



Review Article

Effect of long-term selenium supplementation on mortality: Results from a multiple-dose, randomised controlled trial

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ABSTRACT

Background: Selenium, an essential trace element, is incorporated into selenoproteins with a wide range of health effects. Selenoproteins may reach repletion at a plasma selenium concentration of ~ 125 µg/L, at which point the concentration of selenoprotein P reaches a plateau; whether sustained concentrations higher than this are beneficial, or indeed detrimental, is unknown.

Objective: In a population of relatively low selenium status, we aimed to determine the effect on mortality of long-term selenium supplementation at different dose levels.

Design: The Denmark PRECISE study was a single-centre, randomised, double-blinded, placebo-controlled, multi-arm, parallel clinical trial with four groups. Participants were 491 male and female volunteers aged 60–74 years, recruited at Odense University Hospital, Denmark. The trial was initially designed as a 6-month pilot study, but supplemental funding allowed for extension of the study and mortality assessment. Participants were randomly assigned to treatment with 100, 200, or 300 µg selenium/d as selenium-enriched-yeast or placebo-yeast for 5 years from randomization in 1998–1999 and were followed up for mortality for a further 10 years (through March 31, 2015).

Results: During 6871 person-years of follow-up, 158 deaths occurred. In an intention-to-treat analysis, the hazard ratio (95% confidence interval) for all-cause mortality comparing 300 µg selenium/d to placebo was 1.62 (0.66, 3.96) after 5 years of treatment and 1.59 (1.02, 2.46) over the entire follow-up period. The 100 and 200 µg/d doses showed non-significant decreases in mortality during the intervention period that disappeared after treatment cessation. Although we lacked power for endpoints other than all-cause mortality, the effects on cancer and cardiovascular mortality appeared similar.

Conclusions: A 300 µg/d dose of selenium taken for 5 years in a country with moderately-low selenium status increased all-cause mortality 10 years later. While our study was not initially designed to evaluate mortality and the sample size was limited, our findings indicate that total selenium intake over 300 µg/d and high-dose selenium supplements should be avoided.

1. Introduction

Selenium is a trace element essential for human health. As selenocysteine, it is incorporated into 25 human selenoproteins with a wide range of health effects, most notably the ability to reduce oxidative

stress and inflammation [1,2]. Reinforcing the importance of selenoproteins, single nucleotide polymorphisms in a number of selenoproteins are linked to age-related disorders such as cardiovascular disease (CVD) and a wide range of cancers [1]. The concentration of selenoprotein P, and probably of all selenoproteins, plateaus when plasma

Abbreviations: CVD, cardiovascular disease; ER, endoplasmic reticulum; SD, standard deviation; UPR, unfolded protein response

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selenium concentration reaches $\sim 125 \mu\text{g/L}$ [3]; it is unclear whether selenium supplementation that results in higher plasma concentration, particularly if sustained, has any beneficial, or indeed detrimental, effects.

The US Third National Health and Nutrition Examination Survey (NHANES) showed a U-shaped association between serum selenium and all-cause mortality, with minimal mortality at a concentration of $\sim 135 \mu\text{g/L}$ [4]. Of six other observational studies [2,5–9], five found an inverse association between selenium status or intake and mortality [2,5–8] while one found no effect [9]. These studies are still consistent with a U-shaped dose-response as they were conducted in populations with low [5–7] or relatively low [2,8], serum-selenium concentration or selenium intake and provide no information on mortality at high serum-selenium concentrations [4].

Only two randomised controlled trials of selenium as a single nutrient had a mortality outcome; both were carried out in the US. In the Nutritional Prevention of Cancer trial in 1312 participants, 200 $\mu\text{g/d}$ selenium (as high-selenium yeast) increased mean plasma selenium from 114 to 190 $\mu\text{g/L}$ and resulted in a non-significant 21% reduction in all-cause mortality, a significant 52% reduction in cancer mortality [10], but no effect on cardiovascular mortality [11] over a 6-to-8-year follow-up. In the Selenium and Vitamin E Cancer Prevention Trial (SELECT) conducted in 35,533 men, 200 $\mu\text{g/d}$ selenium (as selenomethionine) increased mean serum selenium from 138 to 252 $\mu\text{g/L}$ with no effect on all-cause or cancer mortality after 5.5 and 8 years of follow-up [12,13]. No randomised trial data are available on the long-term effect of selenium supplementation on mortality in populations of relatively low selenium status; our aim was to provide such data.

We report here the findings of a double-blind, randomised, controlled trial of long-term selenium supplementation and mortality in Denmark, a country of moderately-low selenium status. We randomised participants to treatment with selenium (100, 200, and 300 $\mu\text{g/d}$) or placebo for 5 years and followed them up, post-treatment, for a further 10 years.

2. Subjects and methods

The methods of this pilot study, previously described in detail [14], are summarized below.

2.1. Study design and sample size

The Denmark PRECISE pilot study (ClinicalTrials.gov ID: NCT01819649) was a single-centre, randomised, double-blinded, placebo-controlled, multi-arm, parallel clinical trial with four groups (allocation ratio 1:1:1:1) run from Odense University Hospital, Denmark [14,15]. Denmark PRECISE was one of two pilot studies for the proposed international PRECISE trial of selenium in cancer prevention; the other was carried out in the UK [16–18]. No formal power calculations were performed *a priori*; the sample size was set at 500 participants, which was considered sufficient to assess whether recruitment, adherence, and retention during follow-up would be sufficient for a large trial.

2.2. Change to outcomes

While the corresponding UK trial was terminated after 6 months, as planned, extended funding enabled the Danish pilot study intervention to continue for 5 years in the hope that funding for the proposed international PRECISE trial would become available. Though such funding was never secured, participant follow-up in the Danish cohort was continued for a further 10 years for mortality ascertainment. It should be noted, however, that mortality was not a planned endpoint when the trial was initiated. The trial protocol and subsequent amendments are available on-line [19].

2.3. Participants

From November 1998 to June 1999, 2897 potential participants, males and females aged 60–74 years, from the County of Funen, Denmark were invited to take part in the trial; 630 accepted the invitation to be screened for inclusion at Odense University Hospital. Exclusion criteria were: a Southwest Oncology Group performance-status score > 1 , indicating impairment in general well-being and activities of daily life; active liver or kidney disease; previous diagnosis of cancer (excluding non-melanoma skin cancer); diagnosed HIV infection; receiving immunosuppressive therapy; being unable to understand written/spoken information; and receiving $\geq 50 \mu\text{g/d}$ of selenium supplements in the previous 6 months.

2.4. Randomization

Randomization was computer-generated, blocked and non-stratified [14]. Participating couples living at the same address were allocated to the same intervention. Participants, research staff and investigators were blinded to treatment assignment [14].

2.5. Ethics committee (IRB) approval

The regional Data Protection Agency and Scientific Ethics Committees of Vejle and Funen counties approved the study prior to data collection (Journal nr. 19980186).

2.6. Intervention and procedures

Participants deemed suitable for inclusion provided blood samples and were given yeast tablets for an open-label 4-week placebo run-in phase. Those ($n = 491$) who met the inclusion criteria, displayed good adherence in the placebo run-in phase, and gave written, informed consent, were randomised to 0 (placebo-yeast), 100, 200, or 300 $\mu\text{g/d}$ of Se as Se-enriched yeast (SelenoPrecise[®] Pharma Nord, Vejle, Denmark) (Fig. 1). Participant evaluation was carried out at Odense University Hospital at baseline, 6, 12, 18 months, 2, 3 and 5 years, as previously described in detail [14]. The intervention was delivered for 5 years and participants were followed up, post-treatment, for an additional 10 years for mortality ascertainment.

2.7. Baseline characteristics

Demographic characteristics, smoking status, height, weight, and supplement use were collected at baseline. Medications used were obtained from medical records. Morbidity data were obtained from the Danish National Patient Registry which has records of major and secondary diagnoses for all in-patient discharges since 1977 and all emergency and outpatient contacts since 1995 [20]. The Charlson comorbidity index was computed by adding 19 comorbid conditions diagnosed prior to randomization [21].

2.8. Selenium measurement

As previously described in detail [14], total Se was measured gravimetrically (ng/g) in lithium-heparin plasma at LGC Limited, Teddington, United Kingdom, by inductively coupled-plasma mass spectrometry at baseline, 6-months and 5-years. Analysis of a matrix-certified reference material indicated good accuracy of the method. The intra-assay coefficients of variation (CVs) ranged from 0.5% for samples of high-Se concentration to 3% for samples of low-Se concentration. The inter-assay CV was 3.4%.

2.9. Outcomes: mortality ascertainment

Study participants were followed up for mortality from the date of

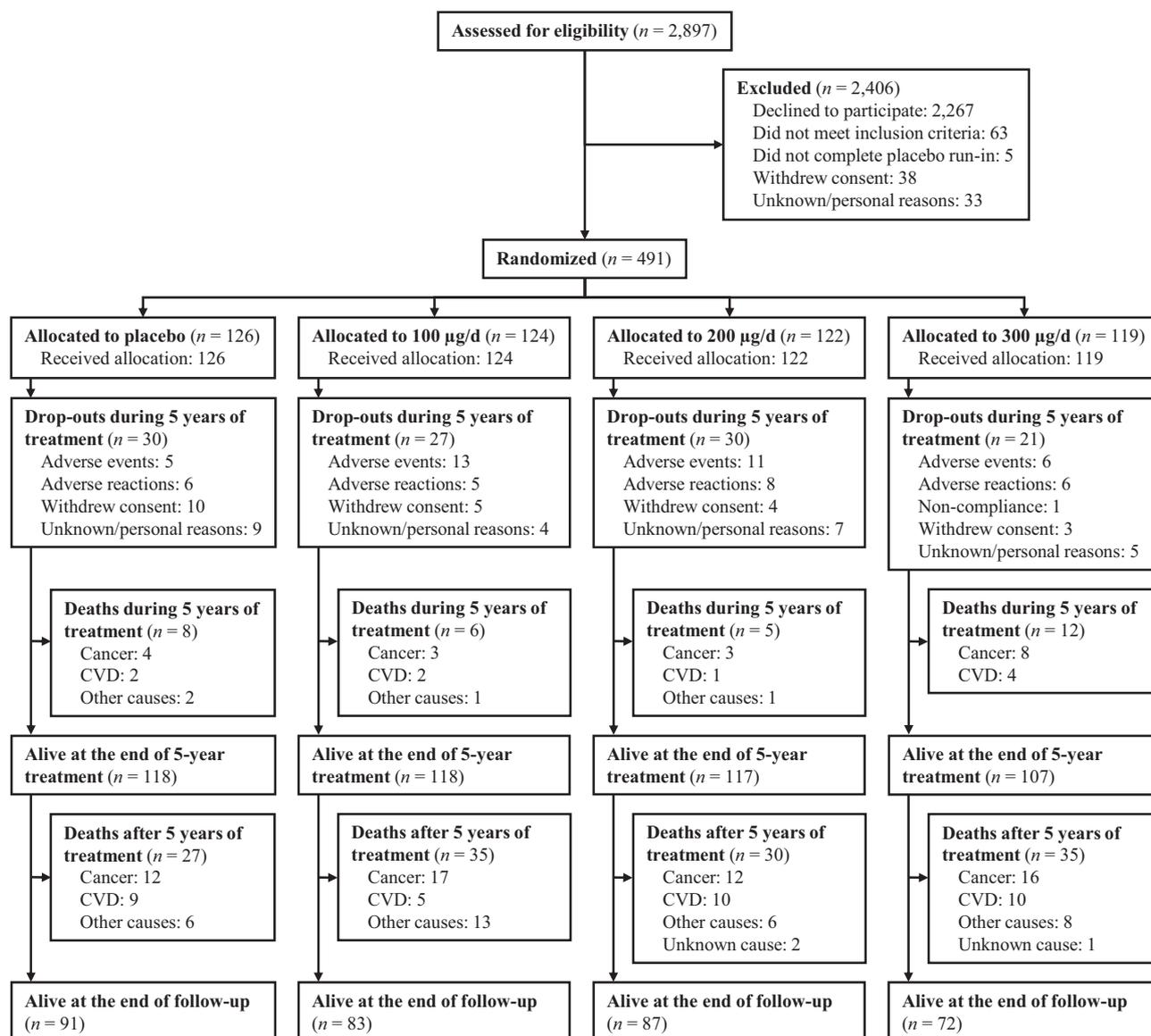


Fig. 1. Study flow diagram. CVD denotes cardiovascular disease.

randomization in 1998–1999 through March 31, 2015. Vital status and date of death were obtained from the Danish Civil Registration System which has recorded all deaths in residents in Denmark since 1968 [22]. Information on the underlying cause of death was obtained from the Danish Registry of Causes of Death [23] through December 31, 2010 and from participant medical charts from January 1, 2011. Cause of death was classified according to the 10th Revision of the International Classification of Diseases as death due to cancer (codes C00–C97), CVD (I00–I99), and all other causes. All participants not found to be deceased were confirmed to be alive and resident in Denmark by March 31, 2015 in the Danish Civil Registration System.

2.10. Power calculations for intention-to-treat mortality analysis

Although no formal sample size determinations were performed *a priori* in the original study design, we carried out *ad-hoc* power calculations for the present intention-to-treat mortality analysis. For the average sample size of 123 participants in each randomised treatment group, the observed cause-specific mortality risks over follow-up in the placebo group (Fig. 2), and an unadjusted two-sided significance level of 0.05, the power to detect underlying increases in risk of 50% and 100% after 5 years of intervention comparing any active treatment

group with placebo was 13.9% and 35.7% for all-cause mortality, 8.7% and 17.6% for cancer mortality, and 8.1% and 15.5% for CVD mortality. After the entire 16-year follow-up period, the power to detect the same underlying risk rose to 62.7% and 99.5% for all-cause mortality, 29.7% and 76.3% for cancer mortality, and 21.0% and 57.1% for CVD mortality. Thus, the study was acceptably powered to detect a 50% increased risk for all-cause mortality and a two-fold increased risk for cancer and CVD mortality over the entire follow-up, but underpowered to detect such risk increases during the initial 5 years of intervention.

2.11. Statistical analysis

All trial participants were assigned to their randomised treatment group irrespective of compliance (intention-to-treat analysis). Cumulative mortality was estimated using Kaplan-Meier methods and compared with the generalized Wilcoxon test. Hazard ratios for mortality and 95% confidence intervals (CIs) comparing the three active treatment groups with placebo were estimated using Cox proportional hazards models. We obtained smooth estimates of cumulative mortality curves by fitting spline-based parametric survival models [24]. These models were used to estimate mortality risks and 95% CIs at 5, 10, and 15 years of follow-up for each treatment group, as well as to calculate

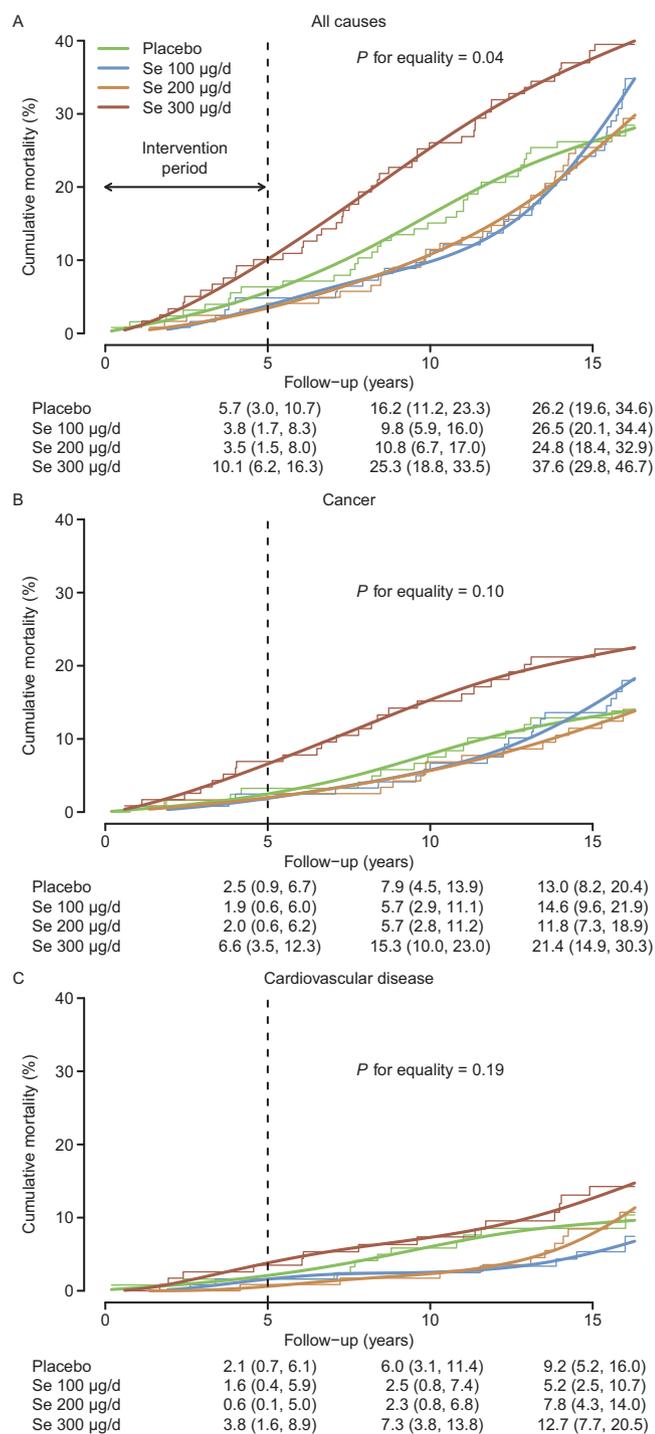


Fig. 2. Cumulative mortality from all causes, cancer, and cardiovascular disease over time by treatment group. Non-parametric cumulative mortality curves (step functions) were estimated from Kaplan-Meier methods and compared with the generalized Wilcoxon test. Parametric cumulative mortality curves (smooth lines) were estimated from spline-based parametric survival models with treatment-specific log cumulative hazards parameterized as natural cubic splines of log time with knots at the 33th and 67th percentiles of the uncensored log-time distribution. Cumulative mortality estimates (95% CIs) at 5, 10, and 15 years of follow-up by treatment group were obtained from spline-based parametric survival models. Se denotes selenium.

cumulative risk differences and cumulative hazard ratios over time, comparing the three active treatment groups with placebo.

We evaluated treatment-effect modification across baseline subgroups specified by age (< 65, ≥ 65 years), sex, smoking status (non-current, current), body-mass index (< 25, ≥ 25 kg/m²), Charlson

comorbidity index (0, 1–2), number of medications (0, 1–3) and plasma selenium concentration (< 80, ≥ 80 ng/g), including main terms and interactions between treatment group and the corresponding covariates in Cox proportional hazards models. We tested for interaction by using joint Wald tests for interaction coefficients. The significance level was set at 0.05; all reported *P* values were two-sided and not adjusted for multiple testing since only three tests were performed in the main analyses to compare simultaneously cause-specific mortality curves for the four treatment groups throughout the entire follow-up period, one test for all-cause mortality and two additional tests for cancer and CVD mortality as major contributing causes. Statistical analyses were performed in Stata, version 14 (Stata Corp) and graphics were produced in R, version 3 (R Foundation for Statistical Computing).

The study was overseen by a safety and monitoring committee with representatives from all organizations involved.

3. Results

The mean (SD) age and plasma selenium concentration of the 491 randomised participants at baseline were 66.1 (4.1) years and 86.5 (16.3) ng/g, respectively (measurements were made gravimetrically, but ng/g can be converted to µg/L by multiplying by 1.027). Only 49 participants (10.0%) presented with comorbid conditions at baseline and 56 (11.4%) were on more than one medication. There were no meaningful imbalances at baseline between treatment groups in plasma selenium concentration or other participant characteristics (Table 1).

A total of 108 participants dropped out before completing the 5-year treatment period due to non-fatal adverse events, adverse reactions to treatment, non-compliance, withdrawal of consent, or unknown/personal reasons (Fig. 1). These 108 non-fatal drop-outs, including 35 with non-fatal adverse events, were equally distributed across treatment groups (*P* = 0.57) [14] and have been captured in the mortality analysis, as mortality registration in Denmark is virtually complete.

After 5 years of supplementation, mean (SD) plasma selenium had risen substantially to 158.3 (28.3), 222.2 (40.6), and 276.5 (78.7) ng/g in the 100, 200, and 300 µg/d treatment groups, respectively, but was unchanged at 87.7 (24.2) ng/g in the placebo group (*P* < 0.001 for homogeneity of changes from baseline to 5 years across the four treatment groups) [14].

The average (range) follow-up among survivors was 15.9 (15.5–16.3) years. During 6871 person-years of follow-up, there were 158 deaths, 31 (18 from cancer, 9 from CVD) during the 5 years of active treatment and 127 (57 from cancer, 34 from CVD) in the 10 years after treatment cessation (Fig. 1).

All-cause cumulative mortality curves differed significantly across treatment groups (*P* = 0.04 for homogeneity of non-parametric curves) (Fig. 2A). Participants randomised to 300 µg selenium/d showed a moderate but non-significant increase in mortality after 5 years of treatment (hazard ratio vs. placebo, 1.62, 95% CI 0.66, 3.96) that was sustained and became significant at the end of the entire follow-up period (hazard ratio 1.59, 95% CI 1.02, 2.46) (Table 2; Supplemental Fig. 1A). The 15-year mortality risk-difference comparing the 300 µg selenium/d group with placebo was 11.3% (95% CI 0.0, 22.6%) (Supplemental Fig. 2A). The 100 and 200 µg/d doses showed non-significant decreases in all-cause mortality during the 5-year intervention, but their effects disappeared progressively after treatment cessation (Table 2; Supplemental Fig. 1A). Adjustment for participant baseline characteristics did not materially change these findings.

The effects of selenium supplementation on cancer mortality (Table 2; Fig. 2B; Supplemental Fig. 2B) and CVD mortality (Table 2; Fig. 2C; Supplemental Fig. 2C) were similar to those on all-cause mortality, though differences in cause-specific mortality among treatment groups were not significant (*P* = 0.10 and 0.19 for homogeneity of cancer and CVD mortality curves, respectively). The hazard ratios (95% CIs) for cancer mortality for 300 µg selenium/d vs. placebo were 2.17 (0.65, 7.21) over the 5-year intervention and 1.78 (0.94, 3.34)

Table 1
Baseline characteristics of trial participants overall and by treatment group.

Characteristic	Overall	Placebo	Selenium dose, µg/d		
			100	200	300
No. of participants	491	126	124	122	119
Age, mean (SD), y	66.1 (4.1)	65.4 (3.8)	66.4 (4.1)	66.3 (4.4)	66.5 (4.1)
Sex, Number (%)					
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, Number (%)					
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)
Body mass index, mean (SD), kg/m ²	26.8 (4.1)	26.5 (4.0)	27.1 (4.0)	27.2 (4.3)	26.5 (4.0)
Comorbid conditions, Number (%)					
Cardiovascular disease ^a	15 (3.1)	2 (1.6)	4 (3.2)	2 (1.6)	7 (5.9)
Chronic pulmonary disease	7 (1.4)	1 (0.8)	2 (1.6)	4 (3.3)	0 (0.0)
Connective tissue disease	6 (1.2)	2 (1.6)	1 (0.8)	2 (1.6)	1 (0.8)
Ulcer disease	14 (2.9)	6 (4.8)	4 (3.2)	0 (0.0)	4 (3.4)
Diabetes mellitus	6 (1.2)	0 (0.0)	4 (3.2)	2 (1.6)	0 (0.0)
Charlson comorbidity index, Number (%)					
0	442 (90.0)	115 (91.3)	108 (87.1)	111 (91.0)	108 (90.8)
1–2	49 (10.0)	11 (8.7)	16 (12.9)	11 (9.0)	11 (9.2)
Prescribed medications, Number (%)					
Lipid-lowering medication	30 (6.1)	8 (6.3)	12 (9.7)	6 (4.9)	4 (3.4)
Diabetes medication	6 (1.2)	1 (0.8)	3 (2.4)	1 (0.8)	1 (0.8)
Antihypertensive medication	124 (25.4)	31 (24.8)	30 (24.2)	27 (22.5)	36 (30.3)
Thyroid medication	14 (2.9)	2 (1.6)	4 (3.2)	5 (4.1)	3 (2.5)
Glucocorticoids	24 (4.9)	4 (3.2)	9 (7.3)	4 (3.3)	7 (5.9)
Osteoporosis medication	81 (16.6)	26 (20.8)	19 (15.3)	16 (13.3)	20 (16.8)
No. of medications, Number (%)					
0	274 (55.8)	72 (57.1)	67 (54.0)	73 (59.8)	62 (52.1)
1	161 (32.8)	37 (29.4)	39 (31.5)	40 (32.8)	45 (37.8)
2–3	56 (11.4)	17 (13.5)	18 (14.5)	9 (7.4)	12 (10.1)
Plasma selenium, mean (SD), ng/g	86.5 (16.3)	86.0 (15.2)	87.5 (16.4)	88.3 (16.2)	83.9 (17.1)

Conversion factor: to convert ng/g values (as measured) for plasma selenium to micrograms per liter, multiply by 1.027.

^a Myocardial infarction, congestive heart failure, peripheral vascular disease, or cerebrovascular disease.

over the entire follow-up, while those for CVD mortality were 2.17 (0.40, 11.85) and 1.51 (0.69, 3.33), respectively.

In subgroup analyses, the increased risk of death with 300 µg/d of selenium supplementation was particularly large in participants, both men and women < 65 years at randomization, but not in older participants ($P = 0.04$ for treatment-by-age interaction; Fig. 3; Supplemental Table 1). The hazard ratio (95% CI) for all-cause mortality over the entire follow-up for 300 µg selenium/d vs. placebo was 3.12 (1.51, 6.44) in participants < 65 years and 0.93 (0.53, 1.63) in those ≥ 65 years. No other interactions were statistically significant across subgroups defined by sex, smoking status, body mass index, Charlson comorbidity index, number of medications, or baseline plasma selenium.

4. Discussion

A dose of 300 µg/d of selenium (as high-selenium yeast) vs. placebo-yeast taken for 5 years resulted in a significant absolute excess risk of all-cause mortality of 11.3% (95% CI 0.0, 22.6%) 10 years later in a country of moderately-low selenium status. Cancer and CVD mortality similarly appeared to increase, but the effects were not statistically significant. Though the 100 and 200 µg/d doses showed a non-significant reduction in mortality during the 5-year treatment period, the effect had disappeared 10 years post-treatment.

Since excess mortality was observed only with the 300 µg/d dose, it is unlikely that high concentrations or activity of selenoproteins are responsible for this adverse effect. Selenoproteins are probably replete at a plasma selenium concentration of ~ 125 µg/L, at which point the concentration of selenoprotein P reaches a plateau [3]; this concentration was exceeded after 6 months of treatment, having reached 152 ng/g (equivalent to 156 µg/L) even at the lowest dose [14]. The

higher plasma-selenium concentrations achieved in our study, particularly in the 200 and 300 µg/d treatment groups, thus reflect predominantly non-specific incorporation of selenomethionine into albumin in place of methionine [3]. We previously obtained interesting results in a speciation study in a small number of PRECISE participants that measured the amounts of selenium present in plasma as selenoproteins, high-molecular-weight selenium and low-molecular-weight selenium after 6 months and 5 years of treatment (Deitrich, Rayman, Moesgard, Goenaga-Infante, unpublished results). While the amount of selenium in albumin increased markedly from 6 months to 5 years, the amount present as selenoproteins fell significantly at the two highest dose levels. This implies that long-term treatment with selenium at the 200 or 300 µg/d dose can lead to selenoprotein depletion, potentially reducing health protection. As supra-nutritional dietary selenium also depressed selenoprotein expression in animal models [25,26], part of the harmful effect of high-dose selenium supplementation may be due to a paradoxical reduction in selenoprotein activity.

The major single component of the selenium-yeast used in our trial was selenomethionine [27]. By analogy with methionine, selenomethionine can be metabolized *via* the methionine cycle and the transsulphuration pathway to the selenols, selenohomocysteine and selenocysteine [28,29], and can be cleaved to methyl selenol by methioninase, an enzyme present in many animal and human tissues [30]. Selenols react with thiols to produce selenyl sulphides/disulphides that can cause undesirable structural and functional changes, including protein aggregation, transcription-factor inactivation, and disruption of redox-regulated cell signalling [31,32]. Selenols, present as selenolates (RSe⁻) at physiological pH, have the capacity to redox-cycle and generate superoxide radicals thus inducing oxidative stress [28,30] and potentially increasing the risk of cancer and CVD [33].

Another potential explanation for the harmful effects of supra-

Table 2
Hazard ratios for all-cause, cancer, and cardiovascular disease mortality after the initial 5-year intervention period and the entire follow-up by Treatment group.

Outcome	Placebo	Selenium dose, µg/d		
		100	200	300
All-cause mortality				
5-year intervention period				
No. of deaths/person-years	8/610.1	6/609.4	5/599.8	12/567.6
Mortality rate ^a (95% CI)	13.1 (6.6, 26.2)	9.8 (4.4, 21.9)	8.3 (3.5, 20.0)	21.1 (12.0, 37.2)
Hazard ratio ^b (95% CI)	1.00 (Reference)	0.75 (0.26, 2.16)	0.64 (0.21, 1.94)	1.62 (0.66, 3.96)
Entire follow-up				
No. of deaths/person-years	35/1766.0	41/1796.8	35/1768.6	47/1539.5
Mortality rate ^a (95% CI)	19.8 (14.2, 27.6)	22.8 (16.8, 31.0)	19.8 (14.2, 27.6)	30.5 (22.9, 40.6)
Hazard ratio ^b (95% CI)	1.00 (Reference)	1.15 (0.73, 1.80)	0.99 (0.62, 1.59)	1.59 (1.02, 2.46)
Cancer mortality				
5-year intervention period				
No. of deaths/person-years	4/610.1	3/609.4	3/589.8	8/562.6
Mortality rate ^a (95% CI)	6.6 (2.5, 17.5)	4.9 (1.6, 15.3)	5.1 (1.6, 15.8)	14.2 (7.1, 28.4)
Hazard ratio ^b (95% CI)	1.00 (Reference)	0.75 (0.17, 3.36)	0.77 (0.17, 3.46)	2.17 (0.65, 7.21)
Entire follow-up				
No. of deaths/person-years	16/1766.0	20/1796.8	15/1748.5	24/1524.6
Mortality rate ^a (95% CI)	9.1 (5.6, 14.8)	11.1 (7.2, 17.3)	8.6 (5.2, 14.2)	15.7 (10.6, 23.5)
Hazard ratio ^b (95% CI)	1.00 (Reference)	1.22 (0.63, 2.36)	0.94 (0.46, 1.90)	1.78 (0.94, 3.34)
Cardiovascular disease mortality				
5-year intervention period				
No. of deaths/person-years	2/610.1	2/609.4	1/589.8	4/562.6
Mortality rate ^a (95% CI)	3.3 (0.8, 13.1)	3.3 (0.8, 13.1)	1.7 (0.2, 12.0)	7.1 (2.7, 18.9)
Hazard ratio ^b (95% CI)	1.00 (Reference)	1.00 (0.14, 7.11)	0.52 (0.05, 5.72)	2.17 (0.40, 11.85)
Entire follow-up				
No. of deaths/person-years	11/1766.0	7/1796.8	11/1748.5	14/1524.6
Mortality rate ^a (95% CI)	6.2 (3.4, 11.2)	3.9 (1.9, 8.2)	6.3 (3.5, 11.4)	9.2 (5.4, 15.5)
Hazard ratio ^b (95% CI)	1.00 (Reference)	0.62 (0.24, 1.61)	1.00 (0.44, 2.31)	1.51 (0.69, 3.33)

^a Mortality rates and 95% CIs per 1000 person-years.

^b Hazard ratios and 95% CIs for the three active treatment groups compared to placebo were obtained from Cox proportional hazards models after the initial 5-year intervention period and the entire follow-up.

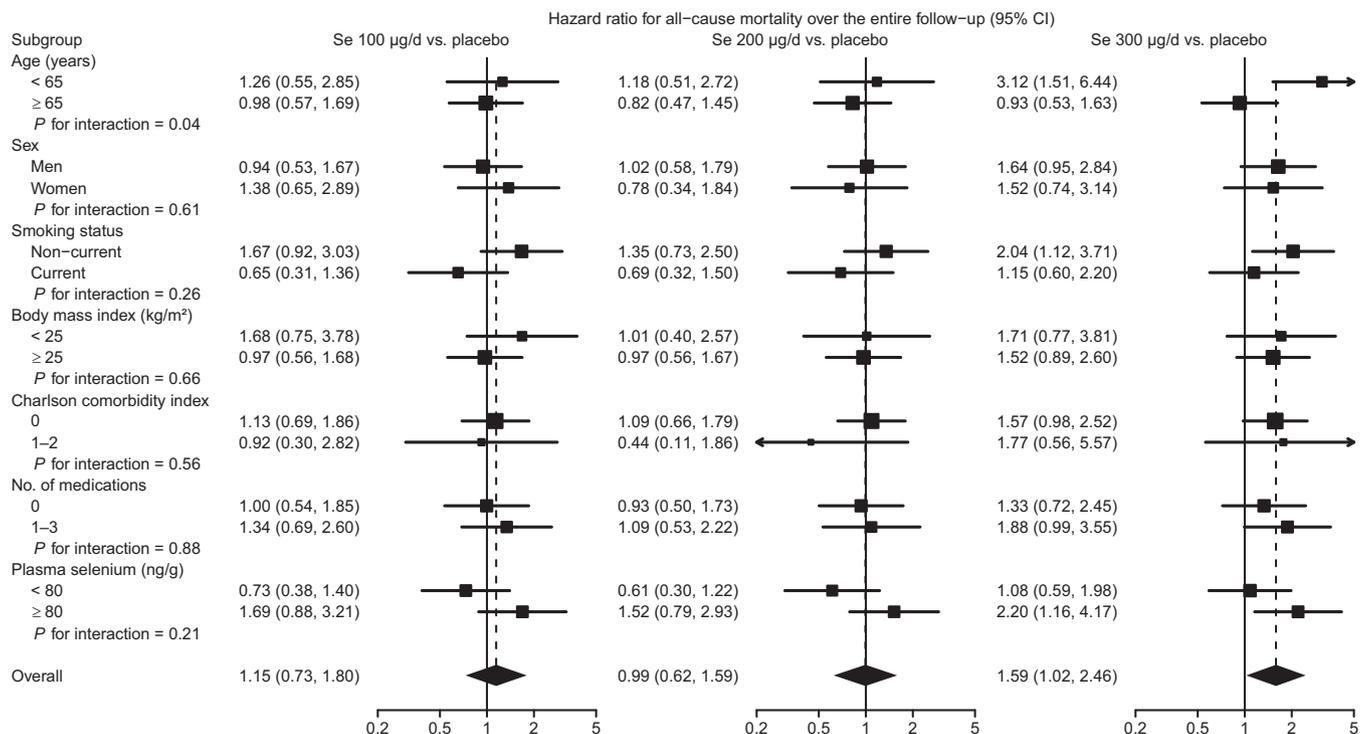


Fig. 3. Hazard ratios for all-cause mortality over the entire follow-up period comparing the three active treatment groups with placebo by baseline subgroup. Subgroup-specific hazard ratios (squares with area inversely proportional to the variance) and their 95% CIs (horizontal lines) were obtained from Cox proportional hazards models with interaction terms between treatment groups and the corresponding subgroup indicator. Se denotes selenium.

nutritional selenium is its effect on protein folding in the endoplasmic reticulum (ER). Selenolates are highly redox active and can effect thiol-disulphide interchange in proteins resulting in protein misfolding or unfolding [31,34], causing ER stress and triggering the unfolded-protein response (UPR), a complex signalling network that attempts to restore homeostasis [35]. Indeed, treatment of cultured endothelial cells with 5 μM (400 $\mu\text{g/L}$) selenium (as selenite), a concentration of the same order of magnitude as the plasma level achieved in the 300 $\mu\text{g/d}$ group (284 $\mu\text{g/L}$), enhanced mRNA expression of several ER-stress markers, reduced nitric-oxide production, induced superoxide generation and impaired angiogenesis [36]. However, the relevance of this comparison is doubtful as the selenium-treatment species were different. As both ER stress and UPR activation are implicated in many human cancer types and UPR activation has recently been shown to be a vital step in cancer development [37], the induction of ER stress and UPR activation might explain the significant excess mortality in the 300 $\mu\text{g/d}$ group. Furthermore, selenium compounds can induce DNA damage and, owing to their reactivity with thiols [31], can interfere with the integrity or function of DNA repair proteins [38], potentially increasing cancer risk.

Kinetic studies have shown that high-dose selenomethionine toxicity persists for ≥ 5 years after supplementation is stopped, as selenomethionine is extensively recycled with a whole-body turn-over time of 363 days [39]. That extended length of exposure resulting from re-utilisation of selenomethionine-selenium may explain the increased mortality 10 years after treatment cessation. Supplementation with sodium selenite, which is either immediately used for selenoprotein synthesis or excreted [40], might not have increased mortality.

In our study, excess mortality with the 300 $\mu\text{g/d}$ selenium dose was concentrated in younger participants. While this may be a chance finding owing to multiple subgroup analyses, there is a possible methodological explanation, and several mechanistic explanations. The methodological explanation is that when a population already elderly at baseline is followed up for a long time, we can expect that mortality curves for the sub-groups will converge at the end of the follow-up. Thus HRs after 15 years will provide downwardly biased estimates of the underlying treatment effects; this ceiling effect would be more marked in older participants. With regard to mechanistic explanations, it is possible that age-related differences that affect methionine/selenomethionine metabolism [29,41], could account for these findings, e.g., changes in gut microbiota [42] that can metabolise selenomethionine [41] or in functional vitamin-B₁₂ status affecting 1-carbon metabolism [43] that is more likely to be identified and addressed in older participants. A further possibility, relates to the known loss of muscle with increasing age (sarcopenia). Whole-body turnover of selenium is determined largely by peripheral tissues including skeletal muscle which contribute 60% to total body selenium content [39]. As muscle mass declines with age [44,45], older participants may be less able to accumulate selenomethionine in muscle tissue, reducing the possibility of its reutilisation, hence lessening exposure. Alternatively, there may simply be stronger competing risk factors in the older age-group.

Our study has limitations: mortality was not the primary endpoint of the Denmark PRECISE Trial but was adopted when further funding enabled the intervention to continue for 5 years, hence the need for cautious interpretation of our findings, as they result from post-hoc analyses; it was conducted in apparently healthy elderly, so the results may not extend to other age groups or participants with different comorbidities; 108 live participants (22.0%) dropped out before completing the 5-year intervention period, though they were equally distributed between treatment groups and captured in the intention-to-treat mortality analysis; there was only a small difference in the number of deaths (35 in the placebo group vs. 47 in the 300 μg group). An additional limitation is the lack of any measurement of selenium status in the final 10 years of follow-up after cessation of supplementation. Importantly, we cannot rule out the role of chance in our findings as the

sample size was small and the study was not originally designed to assess long-term mortality. Nonetheless, as the only long-term, randomised, controlled, trial of selenium as a single agent at different dose levels in a non-selenium-replete population, with complete ascertainment of mortality [22,23], the results make a contribution to the overall evidence base.

The effects of selenium supplementation depend on dose (including dietary intake), selenium species, and duration of treatment. Our results are compatible with a U-shaped dose-response relationship between selenium intake or status and health outcomes, previously shown in observational studies [1]. At low selenium intake, additional selenium may confer protection by increasing the concentration or activity of antioxidant selenoproteins. However, when selenium intake is already adequate, additional selenium can become toxic, at least partly through the generation of superoxide, as described above [29,31,32,36]. A plasma selenium concentration of ~ 125 $\mu\text{g/L}$ is probably sufficient to replete the selenoproteins [3], and a concentration of 228 $\mu\text{g/L}$, the level attained in our study after dosing with 200 $\mu\text{g/d}$ for 5 years, appeared to be without harm and may even have afforded some benefit. A dose of 200 $\mu\text{g/d}$ of selenium, as selenomethionine, in SELECT, where participants had a higher background selenium intake (baseline serum/plasma selenium 136 vs. 91 $\mu\text{g/L}$), resulted in a serum-selenium concentration of 252 $\mu\text{g/L}$ at 4 years and recognised symptoms of selenium toxicity after 5.5 years, i.e., RR (95% CI), alopecia 1.28 (1.01, 1.62) and dermatitis 1.17 (1.0, 1.35), though there was no increase in mortality [12,13]. In our study, a dose of 300 $\mu\text{g/d}$ of selenium, as selenium-yeast (54–60% selenomethionine) [14] raised plasma selenium to the higher value of 284 $\mu\text{g/L}$ at 5 years and significantly increased mortality some 10 years later. These results suggest that a total intake (diet plus selenomethionine-containing supplement) exceeding 300 $\mu\text{g/d}$ for 5 years or more should be avoided.

Our findings may have particular implications for supplement takers who may more readily exceed an intake of 300 $\mu\text{g/d}$. People with deficient or marginally-deficient selenium status may benefit from additional selenium intake [1], depending on their baseline level and dose. While supplementation with 200 μg selenium/d may be safe in Danes or those with similar baseline selenium status, such as many Europeans, the same cannot be assumed in countries with higher baseline status, such as North America. In NHANES 2003–4 [46], a representative survey of the US population, mean serum-selenium concentration was 137 $\mu\text{g/L}$, indicating selenoprotein repletion [4]. Under these circumstances, an additional 200 μg selenium/d in a US adult may result in selenium toxicity [12] and a potentially greater risk of mortality if that intake is continued beyond 5.5 years. It would be interesting to examine mortality in SELECT men in 2018, by which time they will have had 10 years of follow-up, post-treatment, as in Denmark PRECISE. In the meantime, despite their limitations, our findings suggest that the public should avoid high-dose selenium supplements, particularly in countries with an adequate background selenium intake.

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Conflict of interest (COI) statement

None of the authors has any potential conflict of interests.

Author contributions

SC and MPR designed research; KHW, MT and FC and SC conducted research and analysed data; RPB performed statistical analysis; MPR, RP-B, EG wrote the paper with help from KHW and SS; MPR had primary responsibility for final content. All authors read and approved the

final manuscript.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.freeradbiomed.2018.02.015>.

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