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Short communication

Selenium supplementation affects the retention of stable isotopes of selenium in human subjects consuming diets low in selenium

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Twenty-nine women and fifteen men from an area of low Se intake (South Island of New Zealand) consumed 100 μg stable ^{74}Se, as selenate given in water after an overnight fast, and blood was collected for 3 weeks. They were then divided into five groups and supplemented with 0, 10, 20, 30 and 40 μg Se/d (as selenomethionine) for 5 months. After 5 months, they received a second dose of ^{74}Se identical to the first. Supplementation significantly altered retention of ^{74}Se in the plasma, but not in the erythrocytes or platelets. Subjects receiving the placebo retained the greatest amount, and subjects receiving 30 μg supplemental Se/d retained the least ^{74}Se. Supplementation resulted in relatively more isotope being retained in a medium molecular mass protein considered to be albumin, and relatively less in another fraction considered to be selenoprotein P. The lack of many observed changes in retention of stable Se, and the shift in retention among the plasma proteins, suggests that supplemental Se was not being used to replete critical pools of Se, probably because of adaptation to low Se intake.

Selenium: Stable isotopes

Because Se is essential for life, recommended dietary allowances of 55 and 70 μg/d have been established for women and men respectively, in the USA (National Research Council, 1989). However, these requirements are controversial because deficiency problems have been primarily confined to China (Chen *et al*. 1981), and some populations, such as in New Zealand (Robinson, 1988), have consumed less Se than suggested by the recommended dietary allowance without apparent problems. Se supplementation above the recommended dietary allowance may confer cancer protection (Clark *et al.* 1996) and improve psychological function (Finley & Penland, 1996). Thus, it is important to determine whether populations with low Se status and no apparent signs of Se deficiency should increase their Se intake. An initial question is whether supplementing such a population with Se alters their Se status and/or metabolism.

The present report details supplementation (0–40 μg Se/d) of healthy men and women living on the South Island of New Zealand, an area known for low Se intakes. Stable isotopes of Se were used to measure changes in Se retention following supplementation. The hypothesis of this study was that if Se supplementation changed the Se status, or the need for Se of the subjects, such a change would be reflected by changes in the retention of stable isotopes of Se.

Materials and methods

Subjects

All subjects resided in the Otago region of New Zealand, were non-smokers aged 18–45 years and not pregnant.

Abbreviation: GSH-Px; glutathione peroxidase.

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Prospective volunteers were screened for Se status, and subjects with the lowest values were selected.

This study was approved by the Southern Regional Health Authority Ethics Committee, Otago, New Zealand and by the Human Studies Committee of the US Department of Agriculture and followed the guidelines of the Department of Health and Human Services and the Helsinki Doctrine regarding human subjects. All subjects were informed in detail as to the nature of the study and gave their informed consent.

Procedure

Twenty-nine women and fifteen men consumed 100 μg stable ^{74}Se, as selenate in water after an overnight fast. They were given a self-selected breakfast immediately afterwards and blood was collected for 3 weeks. Then subjects were divided into five groups and consumed daily, for 6 months, tablets providing 0, 10, 20, 30 or 40 μg Se/d as L-selenomethionine (Westar Nutrition Inc., Costa Mesa, CA, USA).

Subjects were administered a second dose of stable ^{74}Se, identical to the first, during the last 3 weeks of the supplementation period. Therefore, the retention of stable Se was measured before any supplements were consumed and after 5 months of supplementation.

Analytical procedures

Size exclusion chromatography. Plasma (1 ml) collected 8 and 336 h post-dosing from subjects consuming 0 or 30 μg supplemental Se/d was separated by HPLC (ISCO Inc., Lincoln, NB, USA; Shodex GS-520 preparative size exclusion, 13 μm particle size, 300 000 Da exclusion limit) with a 0·01 M-Tris-HCl + 0·04 M-ammonium acetate, pH 7·80 buffer and a flow-rate of 5·0 ml/min for 20 min. Fractions (5·0 ml) were analysed for stable isotopes. Chromatograms of ^{74}Se concentration were separated into individual protein peaks by a computer program (PeakFit; SPSS Inc., Chicago, IL, USA).

Isotopes and mass spectrometry. ^{74}Se was purchased as Se metal (Advanced Materials Inc., New York, NY, USA) and converted to selenate (Gilbertson & King, 1950). The purity of the preparation as selenate was determined by measuring ^{74}Se concentration in the solution before and after reduction by ascorbic acid.

Samples were prepared for analysis as described previously (Finley *et al*. 1995). Natural abundance SeO_2 (99·99 %; Æsar, Puratronic, Ward Hill, MA, USA) was used as a primary standard. Enriched ^{76}Se (metal, 90·50 atom%, Advanced Materials & Technology, New York, NY, USA) was converted to selenate as described earlier and used as the *in vitro* spike for isotope dilution analysis (Buckley *et al*. 1992). Isotopes were detected by an inductively coupled plasma mass spectrometer as described by Finley *et al*. (1995).

Statistical analyses

Stable Se retention values were adjusted to reflect differences in body weight by the formula:

$$RET_{adj} = RET_{unadj} \times (BW/BW_{avg}),$$

where RET_{adj} is adjusted retention, RET_{unadj} is unadjusted retention, BW is body weight and BW_{avg} is the average body weight of all individuals in the experiment.

Analysis of covariance (Kleinbaum & Kupper, 1978) was used to examine treatment effects on plasma isotope retention. Measures of retention made after supplementation co-varied with the same measure made before supplementation began. This allowed the effect of supplementation to be adjusted for the value before supplementation began, thus removing a large amount of inter-individual variation.

The effect of Se supplementation on the retention of stable isotopes in plasma proteins at two different time points was analysed by *t* test within each time point. Analysis of covariance was not possible because chromatography was only done on samples collected after the supplementation period.

Results

Mean plasma Se concentration before supplementation was 71·3 (SE 12·8) μg Se/ml and whole-blood glutathione peroxidase enzyme (GSH-Px; *EC* 1.11.1.9) activity was 18·3 (SE 3·6) EU/g haemoglobin. These values were significantly affected by supplement level and the mean for supplement groups 20, 30 and 40 μg Se/d increased to 83·0 (SE 6·9) μg/ml for plasma Se and 25·6 EU/g haemoglobin for GSH-Px activity. By contrast, the post-supplementation values for the placebo group were 70·2 (SE 6·9) μg Se/ml for plasma Se concentration and 19·5 (SE 1·7) EU/g haemoglobin for whole-blood GSH-Px activity.

Covariate adjusted plasma retention of ^{74}Se (Fig. 1) was significantly ($P = 0·046$) affected by Se supplementation. The greatest amount of ^{74}Se was retained by the placebo group and the least by the 30 μg Se/d group. The 40 μg Se/d group retained less ^{74}Se than the placebo group at all time points, but more than the 30 μg Se/d group.

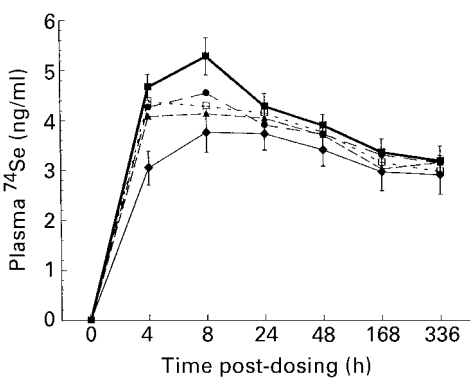

Fig. 1. The effect of supplemental selenium on the retention of 100 μg ^{74}Se in the plasma of men and women who were selected for low selenium status and who resided in a geographical area noted for low selenium intakes. Retention values are mean values (with standard errors represented by vertical bars) for men and women which have been adjusted by analysis of covariance for retention before supplementation began. Supplemental selenium was selenomethionine which was consumed in amounts of 0 (—■—; *n* 9), 10 (- - -●- - -; *n* 9), 20 (- - -▲- - -; *n* 8), 30 (—◆—; *n* 4) or 40 (- - -□- - -; *n* 6) μg/d.

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Table 1. The effect of selenium supplementation on the distribution of natural abundance and stable isotopic selenium between different plasma proteins in men (*n* 15) and women (*n* 29) selected for low selenium status

(Values are means with their standard errors)

	Time post-dosing (h)†							
	8				336			
Se supplement (μg/d) …	0		30		0		30	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Natural abundance peak (ng)								
1	37·0	4·1	47·1	6·0	34·6	3·2	49·6*	6·2
2	1·9	0·5	7·1**	2·7	3·1	0·9	9·7**	1·4
3	0·9	0·6	0·8	0·9	0·6	0·6	1·2	0·7
Stable isotope peak (ng)								
1	3·68	1·17	2·87	0·32	0·86	0·09	0·54*	0·1
2	0·93	0·49	1·10	0·11	0·14	0·07	0·16	0·08
3	0·16	0·10	0·18	0·12	0·03	0·03	0·06	0·03
Stable isotope peak (% natural abundance)								
1	8·63	2·83	5·71	1·45	2·23	0·15	0·91***	0·24
2	2·17	1·26	2·16	0·50	0·34	0·15	0·27	0·15
3	0·33	0·20	0·36	0·21	0·07	0·07	0·08	0·05

Mean values were significantly different (or marginally different: * $P < 0{\cdot}09$) from unsupplemented-group values: ** $P < 0{\cdot}01$, *** $P < 0{\cdot}005$.
† Subjects were given 100 μg ^{74}Se orally as selenate in water after an overnight fast, and thereafter consumed a self-selected diet.

Distribution of ^{74}Se among plasma proteins

Isotopic and natural abundance Se were primarily incorporated into two intermediate molecular mass peaks (peaks 1 and 2), with a smaller and variable amount found in a low-molecular-mass peak (peak 3). Se supplementation significantly ($P = 0{\cdot}01$) increased Se accumulation in peak 2 by almost three-fold (Table 1). The retention of ^{74}Se in peak 1, expressed as a percentage of total Se, was reduced almost 50 % by supplementation with 30 μg Se/d ($P = 0{\cdot}005$), but peak 2 was unaffected.

Discussion

Se deficiency in the Otago region of New Zealand is well documented (Robinson, 1988). Supplemental Se began to be supplied to sheep in the 1950s in order to prevent Se deficiency. Human intakes as low as 20–30 μg/d are not uncommon (Robinson, 1989), resulting in lower Se status in New Zealand residents than in residents of the USA (Robinson, 1989). However, human Se deficiency problems have not been reported except for one patient on total parenteral nutrition who responded to Se supplementation (Van Rij *et al*. 1979). Recently the blood Se status of New Zealanders has gradually risen because of increased importation of Australian wheat with higher Se content (Thomson & Robinson, 1996). Despite this, the mean plasma Se concentration of subjects in the present study was only 60 % of those in a recent study in North America (Finley & Penland, 1996).

The amounts of supplementation chosen represented an intake range from typically low New Zealand intakes to the recommended daily allowance in the USA. Intake was estimated at approximately 30 μg/d (Robinson, 1989), and the supplements were from 0 to 40 μg/d, giving total intakes of 30–70 μg/d.

The conclusion from these data is that Se supplementation had relatively little effect on the retention of stable Se, especially by tissues that reflect long-term intake. Retention of ^{74}Se in plasma was decreased by supplemental Se but the decrease was relatively small. Plasma is considered to be a pool with a fast turnover time that reflects short-term intake. However, supplementation had no effect on retention of ^{74}Se in the platelets and erythrocytes (results not shown), pools with a longer turnover and more indicative of long-term intake. This implies that supplementation at the levels provided had a relatively minor effect on homeostatic controls of Se isotope accumulation in the body. This may be because subjects were adapted to their relatively low Se intake.

One means of adaptation to lower intake may have been through increased retention of Se. Maximal retention of ^{74}Se in plasma (assuming a plasma volume of 3 litres) was 24·4 % by the placebo group, which is much greater than the 15 % maximal retention by North American males (Kasper *et al*. 1984). Reduced urinary excretion may partially explain the difference, as Robinson (1989) noted that greater Se retention by New Zealanders (compared with populations with higher Se intake) was partially a consequence of decreased urinary excretion.

Another means of adapting to low intakes may be redistribution of Se among selenoproteins. Some selenoproteins appear to have priority for Se when it is limiting; for example liver GSH-Px of Se-deficient rats is depleted faster than plasma selenoprotein P (Yang *et al*. 1989). In the present study, increased isotope retention in the plasma of the 30 μg Se/d supplement group was accompanied by significantly decreased isotope accumulation in peak 1, but no significant change in peak 2. When expressed as a percentage of total isotope in the two peaks, supplementation with 30 μg Se/d increased the amount in peak 2 from 13 to 23 %, and decreased the amount associated with peak 1 from 87 % to 77 %. Previous data suggest that peak 1 is

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selenoprotein P and peak 2 is albumin (Finley, 1999), therefore Se supplementation resulted in relatively more isotope going into albumin, and not selenoprotein P. Consequently additional Se may not have increased the production of a high-priority protein (selenoprotein P), because adaptation had already ensured that adequate Se was going into critical pools.

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