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Effect of long-term selenium yeast intervention on activity and gene expression of antioxidant and xenobiotic metabolising enzymes in healthy elderly volunteers from the Danish Prevention of Cancer by Intervention by Selenium (PRECISE) Pilot Study

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Numerous mechanisms have been proposed to explain the anti-carcinogenic effects of Se, among them altered carcinogen metabolism. We investigated the effect of Se supplementation on activities of glutathione peroxidase (GPX), glutathione reductase (GR) and glutathione *S*-transferase (GST) in different blood compartments, and expression of selected phase 1 and phase 2 genes in leucocytes (*GPX1*, *γ-glutamylcysteine ligase* catalytic subunit (*GCLC*), AP-1 transcription factor *Fos-related antigen 1* (*Fra1*), *NAD(P)H:quinone oxidoreductase* (*NQO1*), and *aryl hydrocarbon receptor repressor* (*AhRR*)). Healthy elderly Danes (*n* 105; age 71·3 (SD 4·26) years; 36 % reporting use of multivitamin/mineral supplements) participated and were supplemented daily for 5 years with placebo, 100 μg, 200 μg or 300 μg Se as Se-enriched yeast (SelenoPrecise®). Blood samples were collected after 5 years of intervention. When all four groups were compared we found no effect of Se supplementation on plasma GPX or GR, on erythrocyte GPX, GR or GST, or on thrombocyte GR or GST. We found increased thrombocyte GPX activity at the two highest dosage levels in women only, but not in men. No effects on *GPX1*, *NQO1* or *AhRR* gene expression were found. When all Se-supplemented groups were pooled we found significant down regulation of the expression of some phase 2 genes (*GCLC*, *Fra1*). A significant increase in *AhRR* gene expression with smoking was found but was independent of Se supplementation. Down regulation of phase 2 genes could increase the risk of cancer. However, further studies are needed to establish whether the observed effect in leucocytes reflects a similar expression pattern in target tissues.

Human nutrition: Selenium: Oxidative defence: Electrophile response elements

Se is an essential micronutrient that is incorporated specifically as selenocysteine into the active site of selenoproteins (such as the glutathione peroxidases; GPX). Se as an antioxidant helps to maintain intracellular redox balance, promoting a reducing environment and thereby limiting oxidative damage caused by free radicals. It has been suggested that Se intakes above the level necessary to saturate selenoproteins can reduce the risk of cancer. In the Nutritional Prevention of Cancer Trial published in 1996, a daily supplement of 200 μg Se, as Se-enriched yeast, in patients with a history of basal cell or squamous cell carcinoma of the skin showed a significant increase in recurrence of skin cancer, but a significant reduction in overall cancer risk, in risk of cancers with a more severe prognosis, such as prostate, colorectal and lung cancer, and consequently a decrease in total cancer mortality[1–3]. The strongest effect was found in individuals with a baseline plasma Se (pSe) level below 106 μg/l, while no protective effect was found in the upper tertile[4]. Most of the participants in the Nutritional Prevention of Cancer Trial had initial Se intakes above the level required to saturate selenoproteins, indicating that the observed effects of Se are not limited to antioxidant functions through selenoproteins. Based on results from numerous rodent cancer models, it has also been proposed that alterations in carcinogen metabolism

Abbreviations: AhRR, aryl hydrocarbon receptor repressor; EpRE, electrophile response element; Fra1, Fos-related antigen 1; GCLC, γ-glutamylcysteine ligase catalytic subunit; GPX, glutathione peroxidase; GR, glutathione reductase; GSH, glutathione; GST, glutathione *S*-transferase; NQO1, NAD(P)H:quinone oxidoreductase; pSe, plasma Se; tGPX, thrombocyte glutathione peroxidase; XRE, xenobiotic response element.

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resulting in increased enzymic defence play a role in the cancer-protective effect of Se. But results are far from consistent and may vary with choice of animal model, choice of carcinogen, Se source, Se dosage level and tissue studied[5–7].

Se deficiency has been associated with a decrease in GPX activity as well as in mRNA levels[8]. Besides affecting expression and activity of selenoproteins, Se is also involved in the regulation of other gene products. Se supplementation has been reported to increase blood glutathione (GSH) levels, indicating reduced oxidative stress[9]. Glutathione reductase (GR) is the principal enzyme maintaining GSH in its reduced form. GSH is also a cofactor for GPX and is necessary for GSH conjugation by glutathione *S*-transferases (GST). γ-Glutamylcysteine ligase (GCL) composed of a catalytic (GCLC) and a modulatory (GCLM) subunit is the first step in the *de novo* synthesis of GSH[10,11]. Common for *GCLC* and *GCLM* and other genes encoding phase 2 detoxifying enzymes, including NAD(P)H:quinone oxidoreductase (*NQO1*) and *GST*, is the presence of electrophile response elements (EpRE) in the promoter regions (TGACNNNGC), which mediate the transcriptional up regulation in response to a variety of inducing agents[12]. The AP-1 transcription factor *Fos-related antigen 1* (*Fra1*) may contain an EpRE in its promoter region[13] and is able to down regulate *NQO1* and *GCLC* gene expression[14,15] through embedded AP-1 sites (TGACTCA)[16,17]. Se could induce or repress these gene responses through EpRE.

Up regulation of genes encoding phase 1 xenobiotic metabolising enzymes in the liver has been reported in Se-deficient mice[18]. Some xenobiotics interact with the aryl hydrocarbon receptor (AhR) and transactivate target genes such as cytochrome P450 through the xenobiotic response element (XRE). The aryl hydrocarbon receptor repressor (AhRR) regulates AhR function by a negative feed-back mechanism, thereby down regulating genes that are regulated by AhR[19]. The promoter region of *AhRR* contains 3 XRE binding sites, and could potentially be affected by Se supplementation[20].

In Denmark, the average daily dietary Se intake (excluding supplements) is low compared with other countries[21], being 42 μg/d for men and 33 μg/d for women according to the latest survey[22]. But Se supplementation through multivitamin and mineral supplements is very common, adding on average 25 μg. We assumed that a population like the Danish, with relatively low Se intakes, would be appropriate for studying the biological effects of Se supplementation.

The aim of the present study was to investigate whether the dietary Se intake of elderly Danes is sufficient to obtain saturation of GPX expression in different blood compartments, and whether increased Se intake will affect gene expression and activity of selected phase 2 enzymes and transcription factors containing EpRE or XRE in their promoter, as has been reported in animal studies. We therefore investigated whether a daily Se supplement of 100, 200 or 300 μg as Se-enriched yeast for 5 years would increase activities of GPX, GR and GST in erythrocytes, plasma or thrombocytes, and modulate gene expression of *Fra1*, *GCLC*, *GPX1*, *AhRR* and *NQO1* in leucocytes. The use of three dosage levels enabled us to look closer at dose–response relationships. We also investigated the overall effect of Se supplementation by pooling the supplemented groups and testing them against the placebo group.

Materials and methods

Subjects and study design

In 1999, a cohort of 500 men and women was established in Denmark. Participants were recruited from the county of Funen, were between the ages of 60 and 74 years, had no previous cancer diagnosed, had no severe or life-threatening diseases and a daily intake of Se supplementation not exceeding 50 μg (a typical multivitamin/mineral supplement sold in Denmark contains 40–50 μg inorganic Se). A 1-month run-in period where subjects were supplemented with placebo tablets ensured exclusion of participants who were not compliant. The study was a randomised, double-blinded, placebo-controlled intervention experiment with a parallel design. At recruitment each participant was randomised to one of four groups, a placebo group and three dosage levels: 100, 200 or 300 μg Se as Se-enriched yeast (SelenoPrecise®; Pharma Nord, Vejle, Denmark). The Se-enriched yeast has previously been reported to consist of about 81 % selenomethionine in the fraction liberated by proteolysis[23]. At study entry and at 6, 12, 18, 24, 36 and 60 months the participants visited the centre to donate a blood sample, collect tablets for the following period and answering questions concerning compliance and side effects, such as garlic breath, hair loss and nail brittleness. Based on counting of returned pill packets after years 1, 2 and 3, compliance was >96 %. No serious adverse effects were reported and there was no correlation to Se dose (details will be published elsewhere). The supplementation lasted 5 years. The last blood samples were collected after 5 years of supplementation and the present study was performed on a random sub-sample of 105 participants, selected consecutively among those who attended the centre for the terminal visit within a certain time period. All participants gave oral and written consent according to the second Helsinki declaration. The study was approved by the regional ethical committees of Vejle and Funen (journal no. 19980186).

Blood sampling and storage

After supine rest for 10 min, blood samples were collected from non-fasting participants in 10 ml EDTA-coated tubes (Becton Dickinson, Franklin Lakes, NJ, USA), and centrifuged for 15 min (150 ***g***; room temperature). Thrombocytes were isolated from the thrombocyte-rich plasma by centrifugation (1500 ***g***; room temperature; 5 min). The thrombocyte pellet was washed twice in 0·9 % NaCl and re-suspended in 0·5 ml 0·9 % NaCl. Erythrocytes and buffy coat were isolated by centrifugation (1500 ***g***; room temperature; 10 min). The buffy coat was re-suspended in RLT buffer (QIAgen, Ballerup, Denmark). Erythrocytes were lysed by adding an equal amount of MilliQ water to the tube. Plasma, thrombocytes, buffy coat and erythrocytes were stored at −80°C.

Plasma selenium status

pSe concentration was determined as previously described using an ELAN 6100 ICP-DRC-MS (PerkinElmer SCIEX, Concord, ON, Canada) equipped with an AS-91 autosampler (PerkinElmer, Norwalk, CT, USA)[24]. For every fifteen unknown plasma samples one duplicate sample, one field blank, one certified reference sample and three Se (10 μg/l)

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standard solutions were included. The latter were used to correct for instrumental drift. Based on the determination of field blanks the limit of detection was estimated at 0·1 μg Se/l and the uncertainty was estimated from the differences between double determinations at 2·1 % relative SD. The accuracy was determined by including a reference sample with a known content of 73 (SD 8) μg/l (Seronorm Serum; Nycomed Pharma Diagnostics, Oslo, Norway). We found an Se concentration of 77 (SD 6) μg/l in this sample.

Defence enzyme activities (glutathione peroxidase, glutathione reductase and glutathione S*-transferase)*

Erythrocyte, plasma and thrombocyte GPX and GR activities were determined spectrophotometrically on a Cobas Mira analyser (F. Hoffmann-La Roche Ltd, Basel, Switzerland) according to Wheeler *et al.* [25]. *t*-Butyl hydroperoxide was used as substrate for GPX. GST activity was measured in thrombocytes and erythrocytes as previously described[26]. Enzyme activities measured in erythrocyte lysates were related to the amount of Hb in the sample. Enzyme activities determined in thrombocytes and plasma were related to the amount of total protein. Hb and total protein contents were determined using commercially available kits (catalogue no. HG 980, Randox, Crumlin, Co. Antrim, UK; catalogue no. A11A01669, ABX Diagnostics, Montpellier, France, respectively). Control samples (erythrocytes, plasma and thrombocytes) were included for every tenth sample analysed. Inter- and intraday variations for the control samples were <7 %.

RNA isolation and cDNA synthesis

Total RNA was extracted from the buffy coat using the QIAamp® RNA blood mini kit (QIAgen, Ballerup, Denmark). The concentration and purity of the RNA was determined by measuring the absorbance at 260 and 280 nm using the NanoDrop® ND-1000 UV-Vis spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). A ratio (A_{260}:A_{280}) of $>1·8$ indicated suitable purity. First-strand cDNA was synthesised using 500 ng total RNA in a final volume of 10 μl with Random Hexamer primer and Superscript™ II Rnase H-RT according to the manufacturers (Invitrogen, Taastrup, Denmark).

Primer and probe design

Oligonucleotide primers and TaqMan® probes were designed with Primer Express software v. 1.5 (Applied Biosystems, Stockholm, Sweden), based on sequences from the Genbank database (Table 1). Genomic DNA amplification was excluded whenever possible by designing intron spanning probes.

Leucocyte mRNA quantification of Fos-related antigen 1, glutathione peroxidase 1, γ-glutamylcysteine ligase catalytic subunit, NAD(P)H:quinone oxidoreductase *and* aryl hydrocarbon receptor repressor. Real-time PCR of the five genes was performed on an ABI PRISM® 7900HT Sequence Detection System (Applied Biosystems, Stockholm, Sweden). The PCR reaction was determined in a 20 μl final volume adding 2 ng cDNA, using TaqMan® Universal PCR Master Mix (Applied Biosystems, Stockholm, Sweden). For each target gene, the probe concentration was 0·3 μM and the primer concentrations for the detection of *GCLC*, *AhRR* and *NQO1* genes were 0·3 μM, 0·5 μM for *GPX1*, and 0·9 μM for *Fra1*. The constitutively expressed 18S rRNA primer/probe-set (Applied Biosystems, Stockholm, Sweden) was selected as an endogenous control to correct for potential variation in RNA loading or efficiency of the amplification reaction and used as recommended by the manufacturers.

The PCR amplification was performed in ninety-six-well plates in triplicates using the following cycling protocol: 50°C for 2 min, 95°C for 10 min, forty-five cycles at 95°C for 15 s and 60°C for 1 min. To confirm equal amplification efficiencies, we used the criterion of a regression slope of less than 0·1 for each target gene normalised to 18S rRNA. This confirms that we could use the comparative cycle threshold (Ct) method for the relative quantification of target without running standard curves on the same plate (data not shown). Relative changes in gene expression were calculated

Table 1. Oligonucleotides and TaqMan® fluorogenic probes

Gene	Genbank	Primer/probe	Sequence 5′–3′
GPX1	AY327818	Sense	CCCGTGCAACCAGTTTGG
		Antisense	TGAGGGAATTCAGAATCTCTTCGT
		TaqMan® probe	6-FAM-CATCAGGAGAACGCC-MGB
GCLC	NM_001498	Sense	CGGCACAAGGACGTTCTCA
		Antisense	ACCGGACTTTTTTATTTTCATGATCA
		TaqMan® probe	6-FAM-CGATGAGGTGGAATAC-MGB
NQO1	NM_000903	Sense	CTTCAATCCCATCATTTCCAGAA
		Antisense	GACTCGGCAGGATACTGAAAGTTC
		TaqMan® probe	6-FAM-CATCACAGGTAAACTGAAG-MGB
Fra1	NM_005438	Sense	GCCGCCCTGTACCTTGTATC
		Antisense	CAGTGCCTCAGGTTCAAGCA
		TaqMan® probe	6-FAM-CTTTCCCCAGGGCCT-MGB
AhRR	NM_020731	Sense	GAATCGGAACTGCATGGAAAA
		Antisense	CCAAAACGCCGCTCTCTCT
		TaqMan® probe	6-FAM-CAATTACTCAGCAGGAAGG-MGB

GPX1, glutathione peroxidase 1; 6-FAM, 6-carboxyfluorescein; MGB, minor groove binding; *GCLC*, γ-glutamylcysteine ligase catalytic subunit; *NQO1*, NAD(P)H quinone oxidoreductase 1; *Fra1*, FOS-like antigen 1; *AhRR*, aryl-hydrocarbon receptor repressor.

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by the ΔΔCt method (Applied Biosystems, 2001, User Bulletin no. 2, ABI PRISM 7700 Sequence Detection System, Foster City, CA, USA). Inter-plate variation for a control sample (*n* 27) was <2 % for all genes.

Statistical analysis

Treatment effects were analysed with the general linear model, with sex, smoking and their interactions with treatment included in the model. Weight was significantly higher among men than women, and was therefore included in the model as a covariate. Since a large proportion of subjects reported taking multivitamin/mineral supplements, use of additional supplements was included in the statistical analyses as a covariate. For comparison of two groups, *t* tests were used (placebo against pooled Se-supplemented groups). Data that could not meet the criteria of variance homogeneity and normal distribution after log transformation were analysed by non-parametric tests (Kruskal–Wallis H test or Mann–Whitney *U* test). Group differences for categorical data were analysed using the χ^2 test. For all tests a *P* value less than 0·05 was considered statistically significant. Pearson correlations were used to identify correlating variables. All statistical analyses were performed using SAS (SAS Institute, Cary, NC, USA).

Results

Study population

Baseline characteristics for the study participants are presented in Table 2 as mean values and standard deviations (age, weight) or as percentages (smoking status, sex). The participants were predominantly non-smokers and in the selected sub-sample for the present study women assigned to the highest dosage level were older compared with women in the lowest and medium dosage groups (Table 2). Women had significantly lower body weight compared with men ($P<0·001$). Otherwise, there were no statistically significant differences in baseline characteristics between the groups.

Records of the use of multivitamin/mineral supplements among the participants (registered at time of final blood sampling, year 5) indicate that on average 36 % took additional supplements (as ordinary multivitamin/mineral tablets); however, the percentage of supplement users was about twice as high among participants from the highest dosage group compared with the rest (Table 2). No participants reported use of specific Se supplements.

Compliance

pSe concentration was measured to test for compliance. Se status increased linearly (R^2 0·98; $P<0·0001$) with increasing Se intake (Table 3), indicating a high degree of compliance. Smoking status did not influence pSe ($P>0·05$) (data not shown).

Enzyme activities

There was a statistically significant effect on thrombocyte GPX activity (tGPX) ($P=0·003$), with a statistically significant treatment × sex interaction ($P=0·008$). When subjects were stratified according to sex, we found an effect of Se supplementation on tGPX activity in women only ($P_{women} = 0·006$) (Table 3). tGPX activity was significantly increased in groups supplemented with 200 or 300 μg/d ($P_{placebo-200\,\mu g} = 0·001$; $P_{placebo-300\,\mu g} = 0·008$), while there was a tendency towards increased tGPX activity at the lowest dosage level ($P_{placebo-100\,\mu g} = 0·084$) compared with placebo. We found no statistically significant difference in tGPX between the two highest dosage levels ($P_{200\,\mu g-300\,\mu g} = 0·688$). No effect of Se supplementation on tGPX was observed in men ($P_{men} = 0·401$). We found no effect of Se supplementation on plasma GR or GPX, on erythrocyte GR, GPX or GST, or on GR or GST measured in thrombocytes (Table 3). We found no interaction with smoking on any of the activities.

When all supplemented groups were pooled and tested against the placebo group, only tGPX was significantly increased with Se supplementation ($P=0·027$) and, again, only women responded ($P_{women} = 0·004$; $P_{men} = 0·596$). pSe correlated with plasma GPX activity ($P=0·046$; r 0·212) but not tGPX ($P=0·106$) or erythrocyte GPX ($P=0·162$).

Gene expression

Se supplementation did not affect gene expression of *GPX1*, *Fra1*, *GCLC*, *NQO1* or *AhRR* in leucocytes (Table 4), when all groups were tested against each other. There was a large

Table 2. Baseline characteristics of the study participants
(Mean values and standard deviations or percentages)

	Placebo			100 μg/d			200 μg/d			300 μg/d		
	n	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD
Age (years)	28	70·4	3·8	27	70·3	3·6	23	71·6	4·2	27	73·0	5·0
Weight (kg)												
All subjects	28	78·5	12	27	73·1	12	23	75·6	15	27	80·6	13
Men	13	83·3	8·2	12	80·9	9·1	9	87·7	10	15	81·7	11
Women*	15	74·3	13	15	67·0	9·4	14	67·7	12	12	79·2†	14
Smokers (%)	28	36		27	22		23	22		27	19	
Supplement users (%)	28	29		27	26		23	35		27	56	
Males (%)	28	46		27	44		23	39		27	56	

* Compared with men, women had significantly lower body weight ($P<0·001$; ANOVA).
† Highest dosage group was significantly different from lowest dosage group (100 μg/d) and medium dosage group (200 μg/d) ($P<0·05$; ANOVA).

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Table 3. Effect of selenium supplementation on plasma selenium and activities of glutathione peroxidase (GPX), glutathione reductase (GR) and glutathione *S*-transferase (GST) measured in plasma, erythrocytes or thrombocytes

(Medians and 10th and 90th percentiles and means and standard deviations)

			Placebo	100 μg/d	200 μg/d	300 μg/d
Plasma						
Se (μg/l)	All	*n*	24	22	20	23
		Median	93·0	159*	207*	242*
		10th, 90th percentiles	77·3, 105	134, 217	177, 266	203, 341
		Mean	92	165*	221*	260*
		SD	11·2	31·6	42·3	59·7
GPX (units per g protein)	All	*n*	28	27	23	27
		Median	14·0	15·0	14·2	14·4
		10th, 90th percentiles	11·0, 15·9	12·4, 17·2	12·0, 17·4	12·6, 16·9
		Mean	13·7	14·7	14·5	14·5
		SD	1·95	2·35	2·31	2·10
GR (units per g protein)	All	*n*	28	27	23	27
		Median	0·85	0·85	0·88	0·85
		10th, 90th percentiles	0·72, 1·12	0·71, 1·01	0·65, 1·06	0·74, 0·99
		Mean	0·91	0·85	0·87	0·87
		SD	0·26	0·12	0·17	0·13
Erythrocytes (units per g Hb)						
GPX	All	*n*	28	27	23	27
		Median	92·8	99·5	96·5	99·4
		10th, 90th percentiles	69·3, 110	66·0, 131	78·2, 135	75·0, 124
		Mean	92·7	98·8	104	98·8
		SD	18·0	24·9	28·1	19·0
GR	All	*n*	28	27	23	27
		Median	11·5	11·7	11·5	12·4
		10th, 90th percentiles	9·49, 13·4	9·60, 13·0	10·1, 12·9	10·2, 14·1
		Mean	11·4	11·5	11·4	12·3
		SD	1·6	1·5	1·1	1·5
GST	All	*n*	28	27	23	27
		Median	8·25	7·72	8·05	8·40
		10th, 90th percentiles	5·20, 12·1	5·55, 10·7	4·15, 10·7	6·08, 12·0
		Mean	8·42	8·00	7·66	8·86
		SD	2·9	2·2	2·9	3·2
Thrombocytes (units per g protein)						
GPX	All	*n*	28	27	23	25
		Median	202	224	300	315
		10th, 90th percentiles	80·3, 393	129, 431	84·8, 590	125, 543
		Mean	221	251	323	320
		SD	146	113	217	174
	Men	*n*	13	12	9	14
		Median	224	196	138	208
		10th, 90th percentiles	75·8, 371	130, 343	66·3, 473	86·8, 515
		Mean	218	224	222	283
		SD	126	96·1	194	183
	Women	*n*	15	15	14	11
		Median	155	251	312*	336*
		10th, 90th percentiles	90·8, 427	130, 441	214, 670	187, 553
		Mean	225	274	389*	368*
		SD	165	124	212	157
GR	All	*n*	27	27	22	25
		Median	47·9	41·6	49·0	44·9
		10th, 90th percentiles	28·8, 65·9	20·9, 54·5	20·1, 59·9	21·5, 70·6
		Mean	47·6	40·1	44·1	45·8
		SD	16·4	15·0	14·8	17·7
GST	All	*n*	27	27	22	25
		Median	110	69·5	107	73·8
		10th, 90th percentiles	42·8, 191	33·1, 168	32·9, 233	33·9, 164
		Mean	110	95·4	128	92·2
		SD	57·2	58·3	104	59·5

* Significantly different from placebo group ($P<0·05$; ANOVA).

inter-individual variation in the expression of the transcription factors *Fra1* and *AhRR*, especially in the placebo group (Table 4).

When the Se-supplemented groups were pooled and tested against placebo, we found a statistically significant decrease in expression of *Fra1* and *GCLC* ($P=0·019$ and $P=0·042$, respectively) and a tendency towards a decreased expression of *NQO1* ($P=0·067$) and *AhRR* ($P=0·085$). There was no effect of Se on *GPX1* ($P=0·281$). *AhRR* was the only gene

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Table 4. Effect of selenium supplementation on *glutathione peroxidase 1* (*GPX1*), *γ-glutamylcysteine ligase catalytic subunit* (*GCLC*), *NAD(P)H quinone oxidoreductase 1* (*NQO1*), *FOS-like antigen 1* (*Fra1*) and *aryl-hydrocarbon receptor repressor* (*AhRR*) gene expression normalised to 18S rRNA

(Medians and 10th and 90th percentiles and means and standard deviations)

		Placebo	100 μg/d	200 μg/d	300 μg/d
GPX1	*n*	27	26	23	26
	Median	0·90	0·88	0·82	0·86
	10th, 90th percentiles	0·55, 1·54	0·55, 1·30	0·71, 1·34	0·59, 1·41
	Mean	1·05	0·91	0·95	0·91
	SD	0·58	0·31	0·33	0·34
GCLC	*n*	27	26	23	26
	Median	0·98	0·75	0·86	0·75
	10th, 90th percentiles	0·62, 1·59	0·47, 1·08	0·49, 1·37	0·57, 1·42
	Mean	1·02	0·81	0·88	0·93
	SD	0·39	0·3	0·38	0·55
NQO1	*n*	27	26	23	26
	Median	0·98	0·74	0·81	0·84
	10th, 90th percentiles	0·52, 1·59	0·50, 1·13	0·56, 1·10	0·49, 1·33
	Mean	1·05	0·82	0·86	0·91
	SD	0·54	0·42	0·34	0·41
Fra1	*n*	27	26	23	26
	Median	1·08	0·66	0·44	0·53
	10th, 90th percentiles	0·25, 2·85	0·11, 1·77	0·13, 1·34	0·12, 2·18
	Mean	1·8	0·94	0·83	1·0
	SD	3·0	1·14	1·19	1·36
AhRR	*n*	27	26	23	26
	Median	0·69	0·71	0·65	0·78
	10th, 90th percentiles	0·38, 4·36	0·27, 1·86	0·21, 1·58	0·22, 1·96
	Mean	1·82	1·0	1·07	1·22
	SD	2·39	0·93	1·56	2·05

expression marker influenced by smoking status. Smoking increased the expression of *AhRR* ($P=0·0001$) (Fig. 1), an effect that was independent of Se supplementation.

Discussion

The anti-carcinogenic effects of Se have been linked to increased expression of protective enzymes. In the present study, we investigated the effect of 5 years of Se supplementation on the activity and gene expression of selected selenoproteins and genes involved in carcinogen metabolism. The pSe concentration was determined to test for compliance and we found a statistically significant increase in pSe with increasing Se supplement intake. It has previously been shown in numerous studies that pGPX and eGPX activities increase with Se intake, and that the increase reaches a plateau at intakes corresponding to pSe concentrations of about 70–90 μg/l and higher[27,28]. In the present study pSe concentrations in the placebo group were between 71 and 111 μg/l with a mean of 92 μg/l. If we assume that the pSe in the control group is representative of the mean Se status in the participants before entry into the study, the lack of effect on pGPX and eGPX activities could be explained by these high baseline values, indicating that GPX is saturated in erythrocytes and plasma. We found a statistically significant increase in tGPX activity with Se supplementation, using Se-enriched yeast which consists primarily of the organic Se compound selenomethionine[23]. A sub-group analysis showed that the effect of Se was sex specific. Se supplementation only affected tGPX activity in women, and only at the two highest dosage levels. The responses at these two levels were not statistically different, suggesting that tGPX activity is saturated at pSe concentrations between 165 and 221 μg/l (means of the two groups). This is a bit higher than previously reported where tGPX activity plateaued at pSe concentrations <150 μg/l[27–30]. tGPX activity has been reported to saturate at higher pSe concentrations compared with eGPX and pGPX[31], which could explain the observed positive effect on GPX activity in thrombocytes. We are not aware of other studies reporting this sex-specific difference in tGPX activity in response to Se supplementation, and we cannot exclude that a similar effect would have been observed in men, had

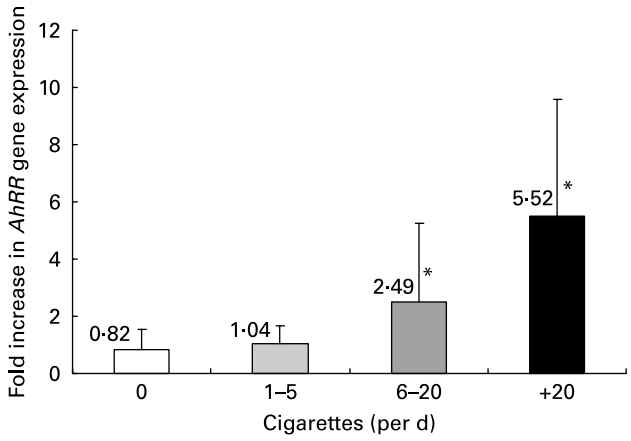

Fig. 1. Effect of smoking on gene expression of *aryl hydrocarbon receptor repressor* (*AhRR*) relative to the mean expression in the placebo group. Values are means, with standard deviations represented by vertical bars. Participants are sub-divided according to number of cigarettes smoked per d: 0 (*n* 77); 1–5 (*n* 8); 6–19 (*n* 11); +20 (*n* 6). Pipe and cigar smokers are placed in the group with smokers of 1–5 cigarettes per d. *Mean value is significantly different from that of non-smokers ($P<0·05$; Kruskal–Wallis).

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the number of participants been higher. Although not statistically significant, we note that GPX activity in all blood fractions increased with Se supplementation. In the present study, five years of Se supplementation did not affect GR activity in plasma, erythrocytes or thrombocytes, or GST activity in erythrocytes or thrombocytes.

Large inter-individual differences in response to Se supplementation have previously been reported[32]. Brown *et al.* found that the lower the baseline activity, the greater was the increase in response to Se supplementation. Thus it is possible that we would have seen effects of Se supplementation on GPX activity in erythrocytes and plasma if we had had baseline measurements available, thereby allowing us to investigate the individual responses, at least in individuals having low baseline levels.

GPX activity was measured in three different blood compartments, and only pGPX correlated weakly with pSe concentration. Nève *et al.* have previously reported significant correlations between pSe and pGPX and tGPX activities[31] but we were not able to show any correlations between pSe and eGPX or tGPX activity. We did not measure Se status in erythrocytes or thrombocytes and it is possible that Se is channelled differently into various blood compartments as GPX synthesis is requested.

Smoking has been reported to decrease Se status and GPX activity[33,34]. We found no interactions with smoking. One possible explanation could be that the group of smokers in the present study was much smaller than the group of non-smokers, and individual smokers were very different regarding smoking habits. Some participants smoked very rarely while others smoked every day.

It has been hypothesised that Se at pharmacological doses would influence expression of phase 1 and phase 2 genes containing XRE or EpRE in their promoters[7]. We found no dose–response relationship on expression of *AhRR*, *GPX1*, *GCLC*, *NQO1* or *Fra1* in leucocytes. Pooling the Se groups and testing them against the placebo group showed decreased expression of *Fra1*, *GCLC* and *NQO1*, although the latter did not reach statistical significance. *Fra1* has been reported to down regulate *GCLC* and *NQO1* [14,15]. If this were the case in the present study, we would have expected to see an up regulation of *Fra1*. Instead we found the opposite. This may indicate that *Fra1* is not involved in the observed down regulation of *GCLC* and *NQO1*. We found increased *AhRR* gene expression with smoking. Cigarette smoke contains carcinogens such as polycyclic aromatic hydrocarbons that are metabolised by cytochrome P450 enzymes. Therefore AhRR is co-induced with cytochrome P450 enzymes via XRE[35]. Whether increased *AhRR* expression in leucocytes is a marker for smoking needs further investigation. None of the other genes were transcribed differently as a function of smoking and there were no significant interactions between smoking and pSe on gene transcription.

Gene expression of phase 2 enzymes has been shown to vary with the studied organ, and results on whether blood expression profiles can be used as surrogate markers of organ expression are conflicting[36,37]. Finnstrom *et al.* found no correlation between blood and liver mRNA expression patterns of *cytochrome P450* genes, but they noted that genes that were highly expressed in the liver at the same time could be expressed at lowered levels in the blood[38]. Rauchy *et al.* on the other hand found good correlation[39]. Results are inconclusive and further studies as to whether a blood sample can be used to determine the expression profile in target organs are needed. Furthermore, leucocytes are a mixture of many different cell types, and it has been shown that the profile of these cells vary from subject to subject based on cell type counts[37]. There might therefore be different expression profiles among different leucocyte cell types. Decreased gene expression of phase 2 enzymes in the blood compartment reported here may thus reflect changed composition of the leucocyte cell pool. It may also reflect altered expression in other tissues. In a study in transgenic mice containing a *GCLC-luc* construct, luciferase activity increased in brain and muscle but decreased in liver following treatments with berry extracts or ellagic acid[36], indicating that complex up and down regulation may take place in different tissues as a response to redox active dietary components. We speculate that Se compounds may elicit similar complex actions in different tissues leading to an overall down regulation of EpRE-regulated genes in blood leucocytes.

We conclude that Se supplementation affects tGPX activity differently in men and women. Se status in healthy elderly Danish women is insufficient to obtain saturation of tGPX activity and higher Se intake might increase oxidative defence in this blood compartment. The present results indicate that Se supplementation can down regulate leucocyte genes having EpRE in their promoter and that *Fra1* does not seem to be involved in down regulation of *GCLC* and *NQO1* with Se. Decreased expression of EpRE-regulated genes could potentially increase the risk of cancer. However, further studies are needed to establish whether this down regulation in leucocytes reflects a similar expression pattern in other tissues.

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