Supplementation with wheat selenium induces a dose-dependent response in serum and urine of a Se-replete population

BY HELLE M. MELTZER[1], GUNNAR NORHEIM[2], ELIN BJØRGE LØKEN[1] AND HALVOR HOLM[1]

[1] *University of Oslo, Institute for Nutrition Research, School of Medicine, PO Box 1046 Blindern, 0316 Oslo 3, Norway*

[2] *Department of Pharmacology and Toxicology, Norwegian College of Veterinary Medicine/National Veterinary Institute, PO Box 8146 Dep, N-0033 Oslo 1, Norway*

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In spite of a rather modest dietary intake of selenium (80 μg/10 MJ), Norwegian serum Se levels are among the highest in Europe. As part of an ongoing study of Se bioavailability, effects of different doses of wheat Se were investigated in eighteen healthy, Norwegian women. The participants were given Se-rich bread providing 100, 200 and 300 μg Se daily for 6 weeks. About 50% of the Se intake was excreted in the urine by week 6, compared with 67% before the intervention started. Serum Se increased by 20, 37 and 53 μg/l respectively, in the three groups ($P < 0.001$). The blood response and renal clearance results compare well with data obtained from less Se-replete populations, and support the hypothesis that selenomethionine from the diet is incorporated into a non-specific amino acid pool. Our study indicates that the intake of wheat Se is the main determinant of blood Se levels in Norway.

Selenium: Wheat selenium: Bioavailability

The dietary intake of selenium varies widely among the various populations of the world. In the Keshan disease areas of China, intakes as low as 7 μg/d were reported (Luo *et al.* 1985). Similar low intake levels (below 10 μg/d) were found among Swedish vegans (Abdulla, 1986). At the other end of the scale, chronic intakes as high as 5 mg/d were reported from seleniferous areas of China (Yang *et al.* 1983), and Venezuelans have a habitual intake around 350 μg/d (Brätter *et al.* 1984).

In general, there does not seem to be any simple relationship between Se intake and blood values at any level of intake: good correlations between intake and blood values have been reported from low-Se areas (Robinson *et al.* 1978; Luo *et al.* 1985). On the other hand, Swedish vegans were found to have normal blood values in spite of their low intake (Åkesson & Øckerman, 1985). As intake increases, the correlation between Se intake and blood values usually weakens, although it has been claimed to persist over a wide range of intakes (Yang *et al.* 1983; Levander & Morris, 1984; Mutanen *et al.* 1985; Abdulla, 1986). From these observed correlations it might be tempting to use blood or plasma Se values as indicators of Se intake. This approach is, however, not justified. For instance, Norwegians probably have the highest average serum Se levels in Europe (120 μg/l), in spite of a daily intake as low as about 80 μg/10 MJ (Meltzer *et al.* 1990). Still more striking are the previously mentioned Swedish vegans. Little is known about the reasons for these variations.

In a previous study it was shown that supplementation with 200 μg Se as selenite or pea (*Pisum sativum*) flour produces only a small effect on blood variables in replete individuals, in spite of good absorption (Meltzer *et al.* 1990). Wheat is the main source of dietary Se

Table 1. *Initial values of serum selenium, urine Se, body mass index (BMI) and age in each group of female subjects*

(Mean values and standard deviations for six subjects)

Group	Dietary Se supplement* (μg/d)	Serum Se (μg/l)		Urine Se (μg/d)		BMI (kg/m²)		Age (years)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	100	115	10	47	10	21	1	25	2
2	200	122	15	45	6	20	3	23	3
3	300	130	13	58	13	21	1	23	2

* For details, see pp. 288–289.

in Norway, owing to the importation of Se-rich wheat from the USA and Canada. The aim of the present study was to investigate the effects of different levels of wheat Se administered to persons from a Se-replete population such as that of Norway.

Establishing a dose–response relationship between wheat Se and blood Se would provide one possible explanation for the high blood values in Norway, and would lend support to the compartment model of Se metabolism proposed by Janghorbani *et al.* (1990) where a large part of body Se is in the non-selenite-exchangeable pool mainly originating from dietary selenomethionine. The present study is the first to assess the effects of different levels of wheat Se in a Se-replete population.

METHODS

Subjects

Eighteen healthy female students 20–26 years of age volunteered to participate. None had taken Se-containing supplements within the 3 months before the start of the study. They were all healthy according to anamnestic information from the subjects. All were non-smokers, and none was pregnant, lactating or dependent on any type of medication, apart from three participants using oral contraceptives. They were living at home and encouraged to maintain their usual daily routines and dietary habits. Written consent was obtained from each subject.

Experimental design

The subjects were randomized into three groups. All participated for a total of 10 weeks. Body mass index, age, and initial serum and urine Se levels are shown in Table 1. For 6 weeks the participants were given Se-rich bread daily, which replaced part of the bread that would otherwise have been consumed during the day. After the 6-week intervention period, a post-intervention period lasted for a further 4 weeks.

Samples

Blood samples, drawn by venepuncture in the morning after a 12 h fast, were taken from the participants at weeks 0, 3, 6 and 10. Urine samples (3 d) were collected initially and after 3, 6 and 10 weeks.

Renal plasma clearances were calculated using concentrations of Se in serum and the amounts excreted in the urine in 24 h by the conventional formula:

$$C_{Se} = \frac{(Se)_U \times V}{(Se)_p},$$

as described by Robinson *et al.* (1985). $(Se)_U$ and $(Se)_p$ are Se concentrations in urine and plasma (serum), expressed in the same units, V is the rate of urine production (ml/min), and $(Se)_U \times V$ is the amount of Se excreted in the urine per min.

Diet

Whole grain wheat containing 10 mg Se/kg was obtained from Mr. R. Marts, Bonesteel, South Dakota. The wheat was ground, mixed with appropriate amounts of ordinary Norwegian flour, and baked into bread giving 1, 2 and 3 mg Se/kg bread respectively.

Weighed food records were obtained for four consecutive days during the intervention period for ten of the subjects. The contents of energy and the major nutrients were calculated according to Norwegian Tables of Food Composition (Norwegian Nutrition Council, 1977). Table 2 shows the mean energy intake and nutritional composition of their diets.

The diet of the participants contained less fat and more total carbohydrates and dietary fibre than normally found in groups of Norwegian women (Blaker *et al.* 1988). In spite of the higher bread and cereal intake of our participants, their initial serum Se levels were identical to those found in other Norwegian surveys (Aaseth *et al.* 1980; Aukrust *et al.* 1983; Blekastad *et al.* 1984; Ringstad *et al.* 1987).

Previous analysis of Se intake in a similar group of Norwegian women, using the duplicate portion technique, indicated a mean dietary Se intake of 80 (range 43–134) μg/10 MJ (Meltzer *et al.* 1990). With an average energy intake of 9·2 MJ/d, baseline average Se intake was estimated to be 75 μg/d. Accordingly, the total daily Se intake in the intervention period was estimated to be 160, 260 and 360 μg respectively for the three groups.

Analytical methods

Serum and urine Se were determined by atomic absorption spectrometry and a hydride generator system (Varian AA-1475, VGA-76) after digestion in a mixture of nitric and perchloric acids (Norheim & Haugen, 1986; Norheim, 1989). The results are expressed as μg/l.

Within groups, the changes in the clinical variables were tested for significance by paired t test. Means for the three groups were subjected to one-way analysis of variance and compared by Duncan's multiple range test; $P < 0{\cdot}05$ was considered statistically significant. The results are expressed as means and standard deviations except for Figs. 1 and 2 where they are shown as means with their standard errors.

RESULTS

At the start of the study serum Se levels did not differ significantly among the three groups of subjects (Table 1). After 3 weeks of the study the serum Se levels of the 300 μg group differed significantly from those of the other groups (Fig. 1). Within all three groups there was a statistically significant increase from week 0 to week 3 and from week 0 to week 6 ($P < 0{\cdot}001$). By week 6 mean serum Se levels had increased approximately 20 (SD 6) μg/l in the 100 μg Se-bread group, and 37 (SD 15) and 53 (SD 8) μg/l in the 200 and 300 μg groups respectively. During the post-intervention period (weeks 6–10) serum Se levels fell in all three groups to 124, 152 and 153 μg/l respectively. In the 200 and 300 μg groups these values were still significantly higher than the initial levels after 10 weeks.

During the first 3 weeks serum Se values rose in a simple dose–response manner: 13 μg/l in the 100 μg group, twice this (25 μg/l) in the 200 μg group and three times (40 μg/l) in the 300 μg group. Between weeks 3 and 6 the serum levels flattened out, especially in the group receiving the highest amount of Se.

Table 2. *Calculated energy and nutrient content of food consumed daily by ten female subjects*

(Mean values and standard deviations)

Dietary component	Mean	SD
Energy (MJ)	9·1	1·5
Protein (% of energy)	14·9	1·9
Fat (% of energy)	24·5	5·9
Carbohydrate (% of energy)	59·6	6·2
Dietary fibre (g)	32	10
Vitamin A (mg)	1·7	0·9
Vitamin D (μg)	5·9	6·7
Thiamin (mg)	1·3	0·3
Vitamin C (mg)	125	52
Calcium (g)	1·2	0·4
Iron (mg)	14·4	2·9
Bread (g)	215	45
Breakfast cereals and flour (g)	45	29
Cakes and biscuits (g)	38	41

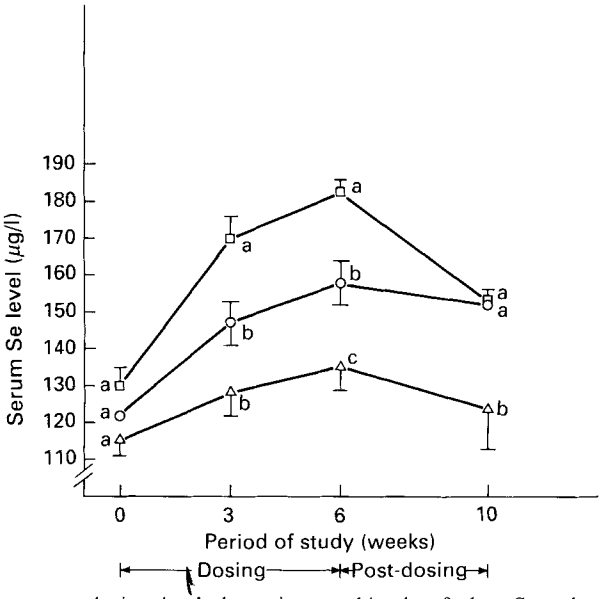

Fig. 1. Changes in serum selenium levels due to increased intake of wheat Se at three different levels (100 (△–△), 200 (○–○) and 300 (□–□) μg/d). Points represents means with their standard errors represented by vertical bars for six subjects per group. Points at any given action week with different superscript letters were significantly different ($P < 0·05$; Duncan's multiple-range test). For details of dietary treatments, see pp. 288–289.

Urine Se levels differed significantly both at 3 and 6 weeks (Fig. 2). In the post-intervention period the levels dropped rapidly and had returned to baseline values by week 10 in all three groups. Renal plasma clearance increased by 53, 116 and 127% in the three groups respectively during the intervention period (Table 3). About 50% of the Se intake was excreted in the urine by week 6 compared with 67% before the intervention started.

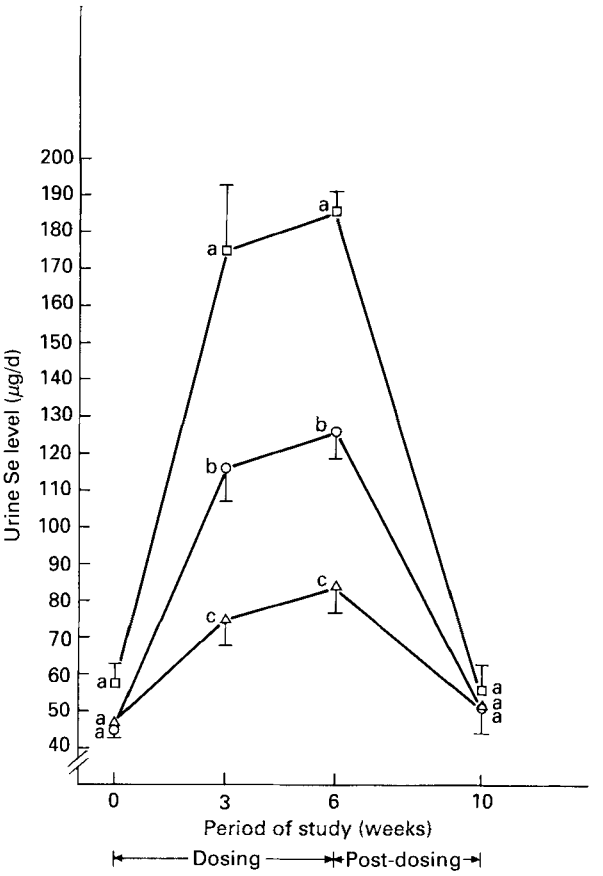

Fig. 2. Changes in urine selenium levels due to increased intake of wheat Se at three different levels (100 (△–△), 200 (○–○) and 300 (□–□) g/d). Points represent means with their standard errors represented by vertical bars for six subjects per group. Points at any given action week with different superscript letters were significantly different ($P < 0.05$; Duncan's multiple-range test). For details of dietary treatments, see pp. 288–289.

Table 3. *Renal plasma clearance (ml/min) of the three groups initially and after weeks 3, 6 and 10*

(Mean values and standard deviations)

	Initial		+3 weeks		+6 weeks		4 weeks after end of intervention	
Group*	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	0·288	0·08	0·416	0·15	0·443	0·13	0·287	0·09
2	0·262	0·04	0·553	0·12	0·559	0·11	0·232	0·05
3	0·307	0·07	0·714	0·17	0·704	0·04	0·252	0·07

* Dietary selenium supplements were 100, 200 and 300 μg/d for groups 1–3 respectively. For details, see pp. 288–289.

DISCUSSION

The present study shows that serum Se levels increase in a dose-dependent manner as a function of the amount of wheat Se in the diet. As far as is known, this is the first human study to assess the response to different doses of wheat Se. However, two previous studies in humans have compared the effect of a single dose of wheat Se with that of other Se forms.

Levander *et al.* (1983) gave 200 μg Se in the form of high-Se-wheat toast, high-Se yeast and selenate to Finnish men with initial serum Se values at 70 ng/ml, i.e. 40% lower than average Norwegian values (Meltzer *et al.* 1990). In both the wheat and yeast groups, plasma Se rose steadily for 11 weeks with no tendency to flatten out after 11 weeks. In a New Zealand trial (Thomson *et al.* 1985) initial plasma Se levels were about 60 μg/l (i.e. about half the initial Norwegian levels), and plasma Se rose to about 170 μg/l after a total of 8 weeks intervention with 200 μg wheat Se/d.

After 6 weeks the New Zealand group had reached almost the same serum level as the 200 μg group in the present study. Finnish men also had comparable levels at this point (145 *v.* 158 μg Se/l).

In both the previous studies and in the present study, 6 weeks supplementation with 200 μg wheat Se raised the serum Se values to comparable levels (145–160 μg/l), irrespective of initial serum Se levels. Thus, a dose–response relationship between wheat Se intake and serum Se levels seems to be confirmed under widely different conditions.

Animal studies have shown that, in Se-depleted rats, wheat Se and selenite given in the same dosage induce the same response in Se and glutathione peroxidase (*EC* 1.11.1.9; GSH-Px) levels in blood and liver, whereas tuna (*Thunnus thynnus*) fish seems to induce a more modest response (Douglass *et al.* 1981; Alexander *et al.* 1983; Mutanen *et al.* 1987).

We have previously shown (Meltzer *et al.* 1990) that at initially high serum levels like those of the Norwegian subjects (120 μg/l) several forms of Se (selenite, pea Se and a special form of yeast Se) at dosages of 200 μg/d have only a marginal effect on serum Se levels. This difference in effect between various forms of Se is consistent with the two-compartment model presented by Burk (1986) and Janghorbani *et al.* (1990). According to their model, selenomethionine is handled by the body as methionine, and thus is able to increase body stores in proportion to the intake. This pool has, however, no known functional significance other than possibly as a storage compartment for Se. All other Se forms, including selenocysteine, seem to be incorporated into the selenite-exchangeable metabolic pool (Se-EMP). If GSH-Px (and possibly other seleno-enzymes) capacity is saturated regarding Se, most trials so far seem to indicate that 'surplus' Se from the Se-EMP is stored in the liver or excreted. Thus, increased intake of Se in forms other than selenomethionine will hardly show any plasma response in a Se-replete population, as long as the excretion capacity of the liver and the kidneys is not exceeded.

So far, the only foods known to contain selenomethionine as a large percentage of the total Se are wheat and yeast (Olson *et al.* 1970; Korhola *et al.* 1986). As yeast is taken in supplement form, we may regard wheat Se as the only known Se-containing foodstuff that directly influences the selenoprotein pool. Plants seem to be the only source of selenomethionine in the diet, but the amounts of selenomethionine in plant foods may also vary considerably, as demonstrated by bioavailability studies of mushroom and pea Se, both of which seem to have low bioavailability in humans (Mutanen, 1986; Meltzer *et al.* 1990). Animal selenoproteins seem to contain the element mainly in the form of selenocysteine (Motsenbocker & Tappel, 1982).

We have previously shown that, irrespective of the form of Se given in the diet, the Se-dependent enzyme GSH-Px does not respond in a Se-replete population like ours (Meltzer *et al.* 1990). Accordingly we did not measure this enzyme in the present study.

Our study indicates that when the Se-EMP is saturated (e.g. as demonstrated by no further increase in activity of GSH-Px), selenomethionine from the diet may be the most important compound able to influence serum Se levels. The slow return to prestudy serum values, after supplementation has been stopped, is indicative of unspecific incorporation into body proteins. The same slow return to initial values has been observed in animal studies with pure selenomethionine supplementation (Moksnes & Norheim, 1983, 1986).

Increases in urinary Se content and plasma clearance rate were similar to those observed when the New Zealanders were given 200 μg wheat Se/d (Thomson *et al.* 1985). In both trials, plasma clearance rates rose to 0·5 ml/min.

In conclusion, our study strongly indicates that selenomethionine in the diet is the main determinant of serum Se in a relatively Se-replete population like the Norwegian population. An almost linear dose–response relationship to wheat Se emerges from the present study, in strong contrast to the marginal effects observed when similar doses of selenite and pea Se were administered to the same type of population (Meltzer *et al.* 1990). This supports the hypothesis that there exist at least two distinct pools of body Se, a selenite-exchangeable pool and selenite non-exchangeable pool. Differences in the amount of selenomethionine ingested may provide a partial explanation for the relatively poor correlation that is often observed between Se intake and serum Se levels.

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