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Research Article

Effect of selenium status and supplementation with high-selenium yeast on plasma homocysteine and B vitamin concentrations in the UK elderly

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The level of plasma total homocysteine (tHcy), long known to be B vitamin dependent, has recently been shown to be inversely associated with plasma selenium (Se) concentration in human subjects. We therefore, chose to investigate the interaction between Se, tHcy and B vitamins in a double-blind, placebo-controlled trial where 501 healthy UK elderly volunteers were randomly allocated to receive 100, 200, or 300 μg Se/day as high-Se-yeast, or placebo-yeast for 6 months. Plasma Se, tHcy, folate, vitamin B-12, pyridoxal-5′-phosphate (PLP) and its catabolite, 4-pyridoxic acid, were measured in all participants at baseline and in samples from the placebo, 100 and 300 μg Se/day groups, at follow-up. At baseline, Se was inversely correlated with tHcy but only in males ($p < 0.001$). Before supplementation, tHcy concentration was significantly lower in the highest compared to the lowest Se tertile in males ($p < 0.05$), and in females when folate concentrations were also in the top tertile ($p < 0.05$). The effect of folate, PLP and vitamin B-12 concentrations on plasma tHcy correlated with Se concentration at baseline. After 6 months of Se supplementation, only Se concentration had changed significantly. Supplementation with Se does not affect tHcy concentration in the UK elderly population.

Keywords: B vitamins / Elderly / Homocysteine / Randomised controlled trial / Selenium

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1 Introduction

Elevated plasma total homocysteine (tHcy) concentrations have been associated with a plethora of complex diseases including cardiovascular disease [1], neurodegenerative disease [2–4], osteoporosis [5, 6], cognitive decline in the elderly [7] and cancer [8–10].

It has been acknowledged for some time that folate and vitamin B-12 are powerful nutritional factors that can reduce plasma tHcy concentration [11, 12] (see Fig. 1). Recently, however, results of a Spanish study [13] suggested that there was a stronger inverse correlation between serum selenium (Se) and tHcy than between folate and tHcy in elderly volunteers (age range 63–86 years). Other examples of a significant inverse association between Se status and plasma tHcy were found in an Inuit population [14], in middle-aged and elderly subjects from Upper Silesia [15] and in the UK National Diet and Nutrition Survey (NDNS) of people aged 65 years and above [16].

Animal studies, however, gave different results: Uthus *et al*. [17] found tHcy concentration to be lower in Se-deficient rats than in rats supplemented with adequate or supranutritional amounts of Se. Following on from these findings and to see if they would be reproduced in humans, a New Zealand group investigated the effect of Se supplementation on tHcy concentrations in healthy volunteers [18]. After 20 wk of supplementing 189 volunteers aged 18–64 years with placebo or 200 μg Se/day as selenomethionine, plasma tHcy was unchanged, neither rising, as might have been expected from the results of Uthus *et al*. [17] nor

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Abbreviations: BHMT, betaine homocysteine methyltransferase; **GCL,** glutamate-cysteine ligase; **GNMT,** glycine-*N*-methyltransferase; **NDNS,** National Diet and Nutrition Survey; **PA,** 4-pyridoxic acid; **PLP,** pyridoxal 5′-phosphate; **PRECISE,** prevention of cancer by intervention with Se; **tHcy,** total homocysteine

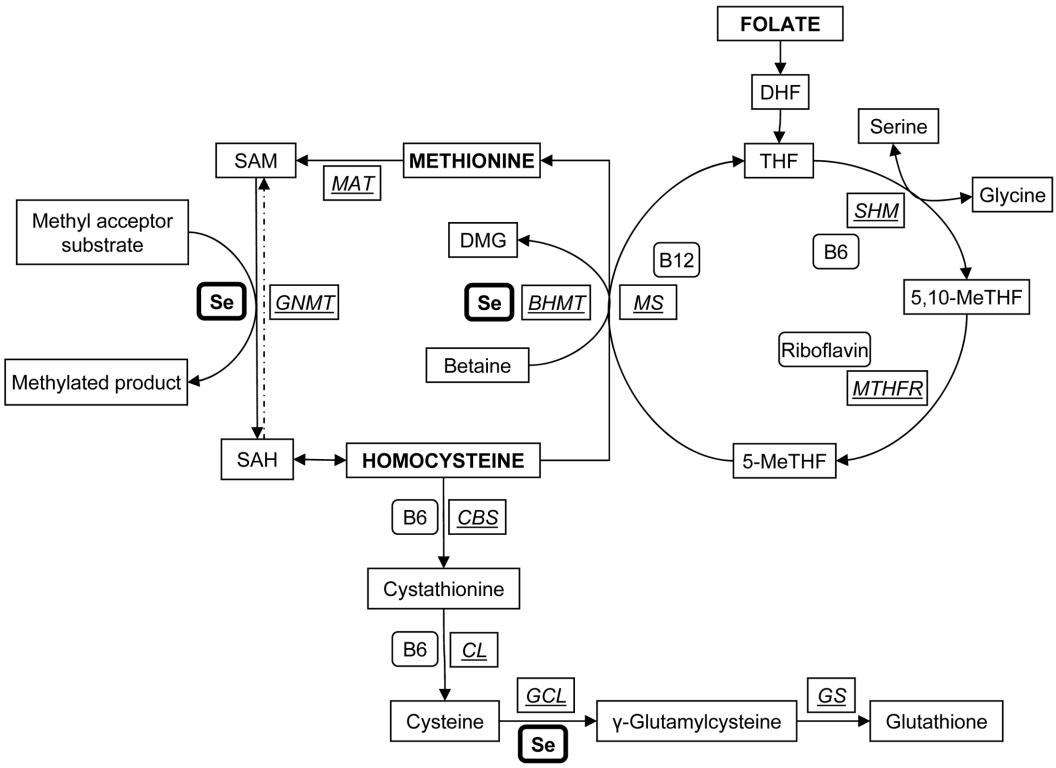

Figure 1. Overview of one-carbon metabolism. Dietary folate is reduced to dihydrofolate (DHF) and tetrahydrofolate (THF) and the transfer of a carbon unit by serine hydroxymethyltransferase (SHM) results in the formation of 5,10-methylenetetrahydrofolate (5,10-MeTHF) and glycine. 5,10-methylenetetrahydrofolate can subsequently be reduced by the riboflavin-dependent methyltetrahydrofolate reductase (MTHFR) to form 5-MeTHF and can be used as a methyl donor for the remethylation of homocysteine back to methionine *via* the B12-dependent enzyme methionine synthase (MS). An alternative route for the re-methylation is *via* the betaine dependent enzyme, BHMT, with dimethylglycine (DMG) as a by-product. *S*-adenosyl methionine (SAM), through the activation of methionine by methionine adenosyl transferase (MAT), can be used by methyltransferases such as GNMT as a methyl donor for biological methylation reactions. The by-product of this reaction, *S*-adenosyl homocysteine (SAH), can be hydrolysed to form homocysteine. The elimination of homocysteine occurs *via* the trans-sulphuration pathway where homocysteine is converted to cystathionine using the vitamin B-6-dependent enzyme cystathionine β-synthase (CBS). Cystathionine, *via* the synthesis of cysteine with the help of cystathionine γ-lyase (CL), can be catabolised to form γ-glutamylcysteine using the γ-GCL enzyme. The trans-sulphuration pathway results in the biosynthesis of glutathione produced by glutathione synthetase (GS).

falling, as might have been predicted from observational studies [13 – 15]. The discrepancy in results between the human and animal studies may be due to the extreme level of Se deficiency attained in the animal studies or to the type of Se administered to the rats, *i.e.* selenite rather than selenomethionine, as in the human study.

An interesting observation in the rat study was that the expression and activity of betaine homocysteine methyltransferase (BHMT), an enzyme that catalyses the transfer of a methyl group from betaine to homocysteine (see Fig. 1), were significantly decreased in Se deficiency resulting in less remethylation of homocysteine to methionine [17]. The authors suggested that if the level of Se is inadequate for BHMT expression, one-carbon metabolism may be shunted towards the trans-sulphuration pathway to reduce the excessive amounts of tHcy. In further rat studies, Se deficiency was also found to reduce the activity of glycine-*N*-methyltransferase (GNMT), a liver enzyme, inhibited by folate, that is thought to provide an alternative route for the conversion of excess *S*-adenosylmethionine to *S*-adenosylhomocysteine [19, 20] (Fig. 1). However, neither gene expression nor activity of BHMT or activity of GNMT was influenced by Se deficiency in mice, demonstrating a species-specific effect [20]. Se may also influence the activity of glutamate-cysteine ligase (GCL), an enzyme that converts cysteine to γ-glutamyl cysteine (on the pathway to glutathione) and may thereby aid removal of tHcy (Fig. 1): its activity has been shown to be dependent on Se status in rats and mice, though again with a species difference [20].

Given the conflicting reports described above, we decided to investigate whether Se supplementation affected plasma tHcy, and if so, to what extent B vitamin status might affect the interaction, particularly given the known requirement for pyridoxal-5′-phosphate (PLP) in selenoamino acid metabo-

lism [21]. For this purpose, we made use of plasma samples generated in the UK PRECISE (PREvention of Cancer by Intervention with Se) pilot study [22]. This was a double-blind, placebo-controlled trial in which 501 UK volunteers aged 60 – 74 were randomly allocated to receive 100, 200, or 300 μg Se/day as high-Se yeast or a placebo yeast. Blood was collected at baseline and 6 months, enabling plasma Se, tHcy, folate, vitamin B-12 and PLP to be measured at these time points in a selection of samples. As it is considered good practice to use more than one index for evaluation of vitamin B-6 status [23], we also measured plasma 4-pyridoxic acid (PA), a catabolite of PLP which gives a complementary measurement of vitamin B-6 status [24].

2 Materials and methods

2.1 Study design

The UK pilot study for the planned international PRECISE trial was setup to assess the viability of conducting the main trial in the UK. It was carried out in four general practices of the Medical Research Council General Practice Research Framework (GPRF) from UK areas with differing demographic characteristics: Guisborough and Linthorpe (North East), Bromsgrove (West Midlands) and Bungay (East Anglia). No formal power calculations were performed for the pilot study and the target accrual (510 subjects in 12 months) was chosen to give sufficient subjects from which to be able to draw reasonable inferences about recruitment, compliance and loss-to-follow-up, while keeping costs within reasonable bounds.

2.2 Subjects and recruitment

Between June 2000 and July 2001, research nurses recruited similar numbers of male and female volunteers from each of three age-bands: 60 – 64, 65 – 69 and 70 – 74 years. Exclusion criteria were: (i) a Southwest oncology group performance status score >1 (*i.e.*, incapable of carrying out light housework or office work); (ii) active liver or kidney disease; (iii) prior diagnosis of cancer (excluding nonmelanoma skin cancer); (iv) diagnosed HIV infection; (v) on immunosuppressive therapy; (vi) diminished mental capacity; (vii) supplementing with ≥ 50 μg/day of Se in the previous 6 months (by patient report). The study had approval from the appropriate UK Local Research Ethics Committees and participants provided written informed consent to participate.

2.3 Protocol

Following a 4 wk placebo run-in, 501 volunteers were randomly assigned to one out of four treatment regimens: placebo, 100, 200, or 300 μg of Se/day for a minimum of 6 months. The intervention agent was high-Se yeast, SelenoPrecise™ (Pharma Nord, Vejle, Denmark) or a placebo-yeast, identical except for Se-content. Central randomisation was by computer-generated permuted blocks stratified by GP practice, gender and age group. Research nurses telephoned the independent randomisation service at the Clinical Trials and Statistics Unit, Institute of Cancer Research, to obtain an anonymous code for each volunteer. Participants collected their corresponding prelabelled tablets from the research nurses. Participants and general practice personnel were blinded to study treatment. Participants provided a blood sample at both baseline and 6 months (when visiting the GP practices for the purpose of the PRECISE pilot). Heparinised plasma was prepared and frozen at the practices. Plasma samples were transferred to the University of Surrey, where they were stored at −80°C. All transfer of samples between centres was carried out on dry ice.

2.4 Measurements

Baseline and 6 month plasma samples from the placebo, 100 and 300 μg/day groups were analysed for Se, tHcy, folate, B-12, PLP and PA. For reasons of cost, B vitamins and tHcy were not measured in follow-up samples from the 200 μg/day group.

Lithium–heparin plasma was analysed for Se at Central Science Laboratory (Sand Hutton, UK) by hydride-generation inductively coupled plasma MS. Weighed plasma samples were prepared by microwave digestion (Multiwave, Perkin-Elmer, Bucks, UK) and reduced to Se (IV), before being made up to volume for analysis. All reagents were of 'Analar' grade (or better) and the water used was of Millipore grade (18 MΩ). Quality-control procedures were accredited under the UK Accreditation Scheme (UKAS). Accuracy was assured by analysis of certified reference materials namely: Seronorm serum, mean value (10 determinations) 85.5 ng/g, RSD 12.7% (certified 86 ng/g); NIST 1598 bovine serum, mean value (16 determinations) 43.5 ng/g, RSD 6.2% (certified 42.4 ± 3.5 ng/g). The LOD was 5 ng/g and the mean recovery 108% (12 determinations).

Plasma folate and vitamin B-12 were measured using microbiological assays as previously described [25, 26] Inter- and intra-assay CVs were ≤8.2% for plasma folate and ≤10.4% for vitamin B-12. Plasma tHcy was measured by fluorescence polarisation using an Abbott IMX instrument [27]. Inter- and intra-assay CVs were ≤2.5%.

Plasma PLP (the main active form of vitamin B-6) and PA (a catabolic B-6 form) concentrations were determined by RP HPLC with fluorescence detection according to the method of Bates *et al*. [28]. Interassay CVs were 3.7 and 4.2% for PLP and PA, respectively.

2.5 Statistical analysis

Data analyses were performed using SPSS version 14.0. Data are generally expressed as means ± SD. Variables not

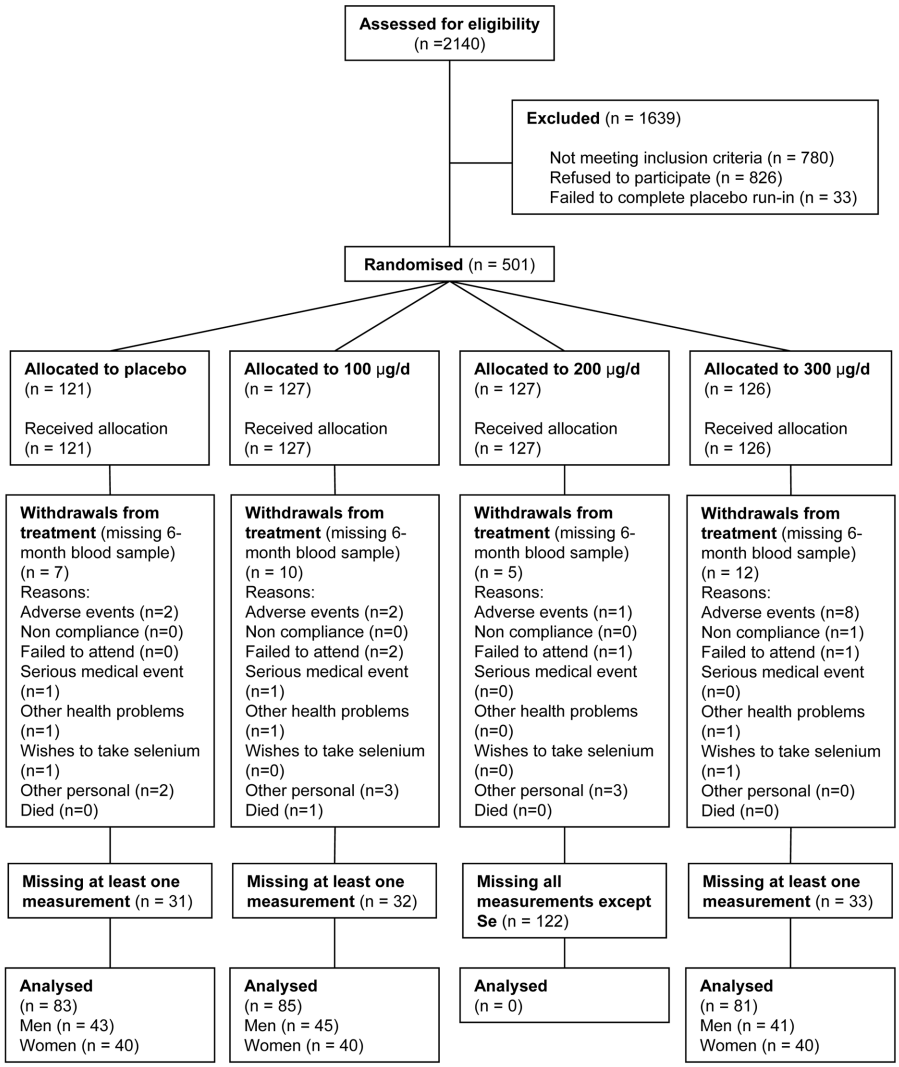

Figure 2. Participant flow through the study.

normally distributed were log transformed. ANOVA, ANCOVA, independent samples *t*-test, Pearson's correlations and linear regression were used to test the relationships among the different variables as appropriate. $p < 0.05$ was taken as the criterion of statistical significance. Baseline linear regression analyses were adjusted for age owing to the known relationship between age, kidney function and tHcy. Baseline and age were included as covariates in the ANCOVA analyses of follow-up values. Statistical parameters presented are β (standardised regression coefficient), R^2 (coefficient of multiple determination) and *r* (Pearson correlation coefficient).

3 Results

3.1 Participants

Five hundred and one participants were recruited between June 2000 and July 2001. All variables were measured in the baseline samples but owing to financial constraints, only samples from the placebo, 100 and 300 μg groups were analysed for all variables at follow-up. Figure 2 shows the flow of participants through the study from which it can be seen that 374 participants in total were allocated to the placebo, 100 and 300 μg groups and that 83, 85 and 81 samples, respectively were analysed for all variables at follow-up.

3.2 Compliance with treatment

Three hundred and sixty-one of the 374 participants (96.4%) randomised to the placebo, 100 and 300 μg/day groups were compliant (missed less than 10% of the total number of tablets they should have taken) according to pill count. Nonprotocol use of over-the-counter Se ('drop-ins') was assessed by inspection of the distribution of plasma Se concentrations in the placebo group at 6 months. One hundred and three out of one hundred and fourteen participants

Table 1. Baseline study population characteristics by sex and *p*-values for differences between men and women

Variables	*n*[a)]	Men[b)]	Women[b)]	*p*
Age, *y*	501	67.05 ± 4.01	66.64 ± 4.17	0.269
Plasma Se (ng/g_	483	88.09 ± 17.78	91.28 ± 20.23	0.066
Plasma tHcy (mmol/L)	454	11.50 ± 4.19	10.17 ± 3.64	<0.001
Plasma folate (ng/mL)	455	11.51 ± 7.47	13.28 ± 8.86	0.023
Plasma PLP (nmol/L)	449	51.17 ± 27.28	49.40 ± 27.08	0.493
Plasma PA (nmol/L)	449	18.79 ± 14.34	16.65 ± 15.23	0.125
Plasma B-12 (pg/mL)	450	340.76 ± 100.34	369.83 ± 130.80	0.009

a) Number of participants who had each measurement at baseline.
b) Values are means ± SD.

Table 2. Correlation matrix of baseline values[a,b)]

	tHcy		Folate		PLP		PA		B-12	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
Se	−0.138	−0.067	0.089	−0.055	0.144	0.077	0.062	0.080	0.089	0.114
	0.034	*0.325*	*0.172*	*0.418*	*0.027*	*0.265*	*0.340*	*0.246*	*0.173*	*0.095*
	(237)	(215)	(237)	(216)	(236)	(211)	(236)	(211)	(233)	(215)
tHcy			−0.297	−0.258	−0.178	−0.110	−0.161	−0.125	−0.363	0.311
			0.000	*0.000*	*0.006*	*0.114*	*0.014*	*0.072*	*0.000*	*0.000*
			(237)	(213)	(234)	(208)	(234)	(208)	(233)	(213)
Folate					0.288	0.428	0.433	0.437	0.072	0.224
					0.*000*	*0.000*	*0.000*	*0.000*	*0.275*	*0.001*
					(234)	(209)	(234)	(209)	(234)	(214)
PLP							0.525	0.385	0.174	0.228
							0.000	*0.000*	*0.008*	*0.001*
							(238)	(211)	(230)	(208)
PA									0.195	0.147
									0.003	*0.035*
									(230)	(208)

a) Variables entered into the Pearson correlation matrix were log transformed.
b) *R* values are in normal script, Pearson correlation values in italics and number of subjects in brackets.

on placebo had follow-up Se measurements; of these, two (1.9%) had an Se status at follow-up more than two SDs above the mean, consistent with the 2.5% expected for a population approximating a normal distribution. We have made the assumption therefore, that drop-ins were rare.

3.3 Withdrawals

Thirty-four out of the initial five hundred and one participants (7%) withdrew from the study within the first 6 months, five of whom had been allocated to the 200 µg group. Thus, there were 29 withdrawals from the 374 participants that were randomised to the placebo, 100 and 300 µg groups, *i.e.* 8% of the total. There was no significant difference in numbers of participants withdrawing from treatment in the different groups (7, 10 and 12 in the placebo, 100 and 300 µg groups, respectively: $X^2 = 1.2$, df = 2, $p = 0.57$). Of these, 12 withdrew because of adverse events, six of which were abdominal/stomach problems. Other reasons for discontinuation appeared unrelated to treatment.

3.4 Baseline data

Table 1 shows the mean concentrations of the different factors assayed prior to treatment. Men had significantly higher total plasma tHcy but lower plasma folate and vitamin B-12 values compared to women. Age was inversely correlated (both sexes combined) with Se ($r = -0.176$, $p < 0.0001$) and positively correlated with tHcy ($r = 0.220$, $p < 0.0001$).

The correlation matrix (Table 2) revealed that tHcy was significantly inversely correlated with all variables though only in males in the case of Se, PLP and PA. Se was also significantly correlated with PLP but again, only in males. PLP and PA were correlated very strongly with each other and with folate and vitamin B-12. In females, tHcy was correlated with folate and vitamin B-12. Folate was also significantly correlated with PLP and PA while vitamin B-12 was correlated with all the other variables with the exception of Se.

Linear regression identified factors that significantly influenced tHcy concentration at baseline (Table 3). The

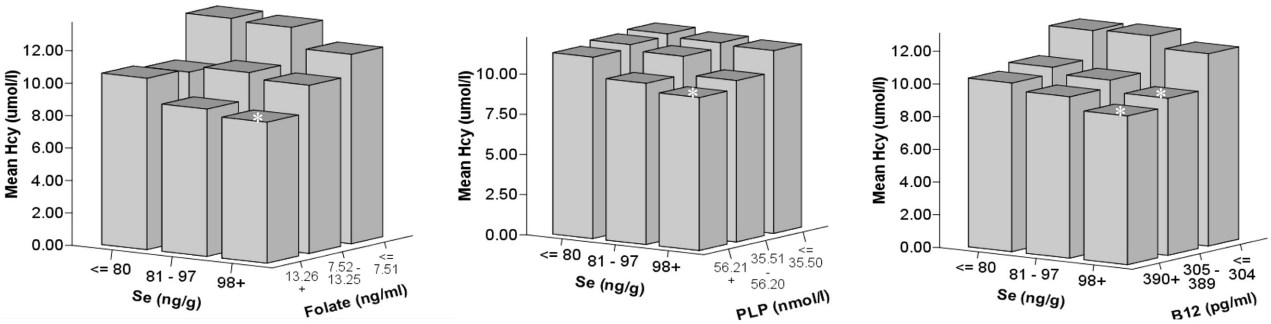

Figure 3. 3-D bar charts showing the interaction of Se, folate, PLP and vitamin B-12 on tHcy concentrations at baseline. A: Se and folate tertiles; B: Se and PLP; C: Se and vitamin B-12. (Asterisks indicate significantly different from the lowest tertile of the *X*-axis variable by ANOVA, $*p < 0.05$).

Table 3. Linear regression analysis of the impact of baseline variables on baseline tHcy

	Independent variable	β Coefficient[b)]	Significance	R^2 change[b)]
Men ($n = 225$)[a)]	Folate	−0.355	<0.0001	17.6
	B-12	−0.361	<0.0001	13.1
	Se	−0.137	<0.05	1.8
Women ($n = 204$)[a)]	Folate	−0.487	<0.0001	29.2
	B-12	−0.169	<0.01	2.6

a) Number of men or women who had measurements of Se, tHcy, folate, PA, vitamin B-12 and B-6 at baseline.
b) Values were adjusted for age.

Table 4. Plasma Se at baseline and after 6 months supplementation and *p*–value for difference[a)]

Treatment	*N*[b)]	Baseline Se (ng/g)	6 months Se (ng/g)	*p*
Placebo	103	89.98	91.36	0.738
100 mg	107	87.85	144.49	<0.0001
300 mg	101	91.33	226.71	<0.0001

a) Plasma Se measurements can be converted from ng/g to μg/L by multiplying by 1.027, the density of plasma [29].
b) Number of subjects with Se measurements at both baseline and follow-up.

tests were performed separately in men and women as there were marked differences between the sexes. All variables from Table 1 were entered into the stepwise linear regression model. Plasma Se concentration was found to have a significant influence on tHcy concentration in males, accounting for 1.8% of the variance, though the effect was considerably smaller than that of folate (17.6%) and vitamin B-12 (13.1%); in females, there was no effect of plasma Se, folate having by far the largest effect (29.2%).

There were significant differences in tHcy concentrations between the lowest (≤78 ng/g) and highest (≥94.01 ng/g) Se tertiles in males (11.73 ± 3.37 *vs.* 10.91 ± 4.97 μmol/L, respectively, $p < 0.05$, independent *t*-test) consistent with linear regression and Pearson correlation results. Female subjects with folate concentrations in the top tertile (≥14.16 ng/mL) had lower tHcy concentrations (8.04 ± 1.56 *vs.* 10.67 ± 5.92 μmol/L, $p < 0.01$, independent *t*-test) when their Se concentrations were also in the top tertile (≥100.01 ng/g) compared to the lowest Se tertile (≤80 ng/g).

Concentrations of tHcy were lowest in those subjects with high folate (≥13.25 ng/mL), PLP (≥56.21 nmol/L) or vitamin B-12 (≥390 pg/mL) who also had high Se (≥98 ng/g) (Fig. 3).

3.5 Se status

Overall mean plasma Se in the 483 participants who had Se measurements at baseline was 89.6 (SD ± 19.0) ng/g (equivalent to 92.0 (SD ± 19.5) μg/L [29]). Three hundred and eleven participants had plasma Se measurements at follow-up from which it could be seen that Se supplementation significantly elevated Se concentrations in all treatment groups except the placebo group (Table 4).

3.6 Effect of the intervention on plasma tHcy

Concentrations of plasma tHcy at baseline and after 6 months are shown separately for males and females in the three treatment groups (Table 5). At baseline, there was no evidence of any difference in tHcy levels between groups (*p*

Table 5. tHcy at baseline and after 6 months supplementation and *p*-value for difference

Sex	N[a)]	Treatment group	tHcy at baseline (±SD) (μmol/L)	tHcy at 6 months (±SD) (μmol/L)	*p*-value (ANCOVA)[b)c)]
Males	45	Placebo	10.94 (±3.56)	11.30 (± 3.77)	–
	48	100 μg Se	11.64 (±4.02)	12.12 (± 4.06)	1.000
	50	300 μg Se	11.42 (±3.17)	12.00 (± 3.36)	0.482
Females	41	Placebo	10.42 (±3.72)	10.07 (± 3.47)	–
	42	100 μg Se	10.32 (±2.86)	10.14 (± 3.07)	0.526
	44	300 μg Se	9.56 (±2.37)	9.51 (± 2.26)	1.000

a) Number of participants who had tHcy measurements at both baseline and follow-up.
b) tHcy at baseline (μmol/L) as dependent variable.
c) Values were adjusted for age.

Table 6. Linear regression analysis of the impact follow-up variables on follow-up tHcy

	N[a)]	Treatment	Independent variable	β Coefficient[b)]	Significance	R^2 change[b)]
Men	51	Placebo	Folate	−0.256	<0.01	5.7
	47	100 μg Se	Folate	−0.178	<0.05	2.4
	46	300 μg Se	Folate	−0.162	<0.05	2.3
Females	39	Placebo	Folate	−0.368	<0.001	10.5
	46	100 μg Se	PA	−0.220	<0.05	4.6
	41	300 μg Se	Folate	−0.231	<0.01	5.0

a) Number of men or women who had measurements of Se, tHcy, folate, PA, vitamin B-12 and B-6 at follow-up.
b) Values were adjusted for age.

> 0.741). When follow-up plasma tHcy concentrations (adjusted for baseline tHcy concentration and age) in the different treatment groups were compared to that of the placebo group, there was no evidence of an effect of Se intervention on plasma tHcy in either males, females (Table 5) or both sexes combined (data not shown). When subjects were grouped according to tertile of plasma Se attained at 6 months, no significant differences were found in tHcy concentrations between tertiles (results not shown). Even subjects in the top quartile of tHcy at baseline, who might have been expected to show tHcy lowering on Se supplementation, did not benefit from Se treatment (data not shown).

A stepwise linear regression model containing all the variables in Table 1 was generated to assess which, if any, of the variables affected tHcy concentration at follow-up. The only variable influencing tHcy concentration in all treatment groups at follow-up was folate, except in the 100 μg/day female group where PA had a larger effect (Table 6).

4 Discussion

Females appeared to have better nutritional status than males, as shown by higher concentrations of folate and vitamin B-12 and lower tHcy concentrations at baseline. The UK NDNS provides a suitable reference group with which to compare our values, as it also investigated nutrient concentrations in elderly men and women [16, 30]. Compared to NDNS subjects, participants in our PRECISE study had higher concentrations of all nutrients investigated, while tHcy was lower.

Specifically, mean plasma Se in our healthy volunteers was 89.6 ng/g (equivalent to 92.0 μg/L) compared to 78.17 μg/L in NDNS subjects [16]. In the past, maximum activity of plasma glutathione peroxidase has been taken as a measure of Se adequacy. By this criterion, the mean status of our study population was borderline adequate [31] though given the median value of 91.4 μg/L, this suggests that almost 50% may not have achieved adequacy.

Despite optimistic predictions from observational human studies [13 – 16], we found no effect of supplementation with 100 or 300 μg/day high-Se yeast on plasma tHcy concentration in our double-blind placebo-controlled trial. Our results are in accordance with a study from New Zealand that found no effect of supplementation with 200 μg/day Se as selenomethionine on tHcy concentration [18]. We did not find the increase in tHcy that might have been predicted by studies in rats [20] though this may be because comparisons are made with states of Se deficiency in animals that are not seen in humans. It may also be relevant that species-specific differences have been seen in the response of Hcy to Se supplementation [20].

Though we saw no effect of the intervention, we did find significant associations between plasma concentrations of Se, tHcy and B vitamins at baseline. Notably, low Se status was significantly associated with high tHcy concentration at baseline, though only in men. In women, ANOVA

showed that those with significantly lower tHcy were in the top tertile both of plasma folate and plasma Se.

Linear regression analysis in males at baseline revealed that plasma Se was associated with 1.8% of the variation in tHcy while folate, at 17.6%, had the largest effect. This is in contrast to the results of Gonzalez *et al*. [13] who reported that for both sexes together, serum folate explained a smaller proportion of plasma tHcy variation than did serum Se (2.2 *vs*. 5.8%), with serum vitamin B-12 accounting for very little of the variance (0.6%). Though PLP was shown by Bates *et al*. [24] to affect tHcy concentration, it did not have a significant effect in our study, although it did correlate with tHcy in males at baseline.

The inverse relationship between Se and tHcy that we saw at baseline has previously been observed by other groups [13 – 15] although they did not separate the sexes in their analysis, nor did they take account of any possible confounding by B vitamin status. In the NDNS study of the UK elderly, folate, PLP and vitamin B-12 were also measured [16]. After adjustment for serum folate, PLP and vitamin B-12, the inverse correlation between plasma Se and tHcy seen in this free-living population became nonsignificant (Dr. Chris Bates, personal communication 2006). It is possible that had the B vitamins been measured in the other studies, the relationship between Se and tHcy would have disappeared or have been attenuated. This is unlikely to be the whole explanation for the relationship between Se and Hcy seen in the Spanish study however, as both folate and vitamin B-12 were taken into account in the analysis.

Another possible explanation for our finding of an inverse association between plasma Se and tHcy is that we were seeing an effect of protein intake. Protein is the main dietary carrier of Se and its intake is inversely correlated with tHcy [32]. However, Gonzalez *et al*. [13] found that the correlation they saw between tHcy and protein intake became nonsignificant after Se was included in the model, suggesting that Se is driving the effect of protein on Hcy concentration. This is therefore an unlikely explanation.

Though we measured the B vitamins and could therefore account for their effects, we were unable to measure creatinine, an indicator of kidney function that in turn is linked to plasma tHcy homeostasis [6]. In the elderly NDNS, plasma creatinine was significantly inversely associated with plasma Se ($n = 700$, $F = 15.3$, $p < 0.0001$, ANOVA) [16]. It is therefore possible that we were observing an effect of kidney function, where low Se is secondary to failing kidneys [33]. This is unlikely to be the whole story, however, as Gonzalez *et al*. measured serum creatinine and though they used it as a covariate in their analysis, were still unable to account fully for the association between Se and tHcy.

Perhaps a more likely explanation for our results and those of Gonzalez *et al*. [34 – 36] relates to a primary effect of pro-inflammatory cytokines on the levels of both plasma/serum Se and tHcy. Selenoprotein P and plasma glutathione peroxidase make a major contribution to the concentration of Se in plasma but during the acute phase response, the expression of selenoprotein P and other selenoproteins can be reduced by pro-inflammatory cytokines [34 – 36]. Se (as selenomethionine) is also incorporated into plasma albumin, a negative acute-phase protein. Together with the decreased selenoprotein expression that occurs with inflammation, lower albumin synthesis in an inflammatory state might account for the lower plasma Se concentration observed in such states. In contrast, the concentration of tHcy has been *positively* correlated with pro-inflammatory cytokines [37]. Thus we could hypothesise that the more prevalent inflammation is in a population, the stronger the inverse correlation between Se and tHcy might be expected to be. In an elderly population, levels of inflammatory mediators such as cytokines and acute phase proteins are two- to four-fold higher than in younger groups [38]. Given that the Spanish study population consisted of nursing home residents of mean age 75 year, it is likely that inflammation was more prevalent than in our free-living, healthy volunteers of mean age 67 year. The fact that tHcy concentrations were higher (by 17 and 26% in males and females, respectively) in the Spanish study population than in our subjects supports that conclusion. We therefore think it likely that inflammation explains the inverse correlation observed between Se and tHcy, and that the degree of inflammation in any given population will predict the strength of such a correlation.

While we think that this may be the most likely explanation for the baseline associations we observed, the small correlation of Se status with tHcy concentration could also be an independent effect. Uthus *et al*. [17, 20] found that Se deficiency affected the activity of BHMT, GNMT and GCL enzymes involved in one-carbon metabolism. We know that Se can react with cysteine-rich regions present within the catalytic domain of enzymes such as protein kinase C where it may induce oxidation of cysteines [39]. As BHMT is a zinc-dependent cysteine-rich enzyme, an altered thiol redox status could influence its activity [17] in much the same way as for protein kinase C. Though our volunteers were not classed as Se-deficient, it is possible that those of higher baseline Se status were better able to use BHMT to catalyse the transfer of a methyl group from betaine to homocysteine for its remethylation back to methionine.

In summary, in our population, baseline Se status (mean 89.6 ± 19.0, range 49 – 177 ng/g) was significantly inversely associated with plasma tHcy concentration, though the magnitude of the association was small amounting only to 1.8% of the variance observed. However, we saw no effect of supplementation with Se at 100 or 300 μg/day for 6 months on plasma tHcy concentration in a randomised, double-blind placebo-controlled trial.

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