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Randomised controlled trial of the effect of long-term selenium supplementation on plasma cholesterol in an elderly Danish population

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Abstract

Although cross-sectional studies have shown a positive association between Se and cholesterol concentrations, a recent randomised controlled trial in 501 elderly UK individuals of relatively low-Se status found that Se supplementation for 6 months lowered total plasma cholesterol. The Danish PRECISE (PREvention of Cancer by Intervention with Selenium) pilot study (ClinicalTrials.gov ID: NCT01819649) was a 5-year randomised, double-blinded, placebo-controlled trial with four groups (allocation ratio 1:1:1:1). Men and women aged 60–74 years (*n* 491) were randomised to 100 (*n* 124), 200 (*n* 122) or 300 (*n* 119) μg Se-enriched yeast or matching placebo-yeast tablets (*n* 126) daily for 5 years. A total of 468 participants continued the study for 6 months and 361 participants, equally distributed across treatment groups, continued for 5 years. Plasma samples were analysed for total and HDL-cholesterol and for total Se concentrations at baseline, 6 months and 5 years. The effect of different doses of Se supplementation on plasma lipid and Se concentrations was estimated by using linear mixed models. Plasma Se concentration increased significantly and dose-dependently in the intervention groups after 6 months and 5 years. Total cholesterol decreased significantly both in the intervention groups and in the placebo group after 6 months and 5 years, with small and nonsignificant differences in changes in plasma concentration of total cholesterol, HDL-cholesterol, non-HDL-cholesterol and total:HDL-cholesterol ratio between intervention and placebo groups. The effect of long-term supplementation with Se on plasma cholesterol concentrations or its sub-fractions did not differ significantly from placebo in this elderly population.

Key words: Selenium: Lipids: CVD: Cholesterol: Selenium supplementation: Randomised controlled trials

Se, an essential trace element, has a wide range of health effects when incorporated as selenocysteine into selenoproteins[1,2]. Early studies in areas of low-Se status have implicated Se in CVD risk[3,4]. In Finland, increased cardiovascular morbidity and mortality were observed in men with low serum Se[3], whereas Se supplementation protected against cardiomyopathy in the Keshan province of China[4]. Cardiovascular benefits of Se could be mediated by the ability of selenoproteins, such as glutathione peroxidase and selenoprotein S, to combat the oxidative modification of lipids, inhibit platelet aggregation and reduce inflammation[5–11]. However the evidence that Se status affects CHD risk is equivocal[12–16], with a recent Cochrane review flagging major gaps in the available trial evidence, especially with regard to long-term Se supplementation trials[16].

Cholesterol and its sub-fractions are recognised as important cardiovascular risk factors[17,18]. Several cross-sectional studies have shown a positive association between higher Se status and elevated concentrations of total and LDL-cholesterol[19–27]. However, higher Se status has also been linked to higher circulating HDL-cholesterol[5,22–25], and results from prospective studies have been inconsistent[28,29].

With the aim of showing the feasibility of conducting a large randomised controlled trial of Se in cancer prevention in European populations of relatively low-Se status, both UK and

Abbreviations: PRECISE, PREvention of Cancer by Intervention with Selenium; TFA, *trans*-fatty acids.

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Denmark set up the PREvention of Cancer by Intervention with Selenium (PRECISE) pilot trials, recruiting elderly subjects from 1998 to 2001, using the same study protocol. Se-enriched yeast was chosen as the intervention agent as it successfully reduced cancer risk in the Nutritional Prevention of Cancer (NPC) trial[30] and behaves similarly to wheat-Se in terms of absorption and retention[31]. In the UK PRECISE pilot trial (*n* 501), 6 months of supplementation with 100, 200 or 300 μg/d Se-enriched yeast had modest but statistically significant beneficial effects on plasma lipids compared with placebo-yeast[32]. Other randomised controlled trials on the effect of Se supplementation on circulating lipid concentrations mostly found no effect[33–39], although several of the trials were small and short term.

On the basis of the results from the UK PRECISE trial, we hypothesised that Se supplementation would have a beneficial effect on cholesterol concentrations in an elderly Danish population with marginal Se deficiency. A considerable benefit over the UK study is that the Denmark PRECISE trial continued for 5 years, enabling the effect of long-term supplementation to be assessed for the first time.

Methods

Study design

The Denmark PRECISE pilot study (ClinicalTrials.gov ID: NCT01819649) was a single-centre, non-stratified, randomised, double-blinded, placebo-controlled, multi-arm parallel clinical trial with four groups (allocation ratio 1:1:1:1). The sample size of this pilot study, before a proposed international trial of Se in cancer prevention in the UK, Denmark and Sweden (PRECISE), was set at 500 participants. This was considered sufficient to draw reasonable conclusions about recruitment, adherence and loss to follow-up while keeping costs within reasonable bounds. Because of these objectives, no formal power calculations were performed *a priori*. The funding necessary to conduct the international PRECISE trial was not secured and therefore it never took place.

Participants were men and women aged 60–74 years from the County of Funen, Denmark. Invitation letters were sent out based on a random sample from the Danish Civil Registration System. From November 1998 to June 1999, we invited 2897 potential participants, of whom 630 accepted the invitation for a visit to Odense University Hospital, where they were screened for inclusion. Exclusion criteria were as follows: (i) a Southwest Oncology Group performance status score >1, indicating impairment in general well-being and activities of daily life; (ii) active liver or kidney disease (alanine aminotransferase, alkaline phosphatase, bilirubin or urea two standard deviations above the normal reference range); (iii) previous diagnosis of cancer (excluding non-melanoma skin cancer); (iv) diagnosed HIV infection; (v) receiving immunosuppressive therapy; (vi) unable to understand written and spoken information; (vii) receiving ≥50 μg/d of Se supplements in the previous 6 months (by patient report).

Participants deemed suitable for inclusion provided blood samples and were given yeast tablets for an open-label 4-week placebo run-in phase. After this, potential participants returned for a second visit for a final evaluation of inclusion and exclusion criteria and of participant adherence and satisfaction during the run-in phase. Good adherence was defined as taking >80 % of the run-in phase tablets assessed by tablet count.

The 491 subjects who met the inclusion criteria, who displayed good adherence in the run-in phase and gave written informed consent, were enroled and randomised to 0, 100, 200 or 300 μg of Se daily. The regional Data Protection Agency and Scientific Ethical Committees of Vejle and Funen counties approved the study (Journal number. 19980186).

Randomisation and interventions

Randomisation was computer-generated, blocked and non-stratified and was performed at the Division of Epidemiology & Biostatistics, University of Arizona, Arizona Cancer Center. A badge number system secured blinding and correct distribution of Se doses. The responsibility of distributing tablets was placed with pharmacists at Odense University Hospital. Participating couples living at the same address were allocated to the same intervention.

The intervention agent was the Se-enriched yeast SelenoPrecise© and tablets were formulated and packaged by Pharma Nord ApS. Analytical data (by inductively coupled-plasma (ICP)-MS) for the three different strengths of SelenoPrecise© are as follows: 100 μg tablets, 93–107 μg Se; 200 μg tablets, 186–214 μg Se; and 300 μg tablets, 279–321 μg Se. Speciation of Se in SelenoPRECISE has been investigated previously, and the selenised yeast, which was used as a source of Se, contained selenomethionine at 54–60 % of the total Se, with unknown seleno-compounds accounting for the remainder[40]. The placebo agent was an inactive spray-dried baker's yeast, comprising 250 mg of yeast placebo, 80 mg of cellulose, 65 mg of dicalcium phosphate and ≤5 mg of other inactive ingredients, identical in appearance to the Se tablets. Both intervention and placebo tablets were coated with titanium oxide in order to obtain identical smell and taste. Tablets were packaged in blister packs of twenty-eight tablets, 7 × 4. Participants, research staff and investigators were blinded to treatment.

Sample and data collection and study outcomes

Participants were evaluated at Odense University Hospital at baseline and at 6, 12, 18, 24, 36 and 60 months. Demographic data and medical history including medication and supplement use were collected at baseline. During each visit, medical status was ascertained, side effects were registered and tablets were counted. Adherence was defined as in the run-in phase. New tablets were handed out except at the 60-month visit. Blood was drawn at 6, 12, 18, 24 and 60 months. Participants were non-fasting. Heparinised plasma, serum, whole blood, erythrocytes and buffy coat were prepared and stored at −80°C. Reasons for participant withdrawal were recorded.

The pre-specified primary outcome of the pilot study was to determine recruitment, adherence and drop-out rate of the volunteers to ascertain the viability of conducting the main PRECISE trial in Denmark. The pre-specified secondary outcomes were as follows: (i) to determine the number of staff necessary to

conduct the main PRECISE study; and (ii) to perfect questionnaires and case report forms used at the participant trial visits.

Biochemical analyses

Total Se at baseline and at the 6-month and 5-year visits was measured in lithium–heparin plasma at LGC Limited, by ICP-MS with external calibration. The sample dilutions were introduced into the plasma via a micro-flow quartz concentric nebuliser, operating in pumping mode at 0·1 rpm, and a Scott double-pass spray chamber cooled to 2°C. The Se isotopes ^{77}Se, ^{78}Se and ^{82}Se were measured in both H_2-mode and He-mode using collision cell ICP-MS (7700×; Agilent Technologies) to reduce the interferences on the Se isotopes. Each analysis comprised three replicate measurements. Germanium was added online as an internal standard to correct for any instrumental drift. In addition, 2 % methanol was mixed online in order to compensate for differences in carbon content between the samples and standards that may cause variances in ionisation efficiency leading to erroneous results. As the Se concentrations calculated for all of the measured isotopes agreed well, only the data for ^{78}Se in H_2-mode were reported throughout. All reagents were of the highest purity. Methanol (Optigrade; LGC) and nitric acid (UltraPure; Romil) were used throughout. A stock solution of 1000 mg/kg Se (Romil) was used to prepare the working calibration standards (0–50 ng/g Se) freshly by gravimetric dilution in 0·5 % (v/v) nitric acid. A matrix-certified reference material, BCR-637 Human Serum, with a certified Se concentration of 81 (SD 7) μg/l Se (density corrected 79·1 ng/g), was used for quality control of the total Se measurements. The Se concentration found for BCR-637 was 78·3 (SD 2·7) μg/l Se (sixteen independent replicates), indicating good accuracy of the method. The intra-assay CV ranged from 0·5 % for samples of high-Se concentration to 3 % for samples of low-Se concentration. The interassay CV was 3·4 %.

Total and HDL-cholesterol concentrations at baseline and at the 6-month and 5-year visits were measured in lithium–heparin plasma at the Department of Clinical Biochemistry, Odense University Hospital, Denmark, using an Architect c16000 analyser (Abbott) with dedicated reagents. Measurements were performed by means of enzymatic colorimetric analysis. Traceability for total cholesterol and HDL-cholesterol was ensured through participation in the National Reference System for cholesterol (NRS/CHOL) established by the Clinical and Laboratory Standards Institute with isotope dilution-MS as the reference method and reference material from the National Institute of Standard and Technology. Intra-assay/interassay CV were 0·6/0·8 % for total cholesterol and 1·0/0·5 % for HDL-cholesterol. As evidence of equivalence in the analytical performance of the cholesterol-oxidase assays in the UK and Denmark, a comparison of total cholesterol on forty-four serum samples produced a limit of variation of 2 %.

Power calculations for intention-to-treat analysis

For the average sample size of 117 participants retained in each treatment group after 6 months of follow-up and an uncorrected two-sided significance level of 0·05, the power to detect underlying differences in 6-month changes of 0·20 mmol/l (0·077 g/l) for total cholesterol, 0·05 mmol/l (0·019 g/l) for HDL-cholesterol, 0·20 mmol/l for non-HDL-cholesterol, and 0·15 for total:HDL-cholesterol ratio, comparing any intervention group with placebo, was 72·6, 64·3, 78·1 and 71·8 %, respectively. Because of the smaller sample size of ninety participants remaining in each group at the end of the 5-year follow-up period and the lower correlation between lipid measurements taken 5 years apart, the power to detect the same underlying differences in lipid changes after 5 years was reduced to 37·8, 30·6, 39·8 and 30·6 %, respectively.

Statistical analysis

For the comparison of randomised groups, all trial participants were assigned to their randomised treatment group, irrespective of compliance (intention-to-treat analysis). The effect of different doses of Se supplementation on changes in total cholesterol, HDL-cholesterol, non-HDL-cholesterol and total:HDL-cholesterol ratio after 6 months and 5 years was estimated by using linear mixed models[32,41] with fixed effects for treatment groups, follow-up times and treatment-by-time interactions, and allowing for random between-subject variations in both baseline lipid levels (intercepts) and lipid changes over follow-up (time slopes). These models provided the mean lipid changes from baseline to 6 months and 5 years for each treatment group, as well as the differences in mean lipid changes for the three active treatment groups compared with placebo (treatment effects). We evaluated treatment-effect modifications by sex, baseline age group (< or ≥ 65 years), category of BMI (< or ≥25 kg/m^2), baseline plasma Se concentration (< or ≥90 ng/g) and baseline lipid concentrations (< or ≥6 mmol/l for total cholesterol, 1·5 mmol/l for HDL-cholesterol, 4·5 mmol/l for non-HDL-cholesterol and 4 for total:HDL-cholesterol ratio) by including all main terms and interactions between treatment group, time and the corresponding covariate as fixed effects in the above mixed models. In sensitivity analyses, we excluded visits after participants received lipid-lowering medications at baseline or during the intervention period.

In addition to the intention-to-treat analysis, we evaluated the cross-sectional association between plasma Se and lipid concentrations at baseline and the longitudinal associations between changes in plasma Se and lipid concentrations after 6 months and 5 years. We used linear mixed models with random intercepts, random time slopes and fixed slopes for baseline Se levels and Se changes at 6 months and 5 years[32,42] to estimate the mean difference in baseline lipid levels per 50 ng/g increase in baseline Se concentrations (cross-sectional association), as well as the mean lipid changes from baseline to 6 months and 5 years for each 50 ng/g change in the corresponding Se concentration (longitudinal associations). We also categorised baseline Se concentrations and Se changes into quartiles in the above mixed model and compared mean baseline lipid concentrations across quartiles of baseline Se and mean lipid changes after 6 months and 5 years across quartiles of Se change. Cross-sectional and longitudinal associations were adjusted for baseline age (continuous), sex, smoking status (never, former, or current), alcohol drinking

(≤2, 3–10, or >10 drinks/week), BMI (continuous) and changes in lipid-lowering medications over time. All reported *P* values were two-sided and not adjusted for multiple testing. Statistical analyses were performed with Stata, version 13 (StataCorp).

Results

Participants

Of the 491 randomised participants, twenty-three dropped out of treatment before 6 months of follow-up, 107 participants dropped out between 6 months and 5 years, and the remaining 361 participants completed the 5-year follow-up period (Fig. 1). The 130 participants who withdrew before the end of the study period were equally distributed across treatment groups ($P = 0{\cdot}91$). Lipid measurements were available for 490 participants at baseline, for 435 at 6 months and for 358 at 5 years (Fig. 1). Participants with and without available lipid measurements at 6 months and 5 years did not differ in their baseline characteristics (data not shown). Three of the ninety participants allocated to placebo (3·3 %) had plasma Se concentrations more than two interquartile ranges above the median at 5 years, and

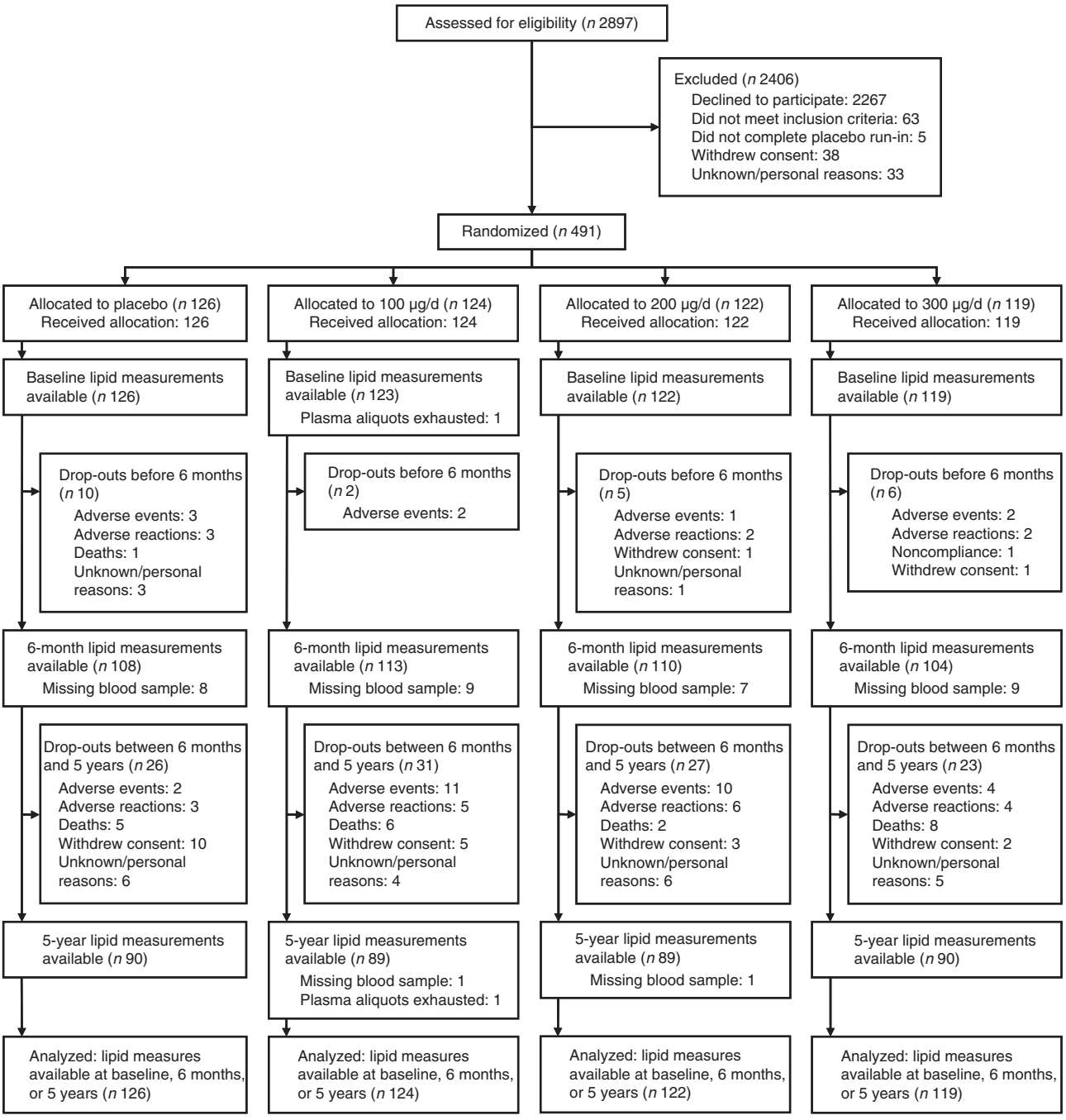

Fig. 1. Study flow diagram.

hence non-protocol use of over-the-counter Se was deemed to be rare. Thirty participants were receiving lipid-lowering medications at baseline and forty-eight at 5 years, with no significant differences between treatment groups at any time ($P = 0{\cdot}20$ and 0·40, respectively).

Intention-to-treat analysis

The mean of age and plasma Se concentration at baseline were 66·1 (SD 4·1) years and 86·5 (SD 16·3) ng/g (88·6 (SD 16·7) μg/l), respectively. Mean baseline levels of total cholesterol, HDL-cholesterol, non-HDL-cholesterol and total:HDL-cholesterol ratio were 6·01 (SD 0·94) mmol/l (2·324 (SD 0·362) g/l), 1·59 (SD 0·38) mmol/l (0·616 (SD 0·147) g/l), 4·42 (SD 0·94) mmol/l (1·708 (SD 0·365) g/l) and 3·95 (SD 1·01), respectively. There were no significant differences between treatment groups at baseline in terms of plasma Se concentrations, lipid concentrations or other participant characteristics (Table 1).

After 6 months of supplementation, plasma Se increased significantly and proportionally to the assigned dose in the three active treatment groups, but remained unchanged in the placebo group (Table 2). Compared with placebo, the mean changes in plasma Se concentrations were 65·7 ng/g (95 % CI 55·7, 75·6 ng/g) after 6 months of Se supplementation at 100 μg/d, 121·7 ng/g (95 % CI 111·7, 131·7 ng/g) at 200 μg/d and 170·7 ng/g (95 % CI 160·5, 180·8 ng/g) at 300 μg/d (P for homogeneity of changes across the four treatment groups <0·01). Lipid concentrations, however, evolved similarly, with no significant differences across the four treatment groups. Compared with placebo, the mean changes in total cholesterol concentrations were -0·07 mmol/l (95 % CI −0·23, 0·09 mmol/l) after 6 months of Se supplementation at 100 μg/d, 0·06 mmol/l (95 % CI −0·10, 0·21 mmol/l) at 200 μg/d and 0·02 mmol/l (95 % CI −0·14, 0·18 mmol/l) at 300 μg/d (P for homogeneity of changes across the four treatment groups = 0·45). The mean changes at 6 months in HDL-cholesterol, non-HDL-cholesterol and total:HDL-cholesterol ratio were also small and not statistically significant (Table 2).

After 5 years of Se supplementation, there were still no significant differences in lipid changes between the three active treatment groups and the placebo group (Table 2). Although results were similar after excluding participants who received lipid-lowering medication during the intervention period, small but

Table 1. Baseline characteristics of trial participants overall and by treatment group (Numbers and percentages; mean values and standard deviations)

					Se dose (μg/d)						
	Overall		Placebo		100		200		300		
Characteristic	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*P**
Participants	491		126		124		122		119		
Age (years)											0·13
Mean	66·1		65·4		66·4		66·3		66·5		
SD	4·1		3·8		4·2		4·4		4·1		
Sex											0·49
Men	255	51·9	60	47·6	70	56·5	66	54·1	59	49·6	
Women	236	48·1	66	52·4	54	43·5	56	45·9	60	50·4	
Smoking status											0·49
Never	160	32·6	35	27·8	42	33·9	40	32·8	43	36·2	
Former	185	37·7	48	38·1	47	37·9	52	42·6	38	31·9	
Current	146	29·7	43	34·1	35	28·2	30	24·6	38	31·9	
Alcohol drinking (drinks/week)											0·76
≤2	170	34·6	47	37·3	41	33·1	40	32·8	42	35·3	
3–10	209	42·6	48	38·1	51	41·1	55	45·1	55	46·2	
>10	112	22·8	31	24·6	32	25·8	27	22·1	22	18·5	
BMI (kg/m^2)											0·40
Mean	26·8		26·5		27·1		27·2		26·5		
SD	4·1		4·0		4·0		4·3		4·0		
Use of lipid-lowering medication	30	6·1	8	6·3	12	9·7	6	4·9	4	3·4	0·20
Total cholesterol (mmol/l)											0·24
Mean	6·01		5·91		6·02		5·98		6·15		
SD	0·94		0·86		0·99		0·93		0·95		
HDL-cholesterol (mmol/l)											0·35
Mean	1·59		1·63		1·58		1·55		1·61		
SD	0·38		0·42		0·34		0·37		0·39		
Non-HDL-cholesterol (mmol/l)											0·18
Mean	4·42		4·28		4·44		4·43		4·54		
SD	0·94		0·90		0·98		0·94		0·94		
Total:HDL-cholesterol ratio											0·37
Mean	3·95		3·83		3·95		4·04		4·01		
SD	1·01		1·03		0·96		1·02		1·04		
Plasma Se (ng/g)											0·17
Mean	86·5		86·0		87·5		88·3		83·9		
SD	16·3		15·2		16·4		16·2		17·1		

* P value for homogeneity of means or proportions across the four treatment groups.

Table 2. Effect of selenium supplementation on changes in plasma lipid and selenium concentrations after 6 months and 5 years*
(Mean values and standard deviations; 95 % confidence intervals)

			Se dose (μg/d)						
	Placebo		100		200		300		
Variable	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*P*†
Total cholesterol (mmol/l)									
At baseline	5·91	0·86	6·02	0·99	5·98	0·93	6·15	0·95	
At 6 months	5·71	0·78	5·75	0·93	5·84	0·95	5·97	0·91	
Change from baseline to 6 months									
Mean	−0·19		−0·26		−0·13		−0·17		
95 % CI	−0·30, −0·08		−0·37, −0·15		−0·25, −0·02		−0·28, −0·05		
Difference in change									0·45
Mean	0		−0·07		0·06		0·02		
95 % CI	Ref.		−0·23, 0·09		−0·10, 0·21		−0·14, 0·18		
P			0·38		0·49		0·80		
At 5 years	5·72	0·84	5·76	1·08	5·64	0·96	5·95	1·19	
Change from baseline to 5 years									
Mean	−0·20		−0·30		−0·40		−0·16		
95 % CI	−0·37, −0·04		−0·46, −0·13		−0·56, −0·23		−0·32, 0·01		
Difference in change									0·19
Mean	0		−0·09		−0·19		0·05		
95 % CI	Ref.		−0·33, 0·14		−0·42, 0·04		−0·18, 0·28		
P			0·43		0·11		0·69		
HDL-cholesterol (mmol/l)									
At baseline	1·63	0·42	1·58	0·34	1·55	0·37	1·61	0·39	
At 6 months	1·62	0·40	1·55	0·31	1·52	0·35	1·56	0·38	
Change from baseline to 6 months									
Mean	−0·02		−0·02		−0·02		−0·05		
95 % CI	−0·05, 0·01		−0·05, 0·01		−0·05, 0·01		−0·08, −0·02		
Difference in change									0·49
Mean	0		−0·01		0·00		−0·03		
95 % CI	Ref.		−0·05, 0·03		−0·05, 0·04		−0·08, 0·01		
P			0·70		0·92		0·16		
At 5 years	1·69	0·45	1·68	0·38	1·56	0·39	1·65	0·42	
Change from baseline to 5 years									
Mean	0·03		0·07		0·00		0·01		
95 % CI	−0·01, 0·08		0·03, 0·12		−0·04, 0·05		−0·04, 0·05		
Difference in change									0·13
Mean	0		0·04		−0·03		−0·03		
95 % CI	Ref.		−0·03, 0·10		−0·09, 0·03		−0·09, 0·04		
P			0·25		0·33		0·41		
Non-HDL-cholesterol (mmol/l)									
At baseline	4·28	0·90	4·44	0·98	4·43	0·94	4·54	0·94	
At 6 months	4·08	0·80	4·20	0·93	4·33	0·94	4·41	0·87	
Change from baseline to 6 months									
Mean	−0·17		−0·23		−0·11		−0·12		
95 % CI	−0·28, −0·07		−0·34, −0·13		−0·22, −0·01		−0·23, −0·01		
Difference in change									0·35
Mean	0		−0·06		0·06		0·05		
95 % CI	Ref.		−0·21, 0·09		−0·09, 0·21		−0·10, 0·20		
P			0·43		0·43		0·49		
At 5 years	4·03	0·84	4·09	0·99	4·08	0·91	4·30	1·11	
Change from baseline to 5 years									
Mean	−0·24		−0·37		−0·40		−0·16		
95 % CI	−0·40, −0·08		−0·53, −0·21		−0·56, −0·24		−0·32, −0·01		
Difference in change									0·13
Mean	0		−0·13		−0·16		0·08		
95 % CI	Ref.		−0·36, 0·09		−0·38, 0·06		−0·15, 0·30		
P			0·25		0·16		0·51		
Total:HDL-cholesterol ratio									
At baseline	3·83	1·03	3·95	0·96	4·04	1·02	4·01	1·04	
At 6 months	3·70	0·94	3·83	0·91	4·01	0·97	4·01	1·00	
Change from baseline to 6 months									
Mean	−0·10		−0·12		−0·06		0·00		
95 % CI	−0·19, −0·01		−0·20, −0·03		−0·15, 0·02		−0·09, 0·09		
Difference in change									0·26
Mean	0		−0·02		0·04		0·10		
95 % CI	Ref.		−0·14, 0·10		−0·08, 0·16		−0·03, 0·22		
P			0·76		0·54		0·12		

Table 2 *Continued*

	Placebo		Se dose (μg/d) 100		200		300		
Variable	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*P*†
At 5 years	3·59	1·00	3·55	0·81	3·78	0·96	3·77	0·99	
Change from baseline to 5 years									
Mean	−0·20		−0·37		−0·29		−0·12		
95 % CI	−0·34, −0·06		−0·51, −0·23		−0·43, −0·15		−0·26, 0·02		
Difference in change									0·06
Mean	0		−0·17		−0·09		0·08		
95 % CI	Ref.		−0·37, 0·02		−0·29, 0·10		−0·12, 0·27		
P			0·08		0·35		0·43		
Plasma Se (ng/g)									
At baseline	86·0	15·2	87·5	16·4	88·3	16·2	83·9	17·1	
At 6 months	85·3	14·2	152·4	23·7	209·1	41·5	253·7	54·1	
Change from baseline to 6 months									
Mean	−0·9		64·8		120·9		169·8		
95 % CI	−8·0, 6·2		57·8, 71·8		113·8, 127·9		162·5, 177·0		
Difference in change									<0·001
Mean	0		65·7		121·7		170·7		
95 % CI	Ref.		55·7, 75·6		111·7, 131·7		160·5, 180·8		
P			<0·001		<0·001		<0·001		
At 5 years	87·7	24·2	158·3	28·3	222·2	40·6	276·5	78·7	
Change from baseline to 5 years									
Mean	1·3		70·8		133·7		192·3		
95 % CI	−8·6, 11·3		60·7, 80·8		123·7, 143·8		182·2, 202·4		
Difference in change									<0·001
Mean	0		69·4		132·4		190·9		
95 % CI	Ref.		55·3, 83·6		118·2, 146·5		176·7, 205·1		
P			<0·001		<0·001		<0·001		

Ref., referent values.
* Results were obtained from linear mixed models with fixed treatment-by-time interactions and random between-subject variations in both baseline lipid levels and lipid changes over time.
† Overall *P* value comparing the three active treatment groups with placebo.

statistically significant decreases ($P=0·04$) in non-HDL-cholesterol and 0·20 ($P=0·02$) in total:HDL-cholesterol ratio were seen at Se supplementation of 100 μg/d (Table 3). There were no significant differences in treatment effects across baseline age groups, sex, category of BMI, or baseline plasma Se concentration or baseline lipid concentrations, after excluding men and women receiving lipid-lowering medications (data not shown).

Association between plasma Se and lipid concentrations

In cross-sectional analyses at baseline, a 50 ng/g increase in plasma Se was significantly associated with mean increases of 0·47 mmol/l (95 % CI 0·24, 0·70 mmol/l; $P_{trend}<0·001$) in total cholesterol levels and 0·41 mmol/l (95 % CI 0·17, 0·64 mmol/l; $P_{trend}=0·001$) in non-HDL-cholesterol levels. HDL-cholesterol and total:HDL-cholesterol ratio were not significantly associated with plasma Se at baseline (Table 4). In longitudinal analyses, a 50 ng/g increase in plasma Se over time was not related to significant changes in total HDL-cholesterol, non-HDL-cholesterol and total:HDL-cholesterol ratio after 6 months or 5 years (Table 5).

Adverse events

Twenty-two participants died during the 5-year follow-up period and thirty-five participants discontinued the study because of non-fatal adverse events (Fig. 1), with no significant differences across treatment groups ($P=0·29$ and 0·14, respectively). Twenty-five participants withdrew because of adverse reactions to treatment (Fig. 1), which were mainly hair loss, skin reactions and grooved nails. These reactions were equally associated with Se or placebo and were independent of Se dose ($P=0·84$).

Discussion

Contrary to our working hypothesis, Se supplementation showed no benefit over placebo on cholesterol concentrations after 6 months or 5 years in this elderly Danish population. Our findings were different from those of the UK PRECISE pilot trial, despite both study populations having similar mean baseline plasma Se concentrations (88·8 ng/g (91·2 μg/l) in UK PRECISE and 86·5 ng/g (88·6 μg/l) in Denmark PRECISE). In the UK study, compared with placebo, total plasma cholesterol and non-HDL-cholesterol concentrations decreased significantly in the 100 and 200 μg/d Se supplementation groups, whereas HDL-cholesterol concentrations increased significantly in the 300 μg/d group. In addition, the total:HDL-cholesterol ratio decreased progressively with increasing Se dose[32]. We could not reproduce the UK PRECISE findings of beneficial effects of Se supplementation on total cholesterol or on the total:HDL-cholesterol ratio at 6 months, as beneficial changes in the intervention groups were matched by similar changes in the placebo group. This was also the case at 5 years, highlighting the internal consistency of the Danish results after both short-term and long-term intervention.

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Table 3. Effect of selenium supplementation on changes in plasma lipid concentrations after 6 months and 5 years in participants not receiving lipid-lowering medications* (Mean values and 95 % confidence intervals)

	Difference in change from baseline								
	Placebo		Se 100 μg/d		Se 200 μg/d		Se 300 μg/d		
Variable	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI	*P*†
Total cholesterol (mmol/l)									
6 months	0	Ref.	−0·13	−0·28, 0·03	0·00	−0·15, 0·16	−0·02	−0·17, 0·14	0·30
P				0·11		0·97		0·82	
5 years	0	Ref.	−0·15	−0·34, 0·04	−0·16	−0·35, 0·03	0·03	−0·16, 0·21	0·10
P				0·11		0·11		0·77	
HDL-cholesterol (mmol/l)									
6 months	0	Ref.	−0·03	−0·07, 0·02	0·00	−0·05, 0·04	−0·03	−0·08, 0·01	0·38
P				0·27		0·91		0·15	
5 years	0	Ref.	0·03	−0·03, 0·10	−0·02	−0·09, 0·04	−0·02	−0·08, 0·04	0·32
P				0·31		0·52		0·53	
Non-HDL-cholesterol (mmol/l)									
6 months	0	Ref.	−0·10	−0·24, 0·04	0·01	−0·14, 0·15	0·02	−0·13, 0·16	0·35
P				0·18		0·93		0·81	
5 years	0	Ref.	−0·19	−0·37, −0·01	−0·13	−0·31, 0·04	0·05	−0·13, 0·22	0·03
P				0·04		0·14		0·58	
Total:HDL-cholesterol ratio									
6 months	0	Ref.	−0·03	−0·15, 0·09	0·00	−0·12, 0·11	0·07	−0·05, 0·19	0·43
P				0·63		0·94		0·28	
5 years	0	Ref.	−0·20	−0·36, −0·03	−0·07	−0·24, 0·10	0·04	−0·12, 0·20	0·03
P				0·02		0·42		0·65	

Ref., referent values.
* Results were obtained from linear mixed models with fixed treatment-by-time interactions and random between-subject variations in both baseline lipid levels and lipid changes over time, excluding visits after participants received lipid-lowering medications.
† Overall *P* value comparing the three active treatment groups with placebo.

Table 4. Cross-sectional association between plasma selenium and lipid levels at baseline*
(Mean values and standard deviations; 95 % confidence intervals)

	50 ng/g increase in baseline Se		Quartile of baseline Se (ng/g)								
			First (43–76)		Second (77–85)		Third (86–95)		Fourth (96–159)		
Variable	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	P_{trend}†
Median baseline Se (ng/g)	85		70		80		89		104		
Participants (*n*)	488		126		129		114		119		
Total cholesterol (mmol/l)											
At baseline	6·01	0·93	5·70	0·94	6·06	0·88	6·11	0·90	6·19	0·94	
Adjusted mean difference											<0·001
Mean	0·47		0		0·33		0·43		0·45		
95 % CI	0·24, 0·70		Ref.		0·13, 0·54		0·23, 0·64		0·24, 0·66		
HDL-cholesterol (mmol/l)											
At baseline	1·59	0·38	1·52	0·37	1·58	0·36	1·64	0·36	1·64	0·41	
Adjusted mean difference											0·16
Mean	0·06		0		0·03		0·09		0·05		
95 % CI	−0·03, 0·15		Ref.		−0·05, 0·11		0·01, 0·17		−0·03, 0·13		
Non-HDL-cholesterol (mmol/l)											
At baseline	4·42	0·94	4·18	0·97	4·48	0·89	4·47	0·92	4·55	0·96	
Adjusted mean difference											0·001
Mean	0·41		0		0·31		0·35		0·40		
95 % CI	0·17, 0·64		Ref.		0·10, 0·51		0·13, 0·56		0·18, 0·61		
Total:HDL-cholesterol ratio											
At baseline	3·95	1·01	3·95	1·10	3·99	0·94	3·87	0·93	3·98	1·06	
Adjusted mean difference											0·17
Mean	0·15		0		0·12		0·03		0·18		
95 % CI	−0·10, 0·39		Ref.		−0·10, 0·33		−0·19, 0·25		−0·04, 0·41		

Ref., referent values.
* Results were obtained from linear mixed models with random between-subject variations in baseline lipid levels and adjusted for baseline age (continuous), sex, smoking status (never, former, or current), alcohol drinking (≤2, 3–10, or >10 drinks/week), BMI (continuous) and use of lipid-lowering medications.
† *P* value for linear trend using an ordinal variable with the median baseline Se level in each quartile.

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Table 5. Longitudinal association between changes in plasma selenium and lipid levels after 6 months and 5 years*
(Mean values and standard deviations; 95 % confidence intervals)

	50 ng/g increase in Se over time		Quartile of Se change (ng/g)								
			First (−48 to 22)		Second (23 to 87)		Third (88 to 146)		Fourth (147 to 468)		
Variable	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	P†
Median Se change (ng/g)	87		−2		60		112		180		
Participants (*n*)											
6 months	432		108		121		106		97		
5 years	356		89		78		92		97		
Total cholesterol (mmol/l)											
At 6 months	−0·18	0·59	−0·20	0·64	−0·24	0·55	−0·09	0·56	−0·20	0·61	
Adjusted difference in change											0·11
Mean	0·02		0		−0·04		0·12		−0·01		
95 % CI	−0·02, 0·05		Ref.		−0·18, 0·10		−0·02, 0·26		−0·16, 0·14		
At 5 years	−0·27	0·82	−0·25	0·81	−0·32	0·78	−0·29	0·89	−0·24	0·80	
Adjusted difference in change											0·65
Mean	0·01		0		−0·10		−0·07		0·00		
95 % CI	−0·03, 0·04		Ref.		−0·29, 0·09		−0·26, 0·11		−0·19, 0·18		
HDL-cholesterol (mmol/l)											
At 6 months	−0·03	0·16	−0·02	0·19	−0·02	0·16	−0·02	0·16	−0·04	0·15	
Adjusted difference in change											0·77
Mean	0·00		0		0·00		−0·01		−0·02		
95 % CI	−0·01, 0·01		Ref.		−0·04, 0·04		−0·05, 0·04		−0·06, 0·02		
At 5 years	0·03	0·23	0·03	0·22	0·03	0·21	0·07	0·25	0·00	0·24	
Adjusted difference in change											0·39
Mean	0·00		0		0·00		0·01		−0·04		
95 % CI	−0·01, 0·01		Ref.		−0·06, 0·06		−0·05, 0·07		−0·10, 0·02		
Non-HDL-cholesterol (mmol/l)											
At 6 months	−0·16	0·55	−0·18	0·58	−0·22	0·53	−0·07	0·54	−0·16	0·57	
Adjusted difference in change											0·08
Mean	0·02		0		−0·04		0·12		0·01		
95 % CI	−0·01, 0·05		Ref.		−0·17, 0·09		−0·01, 0·26		−0·13, 0·15		
At 5 years	−0·31	0·79	−0·28	0·76	−0·36	0·76	−0·35	0·84	−0·24	0·81	
Adjusted difference in change											0·39
Mean	0·00		0		−0·10		−0·08		0·03		
95 % CI	−0·03, 0·04		Ref.		−0·29, 0·08		−0·26, 0·10		−0·14, 0·21		
Total:HDL-cholesterol ratio											
At 6 months	−0·07	0·45	−0·10	0·47	−0·11	0·43	−0·01	0·50	−0·06	0·42	
Adjusted difference in change											0·22
Mean	0·02		0		−0·02		0·09		0·03		
95 % CI	−0·01, 0·04		Ref.		−0·13, 0·09		−0·03, 0·20		−0·09, 0·14		
At 5 years	−0·26	0·70	−0·19	0·61	−0·33	0·69	−0·36	0·77	−0·16	0·70	
Adjusted difference in change											0·10
Mean	0·00		0		−0·13		−0·12		0·04		
95 % CI	−0·04, 0·03		Ref.		−0·30, 0·03		−0·28, 0·05		−0·12, 0·20		

Ref., referent values.
* Results were obtained from linear mixed models with random between-subject variations in both baseline lipid levels and lipid changes over time and adjusted for baseline Se levels (continuous), age (continuous), sex, smoking status (never, former, or current), alcohol drinking (≤ 2, 3–10, or >10 drinks/week), BMI (continuous) and changes in lipid-lowering medications over time.
† Overall *P* value comparing the three highest quartiles of Se change with the lowest quartile.

The different findings in the UK and Danish PRECISE trials cannot be easily explained, as the two populations were of the same age, had similar blood-lipid levels and Se status, and received identical interventions. We could not explore the possibility that the different results might relate to genetic differences, but it seems unlikely, as both populations were almost exclusively Caucasian. Alternatively, there may have been differences in the intake of dietary macro- and micro-nutrients between UK and Danish populations that could have modified the effects of additional Se on blood lipids. For instance, at the time of this intervention there was national concern in Denmark about the potentially adverse effect of high levels of *trans*-fatty acids (TFA) on cholesterol levels. This concern culminated in the introduction of legislation in 2004 to limit the content of industrially produced TFA in foods to <2 % energy[43] Although recruitment to this study took place before this legislation (December 1998 to July 1999), there is evidence from the TRANSFAIR study to suggest that a lower intake of TFA in Denmark over that period might have affected lipid concentrations in Denmark[44]. Indeed, in the Copenhagen City Heart Study[45], total cholesterol concentrations in participants aged 60–74 years decreased between 1991–1994 and 2001–2003 from 6·1 to 5·5 mmol/l in men and from 6·8 to 5·9 mmol/l in women (A Langsted, JJ Freiberg, A Tybjaerg-Hansen, *et al.*, unpublished results). However, secular declining trends in cholesterol levels were also reported in the UK in the period leading up to the PRECISE trial, with population total cholesterol falling 4·2 % from 1981 to 2000[46].

A difference between the Danish and UK populations over this period was that Denmark introduced I fortification in 1998 because of low I intake [47]. In the county of Funen, from where participants were recruited, the average 24 h I excretion in 1988 was reported to be 85 μg, indicative of moderate I deficiency[47,48]. Chronic I deficiency increases thyroid stimulating hormone (TSH) concentration and produces a thyroid hormone pattern consistent with subclinical hypothyroidism, characterised by raised plasma total and LDL-cholesterol concentrations[49,50]. Hence, it is possible that benefits to serum lipids from I fortification over the period of the trial may have camouflaged any beneficial effect of Se supplementation in the Danish cohort[51]. The effect of Se supplementation on thyroid function has been investigated in our study population and minute and dose-dependent decreases in serum TSH and FT4 concentrations were found[52]. This could hypothetically improve the lipid profile[53], but our results suggest that the observed effects on thyroid function were too small to affect lipid metabolism.

In cross-sectional analyses we observed significant positive associations between plasma Se concentrations and total and non-HDL-cholesterol concentrations (Table 4), as found in previous cross-sectional studies[19–27]. Although such observations have previously raised concern that Se intake might have adverse effects on blood cholesterol, our data indicate that these cross-sectional associations are unlikely to be the result of a causal effect of Se intake on lipid concentrations.

This study has several limitations. Participants were invited via a random sample from the Danish Civil Registration System. About 20 % accepted the invitation and this constitutes a potential selection bias. The narrow age range of participants (60–74 years) limits the applicability of our findings to a general population, and the relatively low baseline Se status makes it difficult to extend our finding to populations with higher baseline Se status – for example, the USA. As blood samples were collected in a non-fasting state with no information available on time since last meal, we were unable to assess the concentration of plasma TAG, another CVD risk factor[54]. The lack of comparable dietary data in the Danish and UK PRECISE populations limits the validity of comparisons between the two studies – for example, differences in alcohol intake, which influences lipid metabolism[55,56], were not extracted in the UK PRECISE study. Furthermore, these findings come from *post hoc* analyses of a trial that was not specifically designed and powered to address our study questions, and hence they need to be interpreted with caution. *Post hoc* power calculations, with set absolute differences of 0·20 mmol/l for total and non-HDL-cholesterol, 0·05 mmol/l for HDL-cholesterol and 0·15 for total:HDL-cholesterol ratio, corresponding approximately to the effects detected in the UK PRECISE study, showed that our study was underpowered to find the same differences after 6 months. In addition to the power calculations, 95 % CI are given in the results section, to express the amount of uncertainty about the effect estimates[57]. Meanwhile, the lack of observed effects are not likely because of low bioavailablity of the formulation used, as the Se concentration in whole blood from our trial participants was higher ($P<0{\cdot}001$) than that obtained with synthetic L-selenomethionine in a comparable group of Danes, both groups having been treated with 300 μg Se/d[40]. Despite the limitations mentioned, this is the only randomised, placebo-controlled trial that has examined the effect of long-term Se supplementation on plasma cholesterol and its sub-fractions.

In summary, we conclude that long-term Se supplementation did not alter cholesterol concentrations in this Danish elderly population. Any beneficial effects were matched in the placebo group, and the findings from the UK PRECISE pilot trial could therefore not be reproduced. However, our findings show that long-term supplementation with up to 300 μg/d Se (as Se yeast) had no detectable adverse effects in an elderly population of relatively low-Se status. In view of the potential benefit of raising Se status on some health conditions (e.g. mild Graves' orbitopathy), these findings are reassuring[2,58].

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There are no conflicts of interest to declare.

References

1. Kryukov GV, Castellano S, Novoselov SV, *et al.* (2003) Characterization of mammalian selenoproteomes. *Science* **300**, 1439–1443.
2. Rayman MP (2012) Selenium and human health. *Lancet* **379**, 1256–1268.
3. Salonen JT, Alfthan G, Huttunen JK, *et al.* (1982) Association between cardiovascular death and myocardial infarction and serum selenium in a matched-pair longitudinal study. *Lancet* **2**, 175–179.
4. Anonymous (1979) Observations on effect of sodium selenite in prevention of Keshan disease. *Chin Med J (Engl)* **92**, 471–476.
5. Salonen JT, Salonen R, Seppänen K, *et al.* (1988) Relationship of serum selenium and antioxidants to plasma lipoproteins, platelet aggregability and prevalent ischaemic heart disease in Eastern Finnish men. *Atherosclerosis* **70**, 155–160.

6. Sattler W, Maiorino M & Stocker R (1994) Reduction of HDL- and LDL-associated cholesterylester and phospholipid hydroperoxides by phospholipid hydroperoxide glutathione peroxidase and Ebselen (PZ 51). *Arch Biochem Biophys* **309**, 214–221.
7. Blankenberg S, Rupprecht HJ, Bickel C, *et al.* (2003) Glutathione peroxidase 1 activity and cardiovascular events in patients with coronary artery disease. *N Engl J Med* **349**, 1605–1613.
8. Brigelius-Flohe R, Banning A & Schnurr K (2003) Selenium-dependent enzymes in endothelial cell function. *Antioxid Redox Signal* **5**, 205–215.
9. Curran JE, Jowett JB, Elliott KS, *et al.* (2005) Genetic variation in selenoprotein S influences inflammatory response. *Nat Genet* **37**, 1234–1241.
10. Gao Y, Hannan NR, Wanyonyi S, *et al.* (2006) Activation of the selenoprotein SEPS1 gene expression by pro-inflammatory cytokines in HepG2 cells. *Cytokine* **33**, 246–251.
11. Vunta H, Davis F, Palempalli UD, *et al.* (2007) The anti-inflammatory effects of selenium are mediated through 15-deoxy- Delta12,14-prostaglandin J2 in macrophages. *J Biol Chem* **282**, 17964–17973.
12. Flores-Mateo G, Navas-Acien A, Pastor-Barriuso R, *et al.* (2006) Selenium and coronary heart disease: a meta-analysis. *Am J Clin Nutr* **84**, 762–773.
13. Stranges S, Marshall JR, Trevisan M, *et al.* (2006) Effects of selenium supplementation on cardiovascular disease incidence and mortality: secondary analyses in a randomized clinical trial. *Am J Epidemiol* **163**, 694–699.
14. Stranges S, Navas-Acien A, Rayman MP, *et al.* (2010) Selenium status and cardiometabolic health: state of the evidence. *Nutr Metab Cardiovasc Dis* **20**, 754–760.
15. Bjelakovic G, Nikolova D, Gluud LL, *et al.* (2012) Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases. *Cochrane Database Syst Rev* **3**, CD007176.
16. Rees K, Hartley L, Day C, *et al.* (2013) Selenium supplementation for the primary prevention of cardiovascular disease. *Cochrane Database Syst Rev* **1**, CD009671.
17. Stamler J, Wentworth D & Neaton JD (1986) Is relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded? Findings in 356 222 primary screenees of the Multiple Risk Factor Intervention Trial (MRFIT). *JAMA* **256**, 2823–2828.
18. Prospective Studies Collaboration, Lewington S, Whitlock G, *et al.* (2007) Blood cholesterol and vascular mortality by age, sex and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55 000 vascular deaths. *Lancet* **370**, 1829–1839.
19. Jossa F, Trevisan M, Krogh V, *et al.* (1991) Serum selenium and coronary heart disease risk factors in southern Italian men. *Atherosclerosis* **87**, 129–134.
20. Suadicani P, Hein HO & Gyntelberg F (1992) Serum selenium concentration and risk of ischaemic heart disease in a prospective cohort study of 3000 males. *Atherosclerosis* **96**, 33–42.
21. Gámez C, Ruiz-López D, Artacho R, *et al.* (1997) Serum selenium in institutionalized elderly subjects and relation to other nutritional markers Letter). *Clin Chem* **43**, 693–694.
22. Coudray C, Roussel AM, Mainard F, *et al.* (1997) Lipid peroxidation level and antioxidant micronutrient status in a pre-aging population; correlation with chronic disease prevalence in a French epidemiological study (Nantes, France). *J Am Coll Nutr* **16**, 584–591.
23. Bates CJ, Thane CW, Prentice A, *et al.* (2002) Selenium status and its correlates in a British national diet and nutrition survey: people aged 65 years and over. *J Trace Elem Med Biol* **16**, 1–8.
24. Bleys J, Navas-Acien A, Stranges S, *et al.* (2008) Serum selenium and serum lipids in US adults. *Am J Clin Nutr* **88**, 416–423.
25. Laclaustra M, Stranges S, Navas-Acien A, *et al.* (2010) Serum selenium and serum lipids in US adults: National Health and Nutrition Examination Survey (NHANES) 2003–2004. *Atherosclerosis* **210**, 643–648.
26. Stranges S, Laclaustra M, Ji C, *et al.* (2010) Higher selenium status is associated with adverse blood lipid profile in British adults. *J Nutr* **140**, 81–87.
27. Yang KC, Lee LT, Lee YS, *et al.* (2010) Serum selenium concentration is associated with metabolic factors in the elderly: a cross-sectional study. *Nutr Metab (Lond)* **7**, 38.
28. Stranges S, Tabak AG, Guallar E, *et al.* (2011) Selenium status and blood lipids: the cardiovascular risk in Young Finns study. *J Intern Med* **270**, 469–477.
29. Stranges S, Galletti F, Farinaro E, *et al.* (2011) Associations of selenium status with cardiometabolic risk factors: an 8-year follow-up analysis of the Olivetti Heart study. *Atherosclerosis* **217**, 274–278.
30. Clark LC, Combs GF Jr, Turnbull BW, *et al.* (1996) Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. A randomized controlled trial. Nutritional Prevention of Cancer Study Group. *JAMA* **276**, 1957–1963.
31. Levander OA, Alfthan G, Arvilommi H, *et al.* (1983) Bioavailability of selenium to Finnish men as assessed by platelet glutathione peroxidase activity and other blood parameters. *Am J Clin Nutr* **37**, 887–897.
32. Rayman MP, Stranges S, Griffin BA, *et al.* (2011) Effect of supplementation with high-selenium yeast on plasma lipids. A randomized trial. *Ann Intern Med* **154**, 656–665.
33. Luoma PV, Korpela H, Sotaniem EA, *et al.* (1985) Serum selenium, glutathione peroxidase, lipids, and human liver microsomal enzyme activity. A double-blind controlled trial of selenium supplementation. *Biol Trace Elem Res* **8**, 113–121.
34. Yu SY, Mao BL, Xiao P, *et al.* (1990) Intervention trial with selenium for the prevention of lung cancer among tin miners in Yunnan, China. A pilot study. *Biol Trace Elem Res* **24**, 105–108.
35. Meltzer HM, Mundal HH, Alexander J, *et al.* (1994) Does dietary arsenic and mercury affect cutaneous bleeding time and blood lipids in humans? *Biol Trace Elem Res* **46**, 135–153.
36. Meltzer HM, Folmer M, Wang S, *et al.* (1997) Supplementary selenium influences the response to fatty acid-induced oxidative stress in humans. *Biol Trace Elem Res* **60**, 51–68.
37. Ravn-Haren G, Bugel S, Krath BN, *et al.* (2008) A short-term intervention trial with selenate, selenium-enriched yeast and selenium-enriched milk: effects on oxidative defence regulation. *Br J Nutr* **99**, 883–892.
38. Hawkes WC & Laslett LJ. (2009) Selenium supplementation does not improve vascular responsiveness in healthy North American men. *Am J Physiol Heart Circ Physiol* **296**, H256–H262.
39. Wu J, Salisbury C, Graham R, *et al.* (2009) Increased consumption of wheat biofortified with selenium does not modify biomarkers of cancer risk, oxidative stress, or immune function in healthy Australian males. *Environ Mol Mutagen* **50**, 489–501.
40. Larsen EH, Hansen M, Paulin H, *et al.* (2004) Speciation and bioavailability of selenium in yeast-based intervention agents used in cancer chemoprevention studies. *J AOAC Int* **87**, 225–232.
41. Albert PS (1999) Longitudinal data analysis (repeated measures) in clinical trials. *Stat Med* **18**, 1707–1732.
42. Diggle PJ, Heagerty P, Liang KY, *et al.* (2002) *Analysis of Longitudinal Data*, 2nd ed. Oxford: Oxford University Press.
43. Stender S, Dyerberg J, Bysted A, *et al.* (2006) A trans world journey. *Atheroscler Suppl* **7**, 47–52.

44. Van Poppel G (1998) Intake of trans fatty acids in western Europe: the TRANSFAIR study. *Lancet* **351**, 1099.
45. Langsted A, Freiberg JJ, Tybjaerg-Hansen A, *et al.* (2011) Nonfasting cholesterol and triglycerides and association with risk of myocardial infarction and total mortality: the Copenhagen City Heart Study with 31 years of follow-up. *J Intern Med* **270**, 65–75.
46. Unal B, Critchley JA & Capewell S. (2005) Modelling the decline in coronary heart disease deaths in England and Wales, 1981–2000: comparing contributions from primary prevention and secondary prevention. *BMJ* **331**, 614.
47. Rasmussen LB, Carlé A, Jørgensen T, *et al.* (2008) Iodine intake before and after mandatory iodization in Denmark: results from the Danish Investigation of Iodine Intake and Thyroid Diseases (DanThyr) study. *Br J Nutr* **100**, 166–173.
48. Haas V, Marley M, Green A, *et al.* (1988) Urinary iodine excretion in a geographically stratified Danish population sample not affected by iodination programmes. A change towards higher values. *Acta Endocrinol (Copenh)* **119**, 125–131.
49. Zhao SJ, Ye Y, Sun FJ, *et al.* (2011) The impact of dietary iodine intake on lipid metabolism in mice. *Biol Trace Elem Res* **142**, 581–588.
50. Jung CH, Sung KC, Shin HS, *et al.* (2003) Thyroid dysfunction and their relation to cardiovascular risk factors such as lipid profile, hsCRP, and waist hip ratio in Korea. *Korean J Intern Med* **18**, 146–153.
51. Zimmermann MB, Aeberli I, Melse-Boonstra A, *et al.* (2009) Iodine treatment in children with subclinical hypothyroidism due to chronic iodine deficiency decreases thyrotropin and C-peptide concentrations and improves the lipid profile. *Thyroid* **19**, 1099–1104.
52. Winther KH, Bonnema SJ, Cold F, *et al.* (2015) Does selenium supplementation affect thyroid function? Results from a randomized, controlled, double-blinded trial in a Danish population. *Eur J Endocrinol* **172**, 657–667.
53. Duntas LH & Brenta G. (2012) The effect of thyroid disorders on lipid levels and metabolism. *Med Clin North Am* **96**, 269–281.
54. Sundvall J, Laatikainen T, Hakala S, *et al.* (2008) Systematic error of serum triglyceride measurements during three decades and the effect of fasting on serum triglycerides in population studies. *Clin Chim Acta* **397**, 55–59.
55. Hata Y & Nakajima K (2000) Life-style and serum lipids and lipoproteins. *J Atheroscler Thromb* **7**, 177–197.
56. Wang Z, Yao T & Song Z (2010) Chronic alcohol consumption disrupted cholesterol homeostasis in rats: down-regulation of low-density lipoprotein receptor and enhancement of cholesterol biosynthesis pathway in the liver. *Alcohol Clin Exp Res* **34**, 471–478.
57. Röhrig B, du Prel JB, Wachtlin D, *et al.* (2010) Sample size calculation in clinical trials: part 13 of a series on evaluation of scientific publications. *Dtsch Ärztebl Int* **107**, 552–556.
58. Marcocci C, Kahaly GJ, Krassas GE, *et al.* (2011) Selenium and the course of mild Graves' orbitopathy. *N Engl J Med* **364**, 1920–1931.

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