

## Selenoprotein P and glutathione peroxidase (EC 1.11.1.9) in plasma as indices of selenium status in relation to the intake of fish

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(Received 25 February 1994 – Revised 29 June 1994 – Accepted 7 July 1994)

In Sweden fish is considered to be an important source of dietary Se. Therefore Se status was assessed in forty-one middle-aged men with widely varying fish consumption. Glutathione peroxidase (EC 1.11.1.9) and selenoprotein P in plasma were measured by radioimmunoassay. Plasma Se among the men increased slightly with increasing consumption of fish, but no such increases in the concentrations of glutathione peroxidase and selenoprotein P in plasma were observed. Moreover, no correlation was found between plasma Se and glutathione peroxidase or selenoprotein P. Instead, glutathione peroxidase was significantly correlated with selenoprotein P ( $r$  0.73,  $P$  < 0.001), indicating that both glutathione peroxidase and selenoprotein P were functional indicators of Se status in this group. The proportion of plasma Se located in glutathione peroxidase decreased with increasing plasma Se. The results suggest that the Se consumed from fish had no apparent effect on the amount of Se incorporated into the functional selenoproteins of plasma. It is concluded that in some cases selenoproteins may be better biological markers of Se status than the total concentration of Se.

Dietary fish intake: Glutathione peroxidase: Selenoprotein P: Selenium

Fish is an important component of the diet, being rich in protein,  $n-3$  polyunsaturated fatty acids and certain trace elements and vitamins. In Finland it was calculated that in 1980 about one third of the dietary Se was provided from fish (Koivistoinen, 1980). Less is known about the bioavailability and the metabolic fate of Se from dietary fish. Previous studies showed that subjects with high consumption of fish had higher concentrations of Se in plasma than those with low intake (Svensson *et al.* 1992). Moreover, a change to a diet rich in fish increased the Se concentration in plasma (Robinson *et al.* 1978; Thorngren & Åkesson, 1987). Several studies have shown an association between Se concentration and glutathione peroxidase (EC 1.11.1.9) activity in plasma of healthy adults (Åkesson & Steen, 1987; McMaster *et al.* 1990), but little is known of the relationship of fish intake to specific selenoproteins. In addition to extracellular glutathione peroxidase, selenoprotein P has been demonstrated in plasma (Takahashi *et al.* 1987; Burk, 1991; Åkesson *et al.* 1994). In the present study glutathione peroxidase and selenoprotein P in plasma were studied in relation to consumption of fish, using newly developed radioimmunoassay methods.

\* For reprints.

## METHODS

*Study participants*

From an earlier study group (Svensson *et al.* 1992) drawn from three rural areas of south-east Sweden, forty-one middle aged men were selected because of their differing levels of fish consumption. Twenty were classified as high, twelve as moderate and nine as non-consumers of fish, having mean weekly intakes of 1160, 375 and 0 g. Intakes were calculated from completed questionnaires as already described (Svensson *et al.* 1992).

For comparison, Se status was also studied among sixteen patients with a very wide variation in serum Se concentration (0.1–2.9  $\mu\text{mol/l}$ ) as described previously (Huang & Åkesson, 1993). This heterogeneous group was not characterized further and was selected just to indicate changes in selenoprotein levels at more than ten-fold variation in serum Se.

*Analytical methods*

The radioimmunoassay of glutathione peroxidase and selenoprotein P has been described elsewhere (Huang & Åkesson, 1993; Åkesson *et al.* 1994). The measurement of Se has also been described previously (Svensson *et al.* 1992).

*Calculations*

Correlations were calculated as linear correlation coefficients and Spearman's  $\rho$ . One-way ANOVA and Student's *t* test were used for evaluation of differences among groups.

## RESULTS

*Selenoproteins and selenium in plasma*

The concentration of Se in plasma varied among the groups consuming different amounts of fish (Table 1). It was higher in the group with the high intake of fish compared with the groups with no or moderate consumption. On the other hand, the concentrations of selenoprotein P and glutathione peroxidase did not differ significantly among the three groups.

As reported previously (Svensson *et al.* 1992), plasma Se was correlated with the intake of fish and also with the proportion of *n*-3 polyunsaturated fatty acids in plasma phosphatidylcholine. In the present study no correlation between either selenoprotein P or glutathione peroxidase and the intake of total fish, lean fish or oily fish was found. Neither of the two selenoproteins was correlated with the proportion of *n*-3 fatty acids, which varied several-fold among the groups (Svensson *et al.* 1993).

*Relationship between selenoproteins and selenium in plasma*

The correlation between selenoprotein P and Se was  $-0.29$  (Fig. 1). Similarly, plasma glutathione peroxidase had no positive correlation with plasma Se ( $r = -0.15$ ) (Fig. 2), suggesting that the Se incorporated into plasma selenocompounds due to a large consumption of fish was not primarily incorporated into the selenoproteins. Interestingly enough, plasma glutathione peroxidase was correlated with selenoprotein P ( $r = 0.73$ ,  $P < 0.001$ ; Fig. 3), indicating that although the two indices were not positively related to plasma Se, both reflected another aspect of Se status, maybe functionally active selenoproteins. The association was apparently similar in all three groups, and moreover the data did not suggest that the Se requirement of any of the selenoproteins had reached a saturation level (Fig. 3).

The proportion of plasma Se located in glutathione peroxidase was calculated according to its structure as described previously (Huang & Åkesson, 1993). This proportion

Table 1. Selenium and selenoprotein levels in the plasma of subjects consuming different amounts of fish†

(Mean values with their standard deviations)

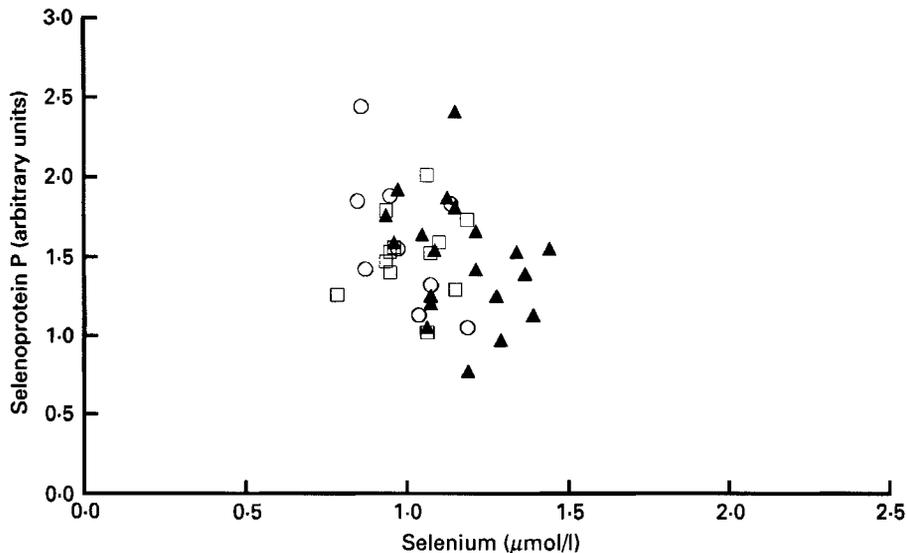
	Fish consumption					
	None (n 9)		Moderate (n 12)		High (n 20)	
	Mean	SD	Mean	SD	Mean	SD
Se ( $\mu\text{mol/l}$ )	0.99	0.12	1.01	0.11	1.17**	0.15
GSHPx (mg/l)	3.51	1.07	3.61	0.85	3.67	1.04
Se%-GSHPx‡	16.7	5.1	16.7	4.2	15.0	5.3
SeP (arbitrary units)	1.61	0.44	1.51	0.26	1.49	0.38

GSHPx, glutathione peroxidase (EC 1.11.1.9); SeP, selenoprotein P.

\*\* Mean value was significantly different from those for moderate and zero consumers,  $P < 0.01$ .

† For details of subjects and procedures, see p. 456.

‡ Percentage of plasma Se located in glutathione peroxidase.

Fig. 1. Relationship between plasma selenium and selenoprotein P concentrations among men with high ( $\blacktriangle$ ), moderate ( $\square$ ) or no ( $\circ$ ) fish consumption ( $r -0.29$ ,  $P = 0.07$ ;  $\rho -0.28$ ,  $P = 0.08$ ).

decreased with increasing plasma Se concentration ( $r -0.56$ ,  $P < 0.001$ ). The (selenoprotein P): Se ratio was calculated as a measure of the proportion of Se located in selenoprotein P, and this variable was also negatively correlated with total Se concentration in plasma ( $r -0.66$ ,  $P < 0.001$ ). These negative associations were similar also within the three groups. The mean percentage of plasma Se located in glutathione peroxidase tended to be lower in the high consumer group. A similar negative association between (selenoprotein P): Se and plasma Se was observed in a study of healthy adults (Marchaluk *et al.* 1995).

Previous studies of the cohort showed positive correlations between Se in plasma and Hg in blood, plasma and erythrocytes (Svensson *et al.* 1992). In the present study, neither

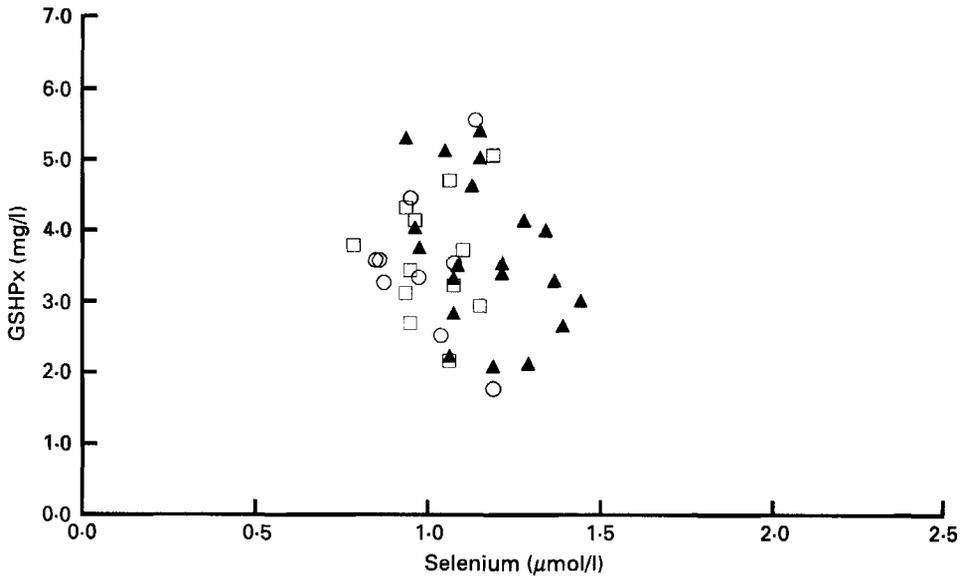


Fig. 2. Relationship between plasma selenium and glutathione peroxidase protein (GSHPx; EC 1.11.1.9) concentrations among men with high ( $\blacktriangle$ ), moderate ( $\square$ ) or no ( $\circ$ ) fish consumption ( $r = -0.15$ ,  $P = 0.36$ ;  $\rho = -0.17$ ,  $P = 0.28$ ).

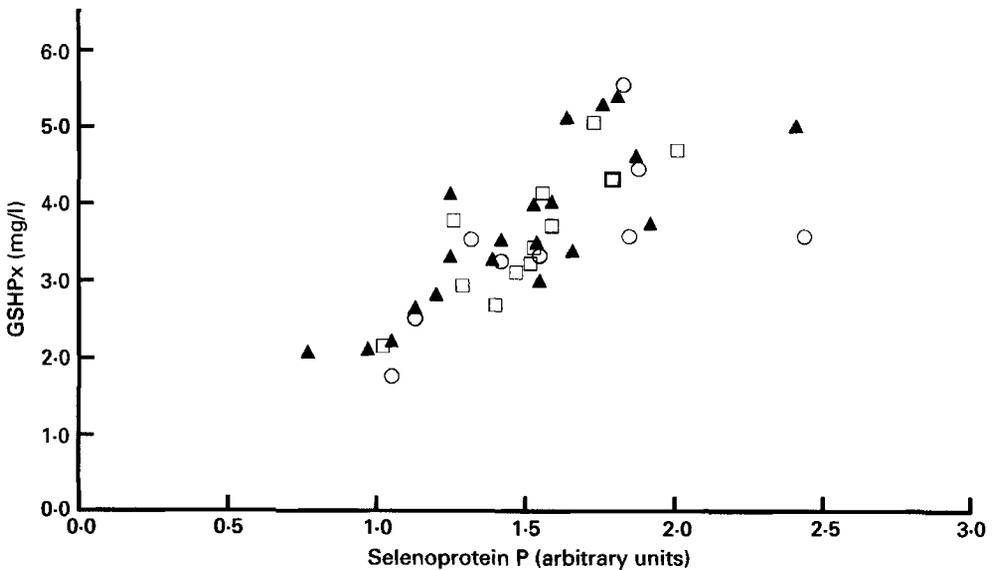


Fig. 3. Relationship between plasma selenoprotein P and glutathione peroxidase protein (GSHPx; EC 1.11.1.9) concentrations among men with high ( $\blacktriangle$ ), moderate ( $\square$ ) or no ( $\circ$ ) fish consumption ( $r = 0.73$ ,  $P < 0.001$ ;  $\rho = 0.79$ ,  $P < 0.001$ ).

plasma glutathione peroxidase nor selenoprotein P was significantly associated with the concentration of Hg in plasma, blood, erythrocytes or urine.

For comparison, Se status in a heterogeneous group of patients with widely varying serum Se concentration was studied (Fig. 4). Among these samples selenoprotein P was

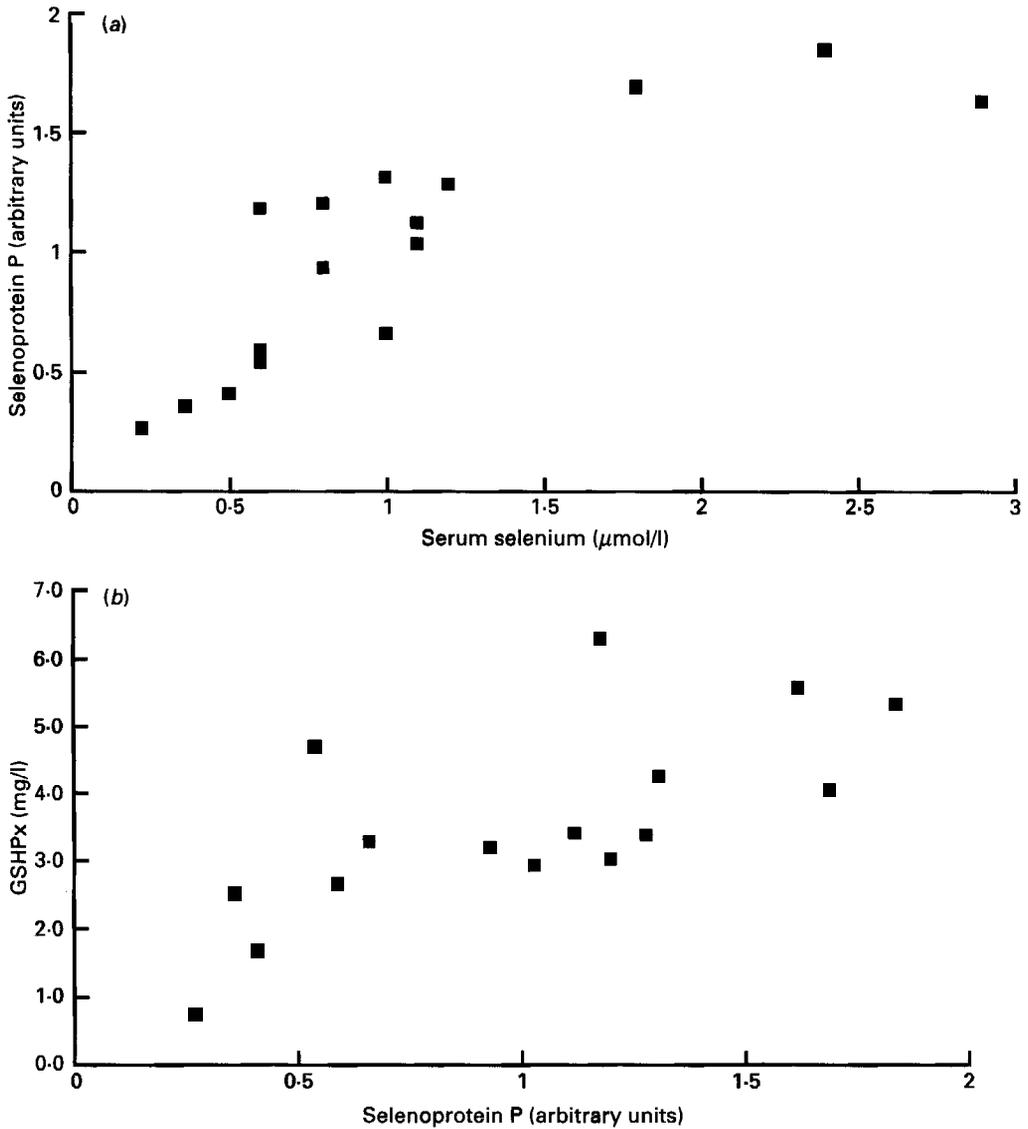


Fig. 4. Associations of plasma selenoprotein P with (a) selenium ( $r$  0.83,  $P$  < 0.001) and (b) glutathione peroxidase protein (GSHPx; EC 1.11.1.9) ( $r$  0.69,  $P$  = 0.003) in patients with widely varying serum selenium concentrations.

significantly correlated both with total Se ( $r$  0.83) and with glutathione peroxidase protein ( $r$  0.69). Similarly, in a group of healthy adults with a more narrow range of serum Se levels the correlation between Se and selenoprotein P was 0.68 (Marchaluk *et al.* 1995).

#### DISCUSSION

Previous studies have shown that plasma Se and glutathione peroxidase activity are positively correlated in healthy subjects with Se status typical for Europe (Åkesson & Steen, 1987; McMaster *et al.* 1990). A similar association was found using an immunochemical

assay of plasma glutathione peroxidase (Huang & Åkesson, 1993). Data in Fig. 4 show a positive correlation also between Se and selenoprotein P in plasma, and similar findings emerge from an ongoing study of healthy European subjects (Marchaluk *et al.* 1995).

The lack of a positive correlation between plasma Se and the two selenoproteins in the men consuming different amounts of fish might have several explanations. It is possible that some of the Se absorbed from ingested fish was in a molecular form that was not utilized for selenoprotein synthesis, but instead accumulated in other compounds, e.g. proteins containing unspecifically incorporated Se (McConnell & Hoffman, 1972). One such possible dietary form of Se is selenomethionine (Yasumoto *et al.* 1988), but this compound has so far not been demonstrated in fish.

Animal studies have indicated that the bioavailability of Se in certain kinds of fish was less than that in other foods (Mutanen, 1986). The bioavailability of Se from different fish in humans has not been studied in detail. One study suggested that the increase in plasma Se after switching to a diet rich in fish was smaller than would have been expected (Thorngren & Åkesson, 1987). Moreover, Meltzer *et al.* (1993) found that a diet rich in fish, containing 50% more Se than a control diet, did not significantly affect serum and platelet Se levels in healthy subjects.

The forms of Se in fish have not been thoroughly studied. Chromatographic studies suggest that the Se compounds in fish differ from those in meat (Åkesson & Srikumar, 1994). Such differences may be one factor explaining differences in Se bioavailability from different foods.

Another possible explanation for the absence of association between fish intake and selenoprotein levels may be that the higher consumption of methylmercury by high fish consumers, as reflected in their higher concentration of Hg in blood, plasma and erythrocytes (Svensson *et al.* 1992), may have interfered with the utilization of Se for selenoprotein biosynthesis. Several reports, especially from studies in animals, indicate that Se interacts with both inorganic Hg and methylmercury, although detailed mechanisms for such interactions are not known (Burk *et al.* 1977; Magos, 1991). In the present study no correlation was found between plasma selenoproteins and Hg in plasma, blood, erythrocytes, and urine, thus giving no support for an interaction between Hg from fish intake and selenoproteins.

Se status affected the distribution of plasma Se among glutathione peroxidase and other proteins. The calculated proportion of Se associated with glutathione peroxidase was inversely correlated with plasma Se among the men with varying fish intake. Gel filtration experiments indicated that the distribution of Se among plasma proteins was affected after supplementation with protein-bound Se (Borglund & Åkesson, 1988). In conclusion, the present study suggests that at least in some cases plasma selenoproteins may be better markers of Se status than the total concentration of Se.

The study was supported by grants from the National Swedish Environmental Protection Agency, the Swedish Medical Research Council (project no. 9873), the Swedish Council for Forestry and Agricultural Research, the Pålsson Foundation, the Swedish Nutrition Foundation, and the Medical Faculty of the University of Lund. Skilful technical assistance was given by Ms Anita Nilsson and Ms Marianne Nilsson.

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