	0.17 ($P = 0.08$)	−0.11	0.22 ($P = 0.044$)	0.21
22:6	0.20 ($P = 0.043$)	−0.13	0.17	0.08
Total n-3	0.20 ($P = 0.048$)	−0.14	0.19 ($P = 0.10$)	0.12
Cholesterol	0.16	0.05	0.03	0.16
HDL cholesterol	−0.02	0.18 ($P = 0.08$)	−0.12	0.05
Triglyceride	0.18 ($P = 0.08$)	−0.13	0.29 ($P = 0.011$)	0.16
Albumin	0.25 ($P = 0.014$)	0.14	0.06	0.09
Creatinine	0.05	−0.34 ($P = 0.001$)	0.27 ($P = 0.016$)	0.45 ($P = 0.016$)
Cobalamin	0.18 ($P = 0.08$)	0.04	0.06	0.25
Folate	0.07	0.05	−0.07	0.15
Body mass index	0.20 ($P = 0.046$)	−0.11	0.16	0.17
Body weight	0.21 ($P = 0.038$)	−0.18 ($P = 0.09$)	0.23 ($P = 0.040$)	0.33 ($P = 0.09$)
Waist-hip-ratio	0.07	−0.34 ($P = 0.001$)	0.12	0.39 ($P = 0.037$)
Age	0.08	−0.05	−0.05	0.27
Women				
α-Tocopherol	0.45 ($P < 0.001$)	−0.17	0.20 ($P = 0.10$)	0.33 ($P = 0.07$)
Retinol	0.32 ($P = 0.001$)	−0.38 ($P = 0.001$)	0.17	0.39 ($P = 0.033$)
Carotenoids	0.02	0.08	0.07	−0.40 ($P = 0.029$)
Ascorbic acid	0.18 ($P = 0.06$)	0.10	0.10	−0.05
18:2	−0.22 ($P = 0.023$)	−0.02	−0.12	−0.47 ($P = 0.008$)
20:4	0.01	0.21 ($P = 0.034$)	−0.01	0.27
Total n-6	−0.24 ($P = 0.013$)	0.11	−0.16	−0.30
20:5	0.25 ($P = 0.012$)	0.05	0.33 ($P = 0.001$)	0.28
22:6	0.08	−0.01	0.12	0.30
Total n-3	0.15	0.00	0.20 ($P = 0.056$)	0.31 ($P = 0.09$)
Cholesterol	0.17 ($P = 0.09$)	−0.17 ($P = 0.09$)	0.13	0.15
HDL cholesterol	−0.03	0.24 ($P = 0.016$)	0.08	−0.32 ($P = 0.09$)
Triglyceride	0.04	−0.33 ($P = 0.001$)	−0.10	0.44 ($P = 0.017$)
Albumin	0.16	−0.00	0.01	0.02
Creatinine	0.01	−0.42 ($P < 0.001$)	0.11	0.19
Cobalamin	0.22 ($P = 0.025$)	0.19 ($P = 0.06$)	0.23 ($P = 0.03$)	0.10
Folate	0.07	0.36 ($P < 0.001$)	0.11	0.01
Body mass index	−0.09	−0.20 ($P = 0.053$)	−0.07	0.37 ($P = 0.046$)
Body weight	−0.09	−0.13	−0.10	0.44 ($P = 0.016$)
Waist-hip-ratio	−0.03	−0.07	−0.05	0.08
Age	0.06	−0.26 ($P = 0.011$)	0.01	0.26

Pearson correlation coefficients of different indices of selenium status to the concentration in serum of other nutrients, lipids and other components, anthropometrical indices, and PUFA composition of serum phosphatidylcholine (expressed as % of total fatty acids and then $\log_e$-transformed). Log_e-transformed data on serum lipids, vitamins and albumin were used. Data on serum levels of selenium components, α-tocopherol, retinol, carotenoids, and ascorbic acid were means from six samples and urinary data were means from eight samples. Other data on serum levels were obtained from the first sampling. The number of subjects (men/women) was 101/103 for serum selenium, 56/70 for eGSHPx, 55/70 for selenoprotein P and 30/30 for urinary selenium. Fatty acids are abbreviated as no. of carbon atoms: number of double bonds. *P* values are given if ≤0.10.

cobalamin, carotenoids (negative), retinol, ascorbic acid and $P = 0.06$ for alanine aminotransferase. When eGSHPx was the dependent variable an adjusted R^2 of 0.20 was obtained with five independent variables (gender ($P = 0.0006(-)$), HDL-cholesterol ($P = 0.025(+)$), folate ($P = 0.012(+)$), retinol ($P = 0.05(-)$), and creatinine ($P = 0.0001(-)$)). Among men, the adjusted R^2 was 0.13 with two variables in the model (albumin ($P = 0.05(+)$) and creatinine ($P = 0.0003(-)$)) and among women 0.29 with three variables remaining (triglyceride ($P = 0.004(-)$), folate ($P = 0.004(+)$), and creatinine ($P = 0.002(-)$)). Finally, when selenoprotein P was the dependent variable, a model with four independent variables (ascorbic acid ($P = 0.01(+)$), cobalamin ($P = 0.04(+)$), retinol ($P = 0.05(+)$), and creatinine ($P = 0.07(+)$) gave an adjusted R^2 of 0.10. Among men, an adjusted R^2 of 0.13 was found with retinol ($P = 0.02(+)$) and α-tocopherol ($P = 0.06(+)$) as explanatory variables, and in women, an adjusted R^2 of 0.10 was found with cobalamin ($P = 0.03(+)$), retinol ($P = 0.06(+)$), and ascorbic acid ($P = 0.08(+)$) as independent variables.

After this search within different categories of variables, the 18 variables most associated to selenium status and the gender variable were entered in the same model as indepen-

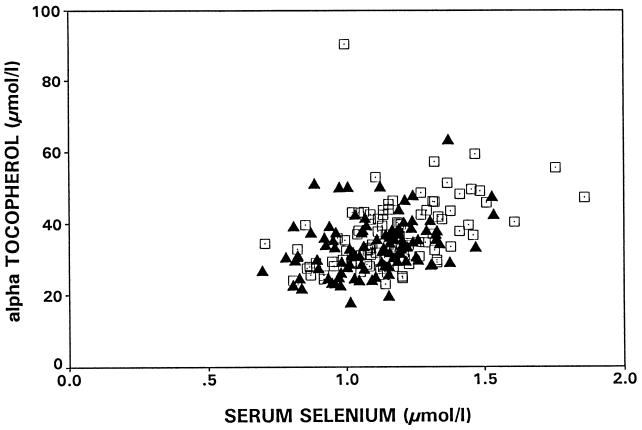

Figure 2 Relation between the concentrations of selenium and α-tocopherol in serum among 206 (101 men/105 women) 50–69-year-old residents of Malmö. The correlation coefficient for men was 0.35, $P = 0.001$ and for women 0.45, $P < 0.001$ (*triangles*, men; *squares*, women).

dent variables (intake of selenium, protein, retinol, α-tocopherol, fibre, energy, meat, egg, fish and fats, serum levels of retinol, cobalamin, α-tocopherol, ascorbic acid, creatinine, albumin and eicosapentaenoic acid, and body mass index). Intake of fish and fats, serum levels of α-tocopherol and retinol and the proportion of eicosapentaenoic acid in serum phosphatidylcholine were the variables significantly associated to serum selenium levels in this model yielding an adjusted R^2 of 0.30 (*Table 4*). For eGSHPx the adjusted R^2 was 0.26 with the following remaining variables, energy and protein intake, serum creatinine and retinol, and gender. For selenoprotein P levels the adjusted R^2 was lower, 0.12, and the remaining variables were intake of energy and α-tocopherol, albumin and retinol levels, and the proportion of eicosapentaenoic acid. Finally, with urinary selenium excretion as the dependent variable an adjusted R^2 of 0.37 was obtained with four variables in the model, serum α-tocopherol, proportion of eicosapentaenoic acid, body mass index, and the gender variable. Thus, the variables most consistently related to more than one variable on selenium status were serum levels of retinol, α-tocopherol and eicosapentaenoic acid, and intake of protein. It would have been expected that the intake of fish or selenium would have been more closely related, but maybe the eicosapentaenoic acid variable reflected these relationships.

Discussion

The established metabolic role of selenium in mammalian species is tied to its presence in selenocysteine residues in specific selenoproteins. One of them, eGSHPx, catalyzes the reduction of different hydroperoxides using glutathione or the thioredoxin and glutaredoxin systems as electron donors.[7,23] The function of the other selenoprotein demonstrated in human plasma, selenoprotein P, is unknown, but it may be involved in the oxidant defense.[24] This study examines the use of the serum levels of these selenoproteins as biomarkers of selenium status based on their relationships to other markers of selenium status, to dietary intake, and to circulating levels of other nutrients.

Table 4 Variables associated to four indices of selenium status in 50–69 year-old subjects using multiple regression analysis

Variables	*B*	95% CI	*P*	Multiple *R*	Adj. R^2
Serum selenium					
Fish intake	0.033	0.002, 0.064	0.035		
Fat intake	−0.012	−0.20, −0.038	0.004		
S-α-Tocopherol	0.31	0.20, 0.41	<0.001		
S-Retinol	0.16	0.037, 0.27	0.010		
S-20:5	0.077	0.025, 0.13	0.004		
				0.56	0.30
eGSHPx					
Gender	−0.52	−0.87, −0.17	0.004		
Energy intake	−1.62	−2.62, −0.62	0.002		
Protein intake	1.96	1.00, 2.92	<0.001		
S-Retinol	−1.11	−1.73, −0.48	<0.001		
S-Creatinine	−0.014	−0.023, −0.0045	0.004		
				0.54	0.26
Selenoprotein P					
Energy intake	−0.22	−0.43, −0.023	0.029		
α-Tocopherol intake	0.15	−0.002, 0.30	0.054		
S-Albumin	−0.76	−1.39, −0.13	0.019		
S-20:5	0.080	−0.015, 0.17	0.098		
S-Retinol	0.38	0.16, 0.59	<0.001		
				0.40	0.12
Urinary selenium					
Gender	−0.051	−0.088, −0.013	0.009		
Body mass index	0.007	0.002, 0.013	0.012		
S-α-Tocopherol	0.21	0.12, 0.30	<0.001		
S-20:5	0.070	0.025, 0.11	0.003		
				0.64	0.37

The prefix S- indicates that the variable is a serum analyte.

In most previous studies on the relation between serum selenium and GSHPx, the latter has been measured as enzymatic activity. In study groups with similar selenium status as that in the present study, serum selenium was correlated to serum GSHPx activity ($r = 0.49$, $P < 0.001$) in 483 Swedish 68-year-old men[25] and in subjects from Northern Ireland ($r = 0.58$ ($P < 0.001$) in 52 men, and $r = 0.36$ ($P < 0.01$) in 48 women).[26] With the immunoassay used in the present study,[9] a larger sample throughout is possible. A significant association between eGSHPx and serum selenium was found by us only in the male group, and this gender difference has no obvious explanation. The associations between biomarkers are usually closer in groups with low or widely varying selenium status as observed in patients on parenteral nutrition[27] and in Chinese subjects.[2,28] In one study, selenium supplementation raised both plasma glutathione peroxidase activity and plasma selenium, but groups with higher plasma selenium obtained a relatively smaller increase in GSHPx than in selenium concentration,[2] indicating that selenoprotein levels will not reflect changes in selenium intake when it has reached a level saturating the requirement for synthesis of the relevant selenoprotein. If it is assumed that steady state levels of selenoprotein reflect selenium intake, their concentration may also be influenced by different responses in their secretion to or elimination from plasma occurring in individuals with changing selenium status because of a differential regulation of the biosynthesis of individual selenoproteins.[11]

We found a higher serum selenium in women than in men, whereas men had higher eGSHPx in serum. The latter finding agrees with results from another study,[26] whereas discrepant findings on the gender difference in plasma selenium have been reported,[26,29–31] indicating that such differences depend on local factors rather than on a general biologic mechanism. No relations of alcohol use or smoking to serum selenium, eGSHPx, and selenoprotein P were found in the present study. Previously, discrepant results on the relation of alcohol consumption to plasma selenium concentration and GSHPx activity have been reported.[32] The absence of a relation between plasma selenium and smoking habits was also reported in other studies using multiple regression analysis or other methods.[30,31,33]

A variable with a major inverse association to eGSHPx was serum creatinine in agreement with the finding that the kidney is the main source of plasma eGSHPx.[34] In principle, this association could disguise a relationship between eGSHPx and other biomarkers of selenium status, but recalculation of some of the correlation coefficients shown in *Table 2* using instead eGSHPx corrected for serum creatinine gave only marginal differences. Also, a parallel study indicated an inverse association between serum creatinine and eGSHPx,[35] and similar results were found using measurement of glutathione peroxidase activity.[36]

Fewer studies have been made on the association between selenoprotein P and selenium in plasma. The highest correlations were also in this case found in studies of patients on parenteral nutrition,[27] of Chinese subjects with varying selenium status,[37] and, moreover, of subjects from several European countries.[38] In most cases, the correlation coefficients between selenoprotein P and selenium were higher than those between plasma selenium and eGSHPx. This may be attributable to selenoprotein P being a larger fraction of plasma selenium than eGSHPx, and selenoprotein P levels being less influenced by factors unrelated to selenium status such as kidney function.

The associations between selenium intake and biomarkers of selenium status found in this study were generally low. Several additional factors may explain such findings, one being the source of error involved in the use of food composition tables to calculate selenium intake. Moreover, intake of different forms of selenium results in discrepant responses in biomarkers of selenium status, and such effects are difficult to account for with the present study design. In analogy with previously discussed arguments, much higher associations between selenium intake and biomarkers of selenium status were found in areas with high selenium intake.[31,39]

One reason for calculating the relations between nutritional status and food intake instead of nutrient intake is that uncertainties regarding nutrient content of food are avoided. In the common Swedish diet, fish accounts for the largest part of dietary selenium (25%), followed by milk products (20%), meat (21%), and eggs (16%).[40] We found some association of the intake of fish and milk products to selenium status among women. In spite of the high selenium content of fish, its contribution to the selenium used by the body is unclear, because its bioavailability may be low. In a study of middle-aged men a positive association was found between fish intake and plasma selenium,[41] but not with individual plasma selenoproteins.[42] Also, in the present study the associations of fish intake to selenium status were, if anything, closer for serum and urinary selenium than for serum selenoprotein levels. On the other hand, eicosapentaenoic acid seemed to be more closely related to selenoprotein P than serum selenium levels (*Table 3*), which is at variance with previous findings.[41,42] In other studies, serum selenium was instead found to be positively associated to plasma n-6 fatty acids[33,43] and negatively to saturated fatty acids but not to n-3 fatty acids of plasma phospholipids.[43] Another study showed a positive relation between plasma GSHPx activity and arachidonic acid,[44] but our data did not show a significant association on this point.

The close correlation found between the daily urinary excretion of selenium and serum selenium levels suggests that both variables are useful for estimating selenium status in these 50- to 69-year-old subjects as also reported by others.[45] Because the selenium intake was in the lower range in our study, the urinary selenium output probably reflects body stores and blood levels more than recent intake.[45] A recent compilation showed a close relationship between dietary and urinary selenium when data from different populations were plotted,[46] and high correlations have been found, especially in subjects with high intake.[39] Moreover, changes in urinary selenium are usually more pronounced than those in plasma selenium after changes in selenium intake[47] because of rapid renal compensatory mechanisms.[48]

The significant association of serum selenium to retinol and α-tocopherol in serum has no obvious explanation. Maybe subjects eating a surplus of retinol and α-tocopherol

also consume selenium in the upper range, although retinol levels are not usually influenced by retinol intake. Previously, a relation between serum selenium and retinol intake was found.[33] Maybe there is an interaction between selenium and α-tocopherol, both participating in the antioxidant defence and sparing the utilization of each other. Further studies of possible biochemical mechanisms for this are necessary. In general, the present study shows that different biomarkers of selenium status are differently associated to variables on dietary intake and nutritional status.

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