Long-term supplementation with selenate and selenomethionine: urinary excretion by New Zealand women

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Thirty-six New Zealand women aged between 18 and 23 years received daily for 32 weeks, 200 μg Se as Se-enriched yeast (selenomethionine, SeMet), or brewer's yeast mixed with selenate, or no added Se (placebo) in a double-blind trial. Mean daily Se excretion increased with both supplements; the selenate group excreted more than the SeMet group, 123 *v.* 66 μg/d respectively at week 2, equivalent to 57 *v.* 27 % of the dose. Thereafter Se output increased for the SeMet group reaching a plateau at about 100 μg/d at week 16, when plasma Se had also plateaued at 190 ng/ml. The selenate group had reached an earlier plateau of 110 ng Se/ml at week 7. There was a close relationship between 24 h urine and plasma Se for the SeMet group but not for the selenate group. Renal plasma clearances showed two distinctly different responses; the clearance of 0·4 ml/min reached by the SeMet group at week 2 plateaued as plasma Se increased almost 2-fold; whereas for the selenate group the clearance varied between 0·8 and 1·1 ml/min whilst plasma Se remained almost constant at 110 ng/ml. Previous studies, also of 200 μgSe/d as Se-rich bread, in New Zealand (NZ) and elsewhere showed similar responses to Se-yeast; the selenite response was intermediate between selenate and Se-yeast (SeMet). The full significance of these studies awaits identification of Se components in plasma, glomerular filtrate and urine; meanwhile renal clearances serve as a pointer to changes in the distribution of Se-containing fractions in the plasma. Trimethylselenonium was detected in basal urines, and was a minor component in urines of supplemented NZ subjects at about 1 % of the total Se.

Selenium: Urinary excretion: Renal plasma clearance: Women

The question of which is the preferred form of Se to raise the Se status of individual persons or of a community continues to challenge workers around the world. Selenite, selenate, selenomethionine, Se-enriched yeast and food Se are effective in short-term Se studies, but there is concern about their use for long-term supplementation, and also about which is the most appropriate criterion for assessing Se status and for alerting to possible toxic effects. The problem is complicated by the scarcity of information about the dietary forms, functions, metabolism and excretion of Se. Because of concern about long-term effects of prolonged supplementation with selenite, attention is now being directed towards selenate as a Se supplement and also to its use as an agricultural fertilizer with effects on

both animal and plant foods, as in Finland (Varo *et al.* 1988). Selenomethionine (SeMet), which is the predominant form in both Se-enriched yeast and Se-rich wheat grown on Se-rich soils, raises blood Se levels more than the inorganic forms and continues to be used as a Se supplement by self-dosers.

We have been seeking to compare the long-term effects of selenate and SeMet on Se metabolism in blood and urine. The response in Se and glutathione peroxidase activity (*EC* 1.11.1.9; GSHPx) in blood components has been reported (Thomson *et al.* 1993), as has the Se distribution in blood fractions (Butler *et al.* 1991). The present study was designed to compare the effects of supplementation with these two forms on urinary excretion of Se. We had noted that New Zealanders and also the Chinese in Keshan disease areas (Luo *et al.* 1985) excreted a smaller proportion of the Se presented to their kidneys, i.e. they had lower renal plasma clearances, than North Americans (Robinson *et al.* 1985). A preliminary report including the response in excretion of trimethylselenonium (TMSe) a Se urinary metabolite, has been presented (Robinson *et al.* 1989). We now suggest that measurements of the renal plasma clearance of Se may help to clarify the distribution of plasma Se between different fractions.

METHODS

Thirty-six women resident in Dunedin, New Zealand, and aged 18–23 years participated in a double-blind supplementation study for 32 weeks in 1987. The subjects were randomly assigned to three groups and received daily tablets containing 200 μg Se as selenate mixed with brewer's yeast (selenate group), as Se-enriched yeast (SeMet group), or as plain brewer's yeast with no added Se (placebo; < 1 μg Se/tablet). The tablets were manufactured by Vita Tech International, Inc., Tustin, CA, USA and supplied by Nutrition 21 (San Diego, CA, USA). The majority of the Se in the Se-enriched yeast (Se-yeast) supplied by this company has been shown to be in the form of SeMet (Beilstein & Whanger, 1986). Each subject filled in a brief questionnaire giving details of residency, dietary habits, nutrient supplementation and other information which might influence Se status. The subjects were requested not to eat Se-rich foods, such as fish, liver, kidney or brazil nuts during the 2 d before the time that urine collections and blood samples were taken. Informed consent was obtained from all subjects, and the study was approved by the ethical committee of the Otago Area Health Board.

Blood and 24 h urine collections were made on the day before supplementation commenced (week 0), at 2 and 4 weeks and monthly for a further 28 weeks. The blood samples were drawn, separated into plasma and other components, analysed, or stored awaiting later analysis as described previously (Butler *et al.* 1991; Thomson *et al.* 1993).

The 24 h urine samples were collected and stored as described previously (Thomson *et al.* 1978). The completeness of urine collections was checked from 24 h creatinine excretions, and five subjects were excluded because their creatinine excretion was 7 mmol/d or less on more than two occasions. Creatinine was measured using the picric acid method (Clarke, 1961) adapted for an auto-analyser.

Total Se was determined in individual urine samples and also in pooled group urines for each stage by the automated fluorimetric method (Brown & Watkinson, 1977). Sensitivity and sample reproducibility of the method were 0·1 μg/l and SD 2·5 % within the range of 0–100 μg/l (Thomson *et al.* 1982). Analysis of the National Bureau of Standards (Gaithersburg, MD, USA) reference material no. 1577, bovine liver, gave a value of 1·11 μg Se/g (certified value $1{\cdot}09 \pm 0{\cdot}10$ μg/g).

Samples of urines were pooled before the five subjects were excluded; there was excellent agreement between the Se contents of the pooled urine and the mean for the individual urines for each group at each stage.

TMSe was determined in pooled group urines for weeks 0, 2, 4, and then 4-weekly, by a dual column ion-exchange method, a modification of the method of Nahapetian *et al.* (1984). The very low concentration of TMSe in normal New Zealand (NZ) urines limited our choice of method because of the large sample size needed for the reineckate method and the problem of adequate desalting for the HPLC technique (Kraus *et al.* 1985). Good agreement was obtained between HPLC and the chosen dual column techniques for TMSe-rich urines collected from a subject after ingesting TMSe (144 ng Se/ml; 139 ng Se/ml urine respectively). Replicate analyses of urines from supplemented subjects gave CV of 10–20 %; mean recoveries of standards and TMSe added to urines varied between 81 and 94 %.

Renal plasma Se clearances (ml/min) were calculated by the conventional formula (Robinson *et al.* 1985) using concentrations of Se in plasma and the amounts of Se excreted in the urine in 24 h:

$$C_{Se} = \frac{(Se)_u . V}{(Se)_p}$$

where $(Se)_u$ and $(Se)_p$ are Se concentrations in urine and plasma, necessarily expressed in the same units, and V is the rate of production of urine in ml/min. The numerator $(Se)_u.V$ is the amount of Se excreted in the urine in 1 min.

Statistical analysis

Differences among groups for urinary Se excretion, plasma Se and renal clearances at each time point were calculated using ANOVA and Student's *t* test after log transformation. Changes with time within each group were calculated using ANOVA for repeated measures after log transformation (Statview, SE + Graphics™, version 1.03, Abacus Concepts Inc., Berkeley, CA, USA).

RESULTS

Baseline information about the subjects is given in Table 1, including height, weight, BMI, plasma Se concentrations, 24 h urinary excretions of Se and creatinine, and renal plasma clearances of Se. Values did not differ among the three groups. However a subject in the selenate group had mistakenly eaten a generous serving of fish on the day of urine collection for week 0 resulting in a 24 h excretion of 88 μg Se/d and a group mean of 17·6 (SD 2·5) μg Se/d (*n* 10); without it the mean became 9·4 (SD 4·1) μg Se/d (*n* 9), close to the means for the other two groups. No differences in plasma Se and urinary Se outputs were detected for the five subjects who described themselves as semi-vegetarians or another two who were lacto-ovo-vegetarians.

Mean daily Se excretions increased with both supplements ($P < 0{\cdot}0001$) and no changes were seen in the placebo group (Fig. 1). At each stage the SEM was greater for the selenate than for the SeMet group and often markedly so. Examination of the individual Se urinary outputs showed that six selenate subjects had excretions over 200 μg Se/d (mega-urines) with two over 300 μg Se/d at week 25. This possibly reflected irregularities in taking the supplement, e.g. late in the evenings instead of the prescribed one capsule in the morning. On the other hand lesser excretions, about 40 μg Se/d occurred on seven

Table 1. *Baseline values for plasma selenium concentrations and urinary excretions of selenium and creatinine in New Zealand women*

(Mean values and standard deviations)

Group*...	Selenate (*n* 10)		SeMet (*n* 12)		Placebo (*n* 19)	
	Mean	SD	Mean	SD	Mean	SD
Age (years)	21	1	21	1	21	1
Height (m)	1·65	0·04	1·66	0·06	1·67	0·08
Weight (kg)	62	7	59	4	62	6
BMI (kg/m^2)	22	1·7	21·4	1·2	22	2·0
Plasma Se (ng/ml)	52	7	53	5	56	6
Urine Se (μg/d)	9·4	4·1	10·9	2·9	11·6	4·2
Creatinine (mmol/d)	9·4	3·2	10·2	1·7	9·4	0·9
Urine Se/creatinine (μg/mmol)	1·01	0·34	1·10	0·3	1·22	0·38
Renal plasma clearance of Se (ml/min)	0·13	0·06	0·14	0·04	0·13	0·04
TMSe† (μg Se/d)	0·9		0·3		0·6	

TMSe, trimethylselenonium; SeMet, selenomethionine.
* For details of dietary groups, see p. 552.
† For pooled urines.

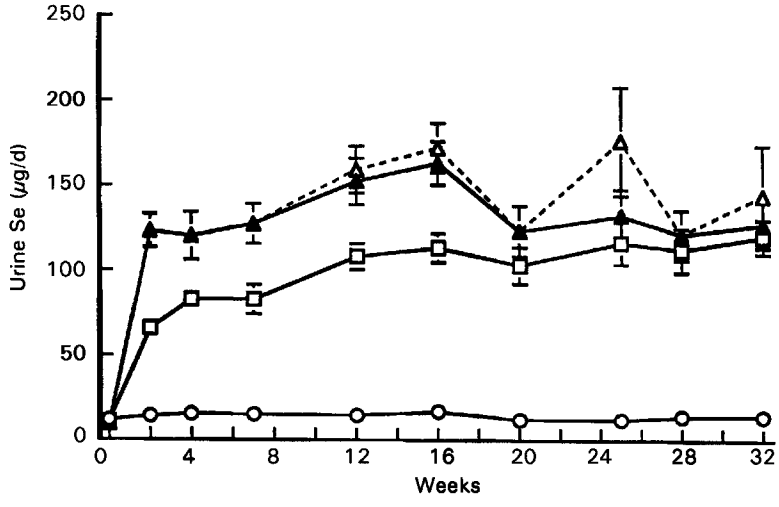

Fig. 1. Urinary selenium excretion by New Zealand women supplemented with selenium either as selenate (▲, *n* 10), or Se-enriched yeast (□, *n* 12) or a placebo (○, *n* 9) for 32 weeks. For details of dietary treatments see p. 552. Values are means with their standard errors represented by vertical bars; (△) overall mean (weeks 12 and 16, *n* 9; weeks 25 and 32, *n* 8) for the selenate groups with subjects excreting mega-urines, >200 μg Se/d, see pp. 553–554.

occasions. The mega-urine outputs were excluded from the excretions for the appropriate selenate group (see Fig. 1). There were no such irregularities for the SeMet group with most falling within the range 50–150 μg Se/d with seven just below and another nine just above this range.

At each stage the selenate group excreted more Se than the SeMet group; at week 2 it accounted for 123 μg Se/d, an increase of 114 μg/d, equivalent to about 57 % of the daily 200 μg Se dose. This was 2-fold greater than for the SeMet group with a urinary output of

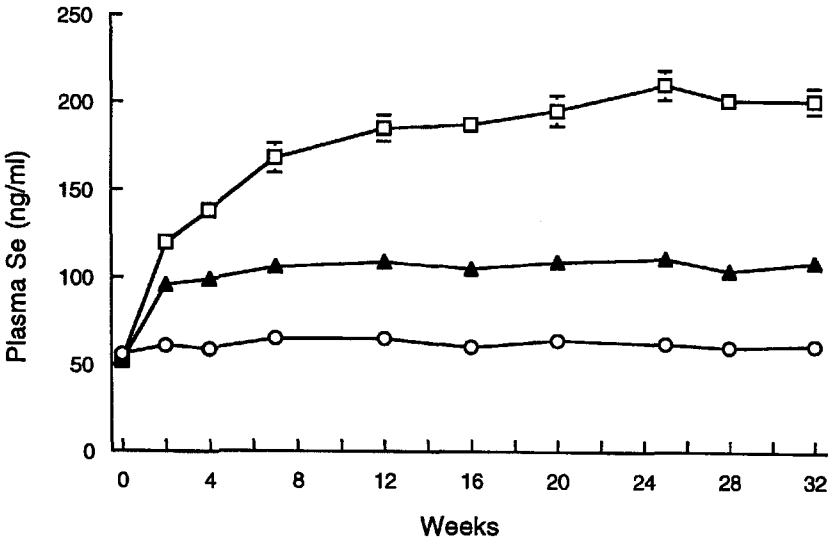

Fig. 2. Selenium concentrations in plasma of New Zealand women supplemented with selenium either as selenate (▲, *n* 10), or Se-enriched yeast (□, *n* 12), or a placebo (○, *n* 9) for 32 weeks. For details of dietary treatment see p. 552. Values are means and their standard errors represented by vertical bars.

66 μg/d, about 27 % of the dose. Thereafter the urinary output of the SeMet group continued to rise, reaching a plateau at about 110 μg Se/d at week 16, and remained a little less than the corresponding selenate outputs for the later stages of the study. Significant differences in mean excretions were apparent among the three groups from week 2 ($P < 0{\cdot}0001$), but the differences between the selenate and the SeMet groups were significant at week 2 ($P < 0{\cdot}001$) and from weeks 7 to 16 only ($P < 0{\cdot}02$).

As pointed out previously (Thomson *et al.* 1993) plasma Se in the selenate group plateaued at about 7 weeks at 110 ng/ml (Fig. 2) but rose further in the SeMet group reaching a plateau of 190 ng/ml at week 16, when urine Se outputs for the SeMet group had also reached a plateau. Significant differences in mean plasma concentrations were apparent among the three groups from week 2 ($P = 0{\cdot}001$), and at the end of the supplementation period plasma Se in the SeMet group was nearly 2-fold greater than in the selenate group. A close relationship was observed for the SeMet group between 24 h urine Se excretion and plasma Se concentration (r 0·96; $P < 0{\cdot}001$) but not for the selenate group (Fig. 3).

Renal plasma clearances

Renal plasma clearance brings together the changes in plasma Se and urinary Se, which had both increased during the first 2 weeks of supplementation, to reach a plateau at about 0·4 ml/min for the SeMet group, but to over twice this value for the selenate group, varying between 0·8 and 1·1 ml/min (excluding the mega-urines) whereas plasma Se remained almost constant. Differences between groups for renal plasma clearances were significant ($P < 0{\cdot}0001$). These two distinctly different responses are shown in Fig. 4 together with the responses from our earlier studies with daily supplements also of 200 μg Se/d taken as selenite for 4 weeks (Robinson, 1988; Thomson *et al.* 1988) and as Se-rich wheat bread for 8 weeks (Robinson *et al.* 1985; Thomson *et al.* 1985). Not surprisingly since SeMet is the major Se form in high-Se wheat, the response to high-Se bread resembled strongly the response to Se-yeast, with renal clearance of about 0·5 ml/min, and plasma Se reaching 166

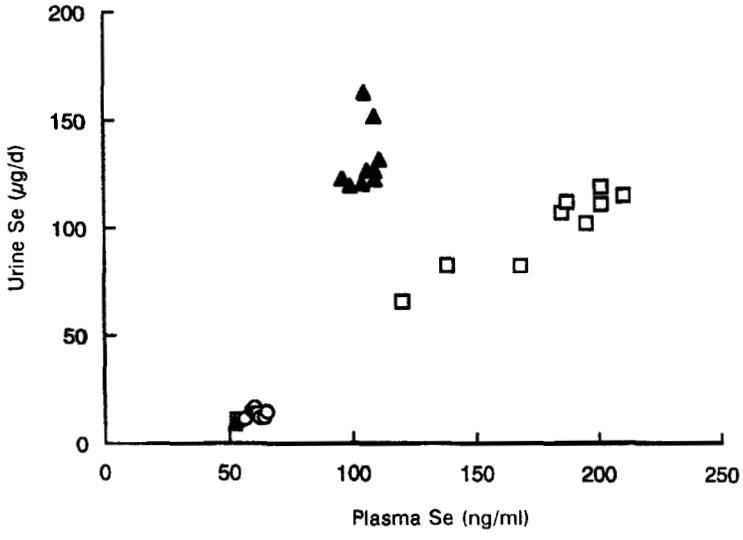

Fig. 3. Relationship between mean urinary excretion of selenium and mean plasma selenium concentration of New Zealand women supplemented with selenium either as selenate (▲), or Se-enriched yeast (□), or a placebo (○) for 32 weeks. For details of dietary treatment see p. 552.

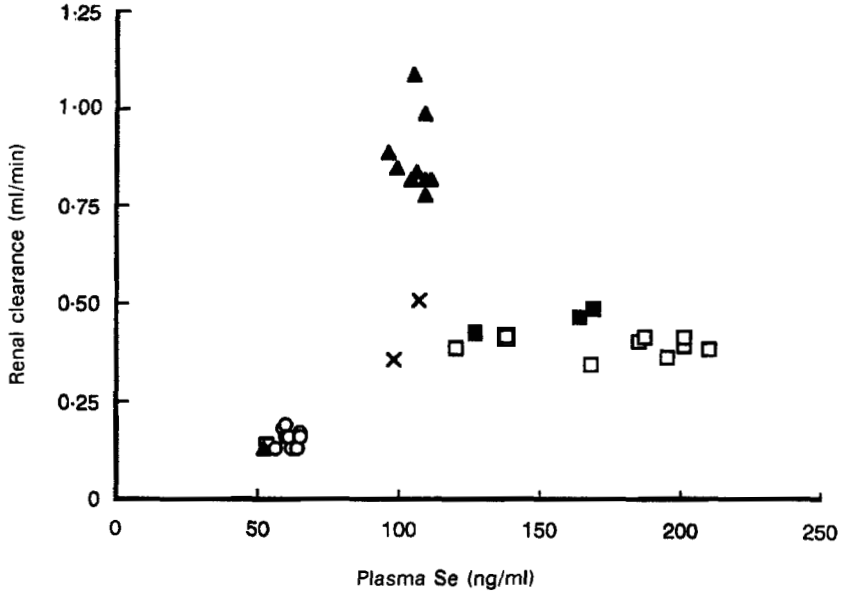

Fig. 4. Relationship between mean renal plasma clearance and mean plasma selenium concentration of New Zealand women supplemented with 200 μg Se/d either as Se-enriched yeast (□), or high-Se wheat bread (■), or selenate (▲), or selenite (×), or a placebo (○). For details of dietary treatment, see p. 552.

ng Se/ml. On the other hand selenite resembled selenate in its plasma response but with only half its renal clearance at 0·5 ml/min.

Trimethylselenonium excretion

Urinary TMSe in the baseline urines was close to the lower limit of sensitivity of the method and appeared to increase with supplementation (Table 2). TMSe output was a little greater for the selenate group than for the SeMet group at each stage of supplementation; expressed as a proportion of total Se excretion, TMSe/total Se fell from 3–5 % to about 1 % and remained there throughout the study.

DISCUSSION

We have again exploited the NZ situation with its low Se status to compare the responses of Dunedin women to increasing the intake with supplements of 200 μg Se/d supplied in the four prime forms of Se as: selenate, SeMet (or Se-enriched yeast in the present study), selenite and food-Se (as high-Se wheat bread in earlier studies).

The urinary output yields information about how the supplement is handled, but its reliability is limited by the completeness of 24 h urine collections. Although none of the subjects in the present study reported errors it was considered from their creatinine excretion that five subjects should be excluded (Bingham & Cummings, 1985). The mean creatinine excretion for each of these subjects was 8 mmol/d or less, with CV > 30 % (Webster & Garrow, 1985; Mills & Hocken, 1986).

Our earlier studies yielded background information about the response to supplements varying between 100 μg and 1 mg Se in the four forms (Robinson, 1988). The supplements had been given as a single dose or daily for periods of 4–17 weeks, whereas in the present study supplementation continued for 32 weeks. Single-supplement studies with 1 mg Se demonstrated the differences in handling Se as selenite, selenate, and SeMet (Thomson & Robinson, 1986). Selenate-Se was more readily absorbed than selenite-Se, reaching a peak within 3 h, and with about 70 % of the amount of Se ingested rapidly excreted within 24 h. Peak excretion was reached 3 h later for selenite-Se with about 20 % recovered within the day. Sandholm (1973) and Burk (1974) have shown that selenite is quickly taken up by

Table 2. *Trimethylselenonium (TMSe) excretion (μg Se/d) in pooled urines of New Zealand women at each stage during daily supplementation with 200 μg Se as selenate or Se-enriched yeast (SeMet) or a placebo for 32 weeks**

Group...	Selenate (*n* 13)		SeMet (*n* 13)		Placebo (*n* 10)	
Weeks	TMSe (μg Se/d)	TMSe/Se† (%)	TMSe (μg Se/d)	TMSe/Se† (%)	TMSe (μg Se/d)	TMSe/Se† (%)
0	0·9	5‡	0·3	3	0·6	5
2	1·8	1·5	0·7	1	0·9	6
12–28§	1·9 (1·7–2·3)	1·4 (1·2–1·5)	1·3 (0·9–1·9)	1·2 (0·8–1·6)	0·7 (0·6–0·9)	5 (4–6)

* For details of procedure and dietary groups, see p. 552–553.
† TMSe excretion as proportion of total Se excretion.
‡ Pooled urine contained 18 μg Se/d.
§ Mean values and ranges for urines collected from week 12.

erythrocytes, metabolized and returned to plasma, becoming bound to protein (Sunde, 1990). SeMet-Se was well absorbed but was mainly retained and only about 22 % recovered in the urine. Similar trends were shown in the initial stages of the present studies with about 57 % and 27 % recovered for selenate and SeMet groups respectively. Without knowledge of the urinary Se components it is uncertain how much of the increase in urinary excretion can be related to the Se content of the dose as such, but it is common to express the increase above the baseline urinary excretion as a proportion of the amount of Se in the dose.

It would have been valuable to identify and determine the proportions of the various inorganic and organic Se components present in the plasma, the glomerular filtrate and the urine after ingestion of these supplements. Our colleagues in Oregon have shown from gel filtration of plasma that those taking SeMet (Se-yeast) revealed two major Se-containing peaks with most of the Se in the second peak (peak IV, albumin) and this increased during the study; whereas for those taking selenate most of the Se went to peak II (selenoprotein P) and the Se content of that peak remained fairly constant (Butler *et al.* 1991). However in order to understand the mechanism of urinary excretion, more information is needed about the 'free Se components' from these supplements in the plasma, glomerular filtrate and urine, and unfortunately there has been little progress in identifying these (Burk, 1976; Robberecht & Deelstra, 1984; Janghorbani *et al.* 1990; Oster & Prellwitz, 1990; Alaejos & Romero, 1993). We have established that TMSe is a minor component in the urine of NZ subjects, and amounts to about 1 % of the total Se in the urines of supplemented subjects. This supports the findings of Sun *et al.* (1987) who found similarly low values for TMSe of about 1 % of the total Se for US residents.

In 1985 we suggested that most of the free SeMet-Se that enters the glomerular filtrate is probably reabsorbed by the proximal tubules, like other amino acids and glucose, by a mechanism shared by methionine (Robinson *et al.* 1985). Thus SeMet should increase the concentration of Se in the plasma and presumably also in the methionine pool; it should increase the excretion and clearances only in so far as the larger store of SeMet yields more Se to the central pool (Burk, 1986). It is generally considered that SeMet follows some of the methionine metabolic pathways, becoming incorporated into protein in place of methionine and thereby contributes to tissue Se (Levander & Burk, 1990). Free inorganic Se compounds that enter the glomerular filtrate are less likely to be reabsorbed and should increase rates of excretion and renal clearance rather than plasma Se concentration, as happened with selenate and possibly also with selenite. It was remarkable how in our various dosing trials since 1975 with daily doses of 90–500 μg Se/d as selenite, and possibly also of selenate, the plasma Se increased only to 100–120 ng/ml where it remained until dosing ceased. This has also been noted by Alfthan *et al.* (1991) in their supplementation trials, also with 200 μg Se/d, in 1981 (Levander *et al.* 1983) and then in 1987 after the Se intake in Finland had been raised by 100 μg/d, as the Se contents of both animal and vegetable products were raised by selenate enrichment of fertilizers (Varo *et al.* 1988).

The same men were studied in both Finnish trials. The basal plasma Se concentration at 70 ng/ml in 1981 had increased in 1987 to 106–114 ng Se/ml, the range for the group means. Selenite, but not selenate, increased the plasma Se to above the placebo to 126 ng Se/ml, whereas SeMet-Se (Se-yeast) reached 169 ng/ml at week 11. Trends in urinary excretions were similar for the Finnish and NZ studies, with greatest amounts excreted for selenate groups. The large SEM could indicate some mega-urines amongst these Finnish men, i.e. > 200 μg Se/d above the baseline output of 64 μg/d. We calculated renal plasma clearances from their data and have listed them with the plasma Se in Table 3 together with

Table 3. *Response in plasma selenium concentration and renal plasma clearance of some Scandinavian, European and New Zealand subjects to daily supplements of 200 μg Se as Se-enriched yeast, Se-rich bread, selenite or selenate*

Form of supplement	Country	Year of study	*n*	Sex	Time* (weeks)	Plasma Se (ng Se/ml)		Renal plasma clearance (ml/min)			Reference
						Initial	Experimental stage	Initial	Experimental stage	Increase	
Se-enriched yeast (selenomethionine)	Finland	1987–8	10	M	11	110	169	0·36	0·65	0·29	Alfthan *et al.* (1991)
	New Zealand	1987	12	F	2	53	120	0·14	0·39	0·25	Present study
					4	53	138	0·14	0·42	0·28	
Se-rich bread	Netherlands	1987	4	M	9	65	138	0·15	0·42	0·27	Van Der Torre *et al.* (1991)
	Norway	NS	18	F	3	122	159	0·26	0·55	0·29	Meltzer *et al.* (1992)
	New Zealand	1982–3	4	F	2	57	127	0·16	0·43	0·27	Thomson *et al.* (1985); Robinson & Thomson (unpublished results)
Selenite	Finland	1987–8	10	M	11	109	126	0·41	0·78	0·37	Alfthan *et al.* (1991)
	Norway	NS	3	F	12	117	NS	0·25	0·76	0·51	Meltzer *et al.* (1990)
	New Zealand	1985	10	F	2	63	98	0·19	0·36	0·17	Robinson (1988);
					4	63	107	0·19	0·51	0·32	Thomson *et al.* (1988)
Selenate	Finland	1987–8	10	M	11	106	103	0·42	1·24	0·82	Alfthan *et al.* (1991)
	New Zealand	1987	10	F	2	52	96	0·13	0·89	0·76	Present study
					4	52	99	0·13	0·85	0·72	

NS, not stated.

* Time of experimental stage when data became available for deriving response to supplementation; see pp. 558–560.

NZ data. Some other groups have also followed the response to supplements of about 200 μg Se/d of one or more of the four prime forms of Se supplement and where available their plasma Se and renal plasma clearances are also given in Table 3. Findings for the supplementation response are given for the initial stage and then at the first stage for which renal plasma clearances could be calculated.

It may not be valid to compare the findings at different stages of supplementation for these groups, but it will be recalled that for the NZ subjects two distinct trends in renal plasma clearances seemed to be established early in supplementation for both the organic and the inorganic Se forms. Table 3 shows similar trends in response to each form of supplement despite different baseline plasma concentrations and renal plasma clearances. It is interesting that the baseline data in Table 3 show the same trends of excretion and renal plasma clearance illustrated in Fig. 1 of our earlier paper (Robinson *et al.* 1985).

For the SeMet studies baseline data for the Finnish subjects were over 2-fold greater than for the NZ subjects, whereas the increases in renal plasma clearances with Se-yeast were almost identical at 0·29 and 0·28 (NZ, week 4) respectively. Likewise for the subjects resident in the Netherlands (Van Der Torre *et al.* 1991), Norway (Meltzer *et al.* 1992), and NZ, on supplements of Se-rich bread, the increases in renal plasma clearance were 0·27, 0·29 and 0·27 respectively. It will be noted that for the Norwegian subjects the basal renal plasma clearance was between the Finnish and NZ values even though their plasma Se was greater than the Finnish value.

Of the inorganic supplements, selenate-Se showed the greatest increases in clearance, with the Finnish value a little greater than the NZ value, 0·82 and 0·72 respectively, possibly reflecting the inclusion of some mega-urines. The selenite response was much less, with clearance increases from 0·32 (NZ, week 4) to 0·51 (Norway; Meltzer *et al.* 1990). The plasma response for both forms reflects the closeness of baseline concentrations to the range of 100–120 ng Se/ml attained with the inorganic supplements.

For further understanding of the very different responses to dietary and non-dietary sources of Se, we need to know the identity of the Se compounds in the urine, the glomerular filtrate and the plasma, as well as their role in the metabolism of Se. The renal plasma clearance is the volume of plasma that would have contained the amount of Se transferred to the urine in 1 min. With an average normal renal plasma flow of about 600 ml/min, a clearance of 1 ml/min means that 0·17 % of the Se in plasma passing through the kidneys is transferred to the urine. The range of clearances in Fig. 4 therefore indicates that between about 0·03 % and 0·2 % of the plasma Se was being excreted. The clearance axis in effect shows the proportion of the total Se in the plasma that was excreted. When the plasma Se was raised beyond 100 ng/ml by ingesting Se-yeast (SeMet), the rate of excretion continued to increase (Fig. 3) but the clearance (Fig. 4) did not. Hence, though the rate of excretion increased, the proportion of plasma Se that was excreted remained the same. In contrast, with selenate, the dramatic increase in the rate of excretion after the plasma Se reached 100 ng/ml occurred with an equally dramatic increase in renal clearance, indicating that a greater proportion of the Se in the plasma was being transferred into the urine.

Not all Se compounds in the plasma are equally likely to be excreted by the kidney; those which are bound to proteins will not pass into the glomerular filtrate; amino acid forms should be almost completely reabsorbed; free inorganic forms which pass into the glomerular filtrate would be expected to be partially reabsorbed like other anions. (The clearance of chloride, for example, 0·2–2 ml/min according to Koushanpour & Kriz (1986) is of similar order to that of Se.) The renal plasma clearance results suggest that large doses of selenate (most of which was quickly excreted) increased the proportion of those

constituents of plasma Se that are freely excreted (presumably free inorganic Se). On the other hand, large doses of SeMet-Se presumably contributed to the central Se pool without much effect on the proportions of the different constituents. Selenite, which showed intermediate behaviour (Fig. 4), is less well absorbed than selenate and is also rapidly taken up into cells before being released to the plasma in protein-bound forms; the modest increase in renal plasma clearance which it produced suggests an intermediate change in the proportions of Se-containing fractions in the plasma. Whilst the separation and identification of these fractions is still lacking, the calculation of renal clearances may, in the mean time, serve to provide a pointer to changes in the distribution of Se-containing fractions in the plasma.

Concluding remarks

All these studies were designed to show how the various forms of Se are handled, and also to give information about which might be the most appropriate and safe form of Se supplement to raise the Se status of people, should this be considered desirable. The striking similarity of the responses of subjects in different parts of the world from NZ to Northern Europe supports their wide applicability.

The continuing discovery of more and more functions of Se emphasizes its importance as a biological trace element but the need for an increased intake has not yet been established. If supplementation should become necessary we have failed to reveal a significant difference in suitability between selenate and SeMet although they were handled quite differently. Supplementation, if required, could be achieved by consuming more Se-rich foods or indirectly by increasing the Se content of natural foodstuffs (as in Finland where selenized fertilizers have been used), rather than by using chemicals as dietary supplements.

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