

Does selenium supplementation affect thyroid function? Results from a randomized, controlled, double-blinded trial in a Danish population

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Abstract

Objective: Selenium is present in the active site of proteins important for thyroid hormone synthesis and metabolism. The objective of this study is to investigate the effect of selenium supplementation in different doses on thyroid function, under conditions of suboptimal dietary selenium intake.

Design: The Danish PREvention of Cancer by Intervention with SEelenium pilot study (DK-PRECISE) is a randomized, double-blinded, placebo-controlled trial. A total of 491 males and females aged 60–74 years were randomized to 100 µg ($n=124$), 200 µg ($n=122$), or 300 µg ($n=119$) selenium-enriched yeast or matching yeast-based placebo tablets ($n=126$). A total of 361 participants, equally distributed across treatment groups, completed the 5-year intervention period.

Methods: Plasma samples were analyzed for selenium and serum samples for TSH, free triiodothyronine (FT₃), and free thyroxine (FT₄) at baseline, and after 6 months, and 5 years of supplementation.

Results: Plasma selenium concentrations increased significantly and dose-dependently in treatment groups receiving selenium ($P<0.001$). Serum TSH and FT₄ concentrations decreased significantly and dose-dependently by 0.066 mIU/l ($P=0.010$) and 0.11 pmol/l ($P=0.015$), respectively, per 100 µg/day increase, with insignificant differences between 6 months and 5 years. No significant effects were found for FT₃ and FT₃:FT₄ ratio.

Conclusions: In euthyroid subjects, selenium supplementation minutely and dose-dependently affects thyroid function, when compared with placebo, by decreasing serum TSH and FT₄ concentrations. Based on these findings, selenium supplementation is not warranted under conditions of marginal selenium deficiency. However, a role for selenium supplementation in the treatment of autoimmune thyroid diseases is still unresolved.

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Introduction

Type 1 and 2 iodothyronine deiodinases (DIO1, DIO2) catalyze the conversion of thyroxine (T₄) to triiodothyronine (T₃), which is the primary mediator of the developmental and metabolic effects of the vertebrate thyroid (1, 2, 3). In addition, the inactivating DIO3 is involved in degradation of the prohormone T₄ and the active hormone T₃. Around 25 years ago, the cloning of DIO1 implicated selenium as the nucleophilic atom in its active site (4). Selenium is a trace element essential to

human health, inserted as selenocysteine residues in the vast majority of 50–70 selenoprotein variants encoded by 25 human genes (5). Several selenoproteins are expressed in thyrocytes, where the most important ones are DIO1, DIO2, and type 3 glutathione peroxidase (GPx3) protecting thyrocytes from excess H₂O₂ generated during thyroid hormone synthesis (6).

In studies contemporary to the efforts toward purifying DIO1, both animal models and human trials suggested

impaired DIO1 activity with low selenium intake (7, 8). In healthy African schoolchildren with combined severe iodine and selenium deficiency, selenium supplementation vs placebo led to decreases in serum thyrotropin (TSH) and reverse T₃ (RT₃) concentrations, and the authors concluded that selenium intake plays a definite role in thyroid hormone metabolism in humans (8). Dietary selenium intake varies considerably between populations (9). In Denmark, selenium status is considered to be borderline low to adequate, with serum selenium concentration of 98.7 ± 19.8 µg/l in a recently investigated adult population (10). Interestingly, serum selenium concentration was inversely associated with thyroid volume and goiter in the same population (11). Similar observations in a study among French women (12) support that the thyroid gland also responds to selenium intake at marginal deficiency, where the activity of at least a subset of selenoproteins is not maximized (13). Subsequent intervention studies of the effects of selenium supplementation on thyroid function have yielded equivocal results. In a randomized controlled trial, enrolling 36 elderly Italians, supplementation with 100 µg/day sodium selenite for 3 months decreased T₄ concentrations (14) when compared with placebo, and in a US intervention study among 28 healthy adults, a small but significant increase in T₃ concentrations was seen in men during supplementation with 200 µg/day selenomethionine for 28 months (15). In contrast, no effects on thyroid hormone concentrations or conversion were found in two larger randomized controlled trials in euthyroid populations and using another selenium compound (16, 17). In the UK, 368 elderly individuals received 100, 200, or 300 µg/day selenium-enriched yeast or placebo-yeast for 6 months (16), and in New Zealand, 172 healthy adults received 200 µg/day selenium-enriched yeast or placebo-yeast for 21 weeks (17). Prioritized expression of DIOs under different degrees of selenium-deficiency may account for the lack of clear-cut effects in healthy individuals (18).

This randomized long-term follow-up study offers an opportunity to add to the ambiguous and limited human trial data on the physiological importance of selenium intake, in a range of doses, for thyroid function in a population with marginally low selenium status.

Subjects and methods

Study design

The PREvention of Cancer by Intervention with Selenium (DK PRECISE) pilot trial (ClinicalTrials.gov ID:

NCT01819649) was a single-center, nonstratified, randomized, double-blinded, placebo-controlled, multi-arm parallel clinical trial with four groups (allocation ratio 1:1:1:1). The sample size was set at 500 participants to draw reasonable conclusions about recruitment, adherence, and loss to follow-up, while keeping the costs within reasonable bounds, in the preparations of a large-scale international PRECISE trial. Due to these circumstances, no formal power calculations were performed.

Participants

Participants were males and females 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, 2897 potential participants were invited. Among them, 630 accepted the invitation for a visit to Odense University Hospital where they were screened for inclusion. Exclusion criteria were: i) a Southwest Oncology Group performance status score >1; ii) active liver or kidney disease (alanine-aminotransferase, alkaline phosphatase, bilirubin, or urea two s.d. above the upper reference range limit); iii) previous diagnosis of cancer (excluding non-melanoma skin cancer); iv) diagnosed HIV infection; v) receiving immunosuppressive therapy; vi) unable to understand written or spoken information; and vii) receiving ≥ 50 µg/day of selenium supplements in the previous 6 months (self reported).

The 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, participant adherence, and satisfaction during the run-in phase. Good adherence was defined as taking more than 80% of the run-in phase tablets assessed by tablet count.

The 491 subjects who met the inclusion criteria displayed good adherence in the run-in phase, gave written informed consent, and were enrolled and randomized to 0, 100, 200, or 300 µg of selenium daily (Fig. 1). The regional Data Protection Agency and Scientific Ethical Committees of Vejle and Funen counties approved the study (journal no. 19980186).

Randomization and interventions

Randomization was computer-generated, blocked, and nonstratified, and was performed at the Division of

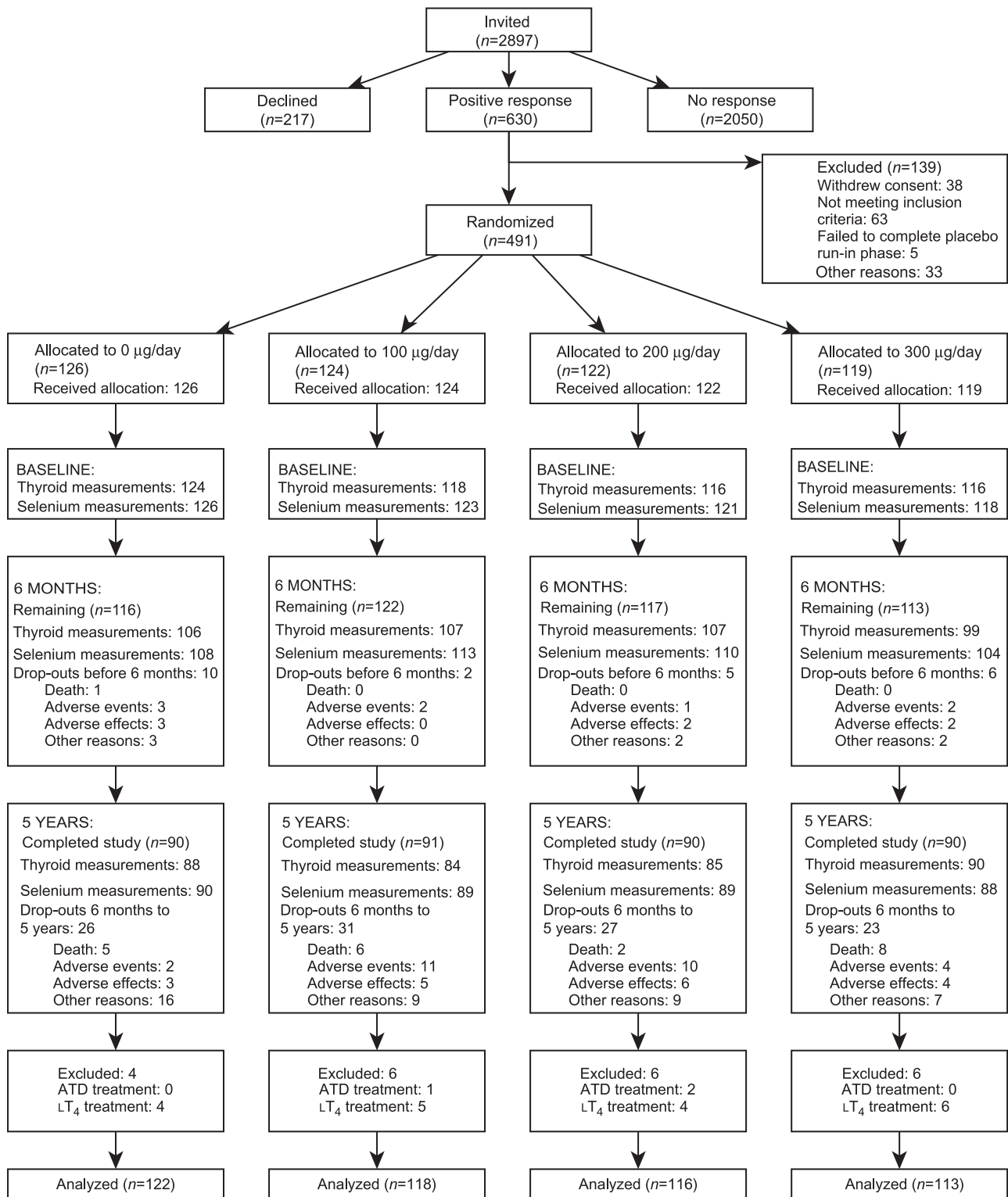


Figure 1

Study flow diagram.

Epidemiology and Biostatistics, University of Arizona, Arizona Cancer Center. A badge number system secured blinding and correct distribution of selenium doses. The responsibility of distributing tablets was placed with pharmacists at Odense University Hospital.

The intervention agent was the selenium-enriched yeast SelenoPrecise and tablets were formulated and packaged by Pharma Nord ApS, Vejle, Denmark. The placebo agent was an inactive spray-dried baker's yeast, comprising 250 mg yeast placebo, 80 mg cellulose, 65 mg dicalcium phosphate, and ≤ 5 mg other inactive ingredients, identical in appearance to the selenium 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 28 tablets, 7 \times 4. The participants, research staff, and investigators were blinded to treatment.

Sample and data collection

The participants were evaluated at Odense University Hospital. Blood was drawn at baseline, 6, 12, 18, 24, and 60 months. Participants were at nonfasting. Heparinized plasma and serum were prepared and stored at -80°C . Reasons for participant withdrawal were recorded.

Biochemical analyses

Total selenium concentrations at baseline and at the 6-month and 5-year visits were measured in lithium-heparin plasma at LGC Limited, Teddington, UK, between July 2012 and March 2013, using inductively coupled-plasma mass spectrometry with external calibration. A matrix certified reference material, BCR-637 Human Serum, with a certified selenium concentration of 81 ± 7 $\mu\text{g/l}$ selenium (density corrected 79.1 ng/g), was used for quality control of the total selenium measurements. The selenium concentration found for BCR-637 was 78.3 ± 2.7 $\mu\text{g/l}$ Se (16 independent replicates) indicating good accuracy of the method. The intra-assay coefficients of variation (CV values) ranged from 0.5% for samples of high to 3% for samples of low selenium concentration. The inter-assay CV was 3.4%.

Serum TSH, free T_3 (FT₃), free T_4 (FT₄), and thyroid peroxidase antibodies (TPO-Ab) concentrations at baseline and at the 6-month and 5-year visits were measured at the Department of Clinical Biochemistry, Odense University Hospital, Denmark, between September 2013 and March 2014. All measurements were serial to avoid interassay variation. TSH concentrations were measured

with a chemiluminescence microparticle immunoassay. The analysis was performed on an Architect i2000 (Abbott, Wiesbaden, Germany) with a documented limit of detection (LOD) ≤ 0.01 mIU/l. The intra-assay variation coefficient was $<2\%$ and the reference range was 0.3–4.0 mIU/l. FT₃, FT₄, and TPO-Ab concentrations were analyzed using time-resolved fluoroimmunoassays. The analyses were performed on AutoDELFIA equipment (Wallac, Turku, Finland) and LOD was 1.5 pmol/l (FT₃), 2 pmol/l (FT₄), and 1 mIU/l (TPO-Ab). The intra-assay variation coefficients were $<5\%$ (FT₃), $<2\%$ (FT₄), and 3.4% (TPO-Ab), and the reference ranges were 4.3–7.4 (FT₃), 9.9–17.7 pmol/l (FT₄), and <15 mIU/l (TPO-Ab) (19, 20).

Statistical analysis

The effect of different doses of selenium supplementation was investigated by regression analysis for five different outcomes: selenium, TSH, FT₃, FT₄ and FT₃:FT₄ ratio.

For this, we applied a population averaged procedure assuming a linear relationship between outcome and covariates, by use of population-averaged generalized estimating equations (PA-GEE) combined with a sandwich estimator (21). This approach accommodates repeated measurements without requiring a constant within-subject variation, and encompasses continuous non-normal outcomes. Each outcome was analyzed in two analyses, with one analysing active treatment vs placebo as a binary covariate, and another analysing intervention dose as a continuous covariate (with values 0, 100, 200, or 300 $\mu\text{g/day}$ selenium-enriched yeast). Other included covariates were the baseline values of the respective outcome, age at baseline, gender, and a binary variable indicating the actual duration of the treatment (5 years vs 6 months). In addition, we included an interaction analysis between the treatment variable (either continuous or binary) and the treatment duration. To obtain our final analyses, we used a stepwise regression selection approach. The analyses underwent reduction and extension iterating between, firstly, removing explanatory variables one at a time, which no longer contributed significantly to the model (P value >0.05), and secondly, screening explanatory variables not contained in the current model one at a time and adding the most significant one (P value ≤ 0.05). Observations were analyzed irrespective of compliance (intention-to-treat analysis). For significant interactions, main effects were forced into the model. All reported P values were two-sided. Statistical analyses were

performed with Stata, version 13 (StataCorp, College Station, TX, USA).

Results

Participants

Of the 491 randomized participants, 23 dropped out of treatment before 6 months of follow-up, 107 participants dropped out between 6 months and 5 years, and the remaining 361 (74%) 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 ($P=0.91$). Twenty-two participants receiving thyroid medication at baseline or after 5 years, supplementation, with no significant differences between treatment groups at any time ($P=0.20$ and 0.40 respectively), were excluded from the statistical analysis. Thyroid function measurements were available for 467 of the remaining 469 participants at baseline, for 410 at 6 months, and for 338 at 5 years (Fig. 1). Participants with and without available thyroid function measurements at 6 months and 5 years did not differ in their baseline characteristics (data not shown). Three participants allocated to placebo had plasma selenium concentrations more than two inter-quartile ranges (IQRs) above the median at 5 years (3.3%), so drop-ins were deemed rare.

Adverse events

Twenty-two participants died during the 5-year follow-up period and 35 participants discontinued the study due to nonfatal adverse events (Fig. 1), with no significant differences across treatment groups ($P=0.29$ and 0.14 respectively). Twenty-five participants withdrew due to adverse reactions to treatment (Fig. 1), which were mainly hair loss, skin reactions, and grooved nails. These reactions were equally associated with selenium and placebo and were independent of selenium dose ($P=0.84$). Plasma selenium concentrations for participants dropping out due to alleged adverse reactions were within IQRs for respective treatment groups before drop out, except for two participants in the 300 µg/day group who had plasma selenium concentrations below the group IQR before dropout (data not shown). This indicates that adverse reactions reported were unlikely to result from excessive use of trial medication or other selenium supplements.

Baseline characteristics

There were no significant differences between treatment groups at baseline in TPO-Ab positivity, or other participant characteristics (Table 1). The overall median (IQR) plasma selenium concentration at baseline was 85 (19) ng/g with no significant differences across treatment

Table 1 Baseline characteristics of trial participants overall and by treatment group. Data are presented as means (s.d.) or numbers (%).

Characteristic	Overall	Placebo	Selenium dose (µg/day)			P value ^a
			100	200	300	
Participants	491	126	124	122	119	
Age (years)	66.1 (4.1)	65.4 (3.8)	66.4 (4.2)	66.3 (4.4)	66.5 (4.1)	0.13
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 status (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)	26.8 (4.1)	26.5 (4.0)	27.1 (4.0)	27.2 (4.3)	26.5 (4.0)	0.40
Use of thyroid medication ^b	14 (2.9)	2 (1.6)	4 (3.2)	5 (4.1)	3 (2.5)	0.20
TPO-Ab (units/ml)						0.33
< 15	409 (87.6)	111 (91)	98 (83.1)	101 (86.3)	99 (87.6)	
≥ 15	61 (12.4)	11 (9)	20 (16.9)	16 (13.7)	14 (12.4)	

TPO-Ab, thyroid peroxidase antibodies.

^aP value for homogeneity of means (one-way ANOVA tests) or proportions (χ^2 tests) across the four treatment groups.

^bThyroid hormone substitution or antithyroid medication.

Table 2 Plasma selenium concentrations and thyroid function at baseline and after 6 months and 5 years of selenium supplementation. *P* values are from Kruskal–Wallis tests comparing the four intervention groups at baseline.

Variable	Overall	Selenium dose (µg/day)			<i>P</i> value	
		Placebo 100	200	300		
Plasma selenium (ng/g)						
Median at baseline (IQR)	85 (19)	85 (20)	86 (18)	88 (22)	84 (19)	0.22
Median at 6 months (IQR)		87 (22)	149 (28)	201 (58)	246 (70)	
Median at 5 years (IQR)		85 (16)	157 (33)	217 (46)	271 (106)	
Serum TSH (mIU/l)						
Median at baseline (IQR)	1.20 (0.90)	1.21 (0.81)	1.28 (0.94)	1.14 (0.95)	1.18 (0.89)	0.68
Median at 6 months (IQR)		1.19 (0.76)	1.20 (1.01)	1.26 (0.84)	1.09 (0.82)	
Median at 5 years (IQR)		1.22 (1.02)	1.17 (0.79)	1.32 (0.84)	1.06 (0.85)	
Serum FT ₃ (pmol/l)						
Median at baseline (IQR)	5.53 (0.76)	5.44 (0.77)	5.56 (0.71)	5.54 (0.68)	5.56 (0.83)	0.39
Median at 6 months (IQR)		5.52 (0.88)	5.59 (0.81)	5.55 (0.77)	5.55 (0.81)	
Median at 5 years (IQR)		5.54 (0.95)	5.59 (0.71)	5.58 (0.78)	5.53 (0.78)	
Serum FT ₄ (pmol/l)						
Median at baseline (IQR)	13.21 (2.49)	12.88 (2.67)	13.06 (2.8)	13.43 (2.28)	13.43 (2.38)	0.13
Median at 6 months (IQR)		12.91 (2.97)	13.00 (2.6)	13.43 (2.3)	12.82 (2.2)	
Median at 5 years (IQR)		13.32 (3.07)	13.52 (2.88)	13.24 (2.08)	13.25 (2.12)	
Serum FT ₃ :FT ₄ ratio						
Median at baseline (IQR)	0.42 (0.08)	0.43 (0.07)	0.43 (0.10)	0.42 (0.07)	0.42 (0.07)	0.19
Median at 6 months (IQR)		0.42 (0.08)	0.43 (0.09)	0.41 (0.06)	0.42 (0.09)	
Median at 5 years (IQR)		0.42 (0.09)	0.42 (0.09)	0.42 (0.07)	0.42 (0.08)	

IQR, interquartile range; TSH, thyrotropin; FT₃, free triiodothyronine; FT₄, free thyroxine.

groups (Table 2). The overall medians (IQRs) for thyroid function parameters at baseline were TSH 1.20 (0.90) mIU/l, FT₃ 5.53 (0.76) pmol/l, FT₄ 13.21 (2.49) pmol/l, and calculated FT₃:FT₄ ratio 0.42 (0.08) with no significant differences across treatment groups (Table 2).

Intention-to-treat analysis

The results of the PA-GEE regression analyses are summarized below and in Table 3 and Fig. 2.

Plasma selenium ► As expected, selenium supplementation significantly increased plasma selenium concentrations compared with placebo in the binary covariate model ($P < 0.001$) as well as in the dose model ($P < 0.001$) (Table 3). In both models, treatment duration enhanced the effect ($P = 0.008$ and 0.025 respectively). Regression slopes based on the dose model estimate the magnitude of this effect, with a dose increase of 100 µg/day selenium-enriched yeast increasing plasma selenium concentrations by, on average, 57 ng/g at 6 months and 64 ng/g at 5 years' intervention (Table 3 and Fig. 2A). Female gender increased plasma selenium concentrations significantly in both models ($P < 0.001$, Table 3).

Serum TSH ► Baseline TSH concentration affected outcome TSH concentrations with an increase of 1.0 mIU/l at baseline, increasing TSH by 0.65 mIU/l at later time points ($P < 0.001$, Table 3). In the binary model of active treatment vs placebo, we could not detect any impact of selenium supplementation on TSH ($P = 0.61$, Table 3), but in the dose model, increasing the selenium dose affected TSH ($P = 0.010$, Table 3). A time-independent regression slope quantified that each dose increase of 100 µg/day decreased serum TSH concentration by, on average, 0.066 mIU/l (Table 3 and Fig. 2B). Subsequently, in a binary model of 300 µg selenium-enriched yeast/day vs other treatment groups, the highest selenium dose significantly decreased serum TSH by, on average, 0.21 mIU/l ($P = 0.003$). Other covariates were found not to have a significant impact.

Serum FT₃ ► Baseline FT₃ concentrations, as for TSH, affected outcome concentrations. An increase of 1.0 pmol/l at baseline increased FT₃ by, on average, 0.49 pmol/l at later time points ($P < 0.001$, Table 3). FT₃ concentrations decreased at later time points in female participants ($P = 0.021$ and 0.031 , Table 3). Neither in the binary model ($P = 0.48$, Table 3) nor in the dose model ($P = 0.51$, Table 3) did we find an effect of selenium

Table 3 Population averaged general estimating equations (PA-GEE) regression analyses results. For each outcome variable, covariates included in the final binary and dose analyses respectively are reported with estimated regression coefficients β , corresponding s.e.m., and nominal *P* values.

Outcome	Binary analysis			Dose analysis		
	Covariates	Estimated β (s.e.m.)	Nominal <i>P</i> value	Covariates	Estimated β (s.e.m.)	Nominal <i>P</i> value
Selenium (ng/g)	Constant	69.8 (3.3)	<0.001	Constant	79.0 (2.5)	<0.001
	Female gender	29.2 (5.3)	<0.001	Female gender	23.8 (3.6)	<0.001
	Treatment time	9.4 (4.2)	0.025	Treatment time*	6.7 (2.5)	0.008
	Active treatment	120.9 (3.8)	<0.001	Selenium dosage	57 (1.7)	<0.001
TSH (mIU/l)	Constant	0.53 (0.21)	0.013	Constant	-0.60 (0.21)	0.004
	Baseline TSH	0.65 (0.15)	<0.001	Baseline TSH	0.65 (0.15)	<0.001
	Active treatment	-0.035 (0.071)	0.611	Selenium dosage	-0.066 (0.026)	0.010
FT ₃ (pmol/l)	Constant	3.0 (0.22)	<0.001	Constant	2.9 (0.22)	<0.001
	Baseline T ₃	0.49 (0.037)	<0.001	Baseline T ₃	0.49 (0.037)	<0.001
	Female gender	-0.14 (0.059)	0.021	Female gender	-0.13 (0.061)	0.031
	Active treatment	-0.64 (0.91)	0.484	Selenium dosage	-0.02 (0.03)	0.508
FT ₄ (pmol/l)	Constant	6.3 (1.3)	<0.001	Constant	6.2 (1.2)	<0.001
	Baseline FT ₄	0.69 (0.059)	<0.001	Baseline FT ₄	0.69 (0.058)	<0.001
	Baseline age	-0.031 (0.014)	0.023	Baseline age	-0.031 (0.013)	0.022
	Treatment time	0.17 (0.083)	0.036	Treatment time	0.18 (0.083)	0.035
	Active treatment	-0.21 (0.12)	0.096	Selenium dosage	-0.11 (0.047)	0.015
T ₃ :T ₄	Constant	0.14 (0.026)	<0.001	Constant	0.13 (0.026)	<0.001
	Baseline T ₃ :T ₄	0.68 (0.058)	<0.001	Baseline T ₃ :T ₄	0.68 (0.058)	<0.001
	Active treatment	-0.068 (0.83)	0.934	Selenium dosage	-0.0011 (0.0027)	0.692

TSH, thyrotropin; FT₃, free triiodothyronine; FT₄, free thyroxine.

supplementation, or any other covariates, on FT₃ (Fig. 2C).

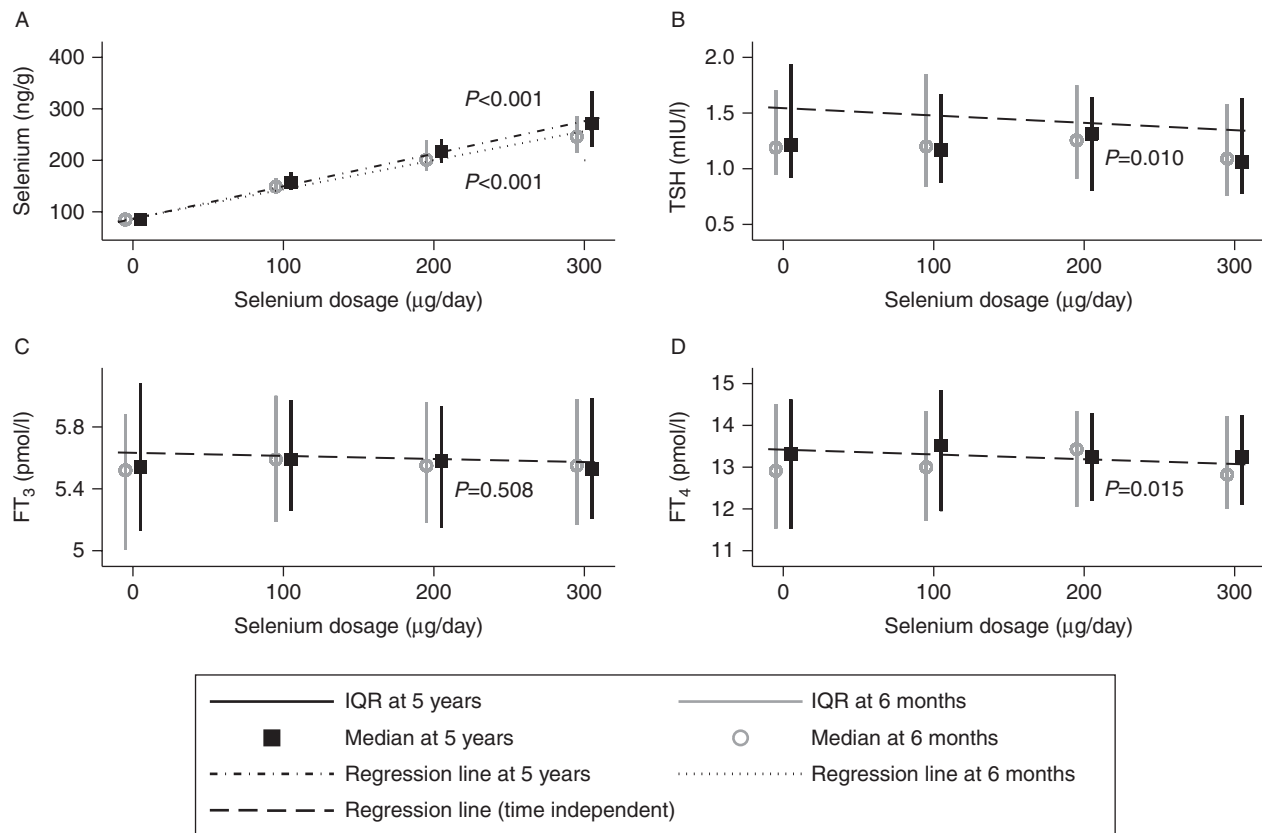
Serum FT₄ ► Baseline FT₄ concentrations affected outcome concentrations, with an increase of 1.0 pmol/l at baseline increasing FT₄ by average 0.69 pmol/l at later time points ($P<0.001$, Table 3). FT₄ concentrations at 5 years were found to be on average 0.17 and 0.18 pmol/l higher than at 6 months ($P=0.036$ and 0.035 respectively, Table 3). In line with the results for TSH, no effect of selenium supplementation on FT₄ was observed in the binary model ($P=0.10$, Table 3), while a significant effect was found in the dose model ($P=0.015$, Table 3). Thus, a dose increase of 100 μ g selenium/day decreased serum FT₄ by average 0.11 pmol/l (Fig. 2D). Other covariates with significant impacts were age at baseline, which lowered FT₄ on average by 0.031 pmol/l for each 1-year increase in age in both the binary and continuous dose covariate models ($P=0.023$ and 0.022 , Table 3) and the treatment duration.

Serum FT₃:FT₄ ratio ► Baseline FT₃:FT₄ ratio affected outcome ratios, with an increase of one > at baseline, increasing later time point ratios on average by 0.68 ($P<0.001$, Table 3). No significant effects of other covariates were found, including selenium supplementation ($P=0.93$ and 0.69 , Table 3).

Discussion

In this prospective, randomized, double-blinded, controlled trial among elderly Danes, selenium supplementation affected thyroid function by dose dependently decreasing serum TSH and FT₄ concentrations. Our trial also provided additional data on selenium status in Denmark. The median overall baseline plasma selenium concentration of 85 ng/g can be converted to 87.3 μ g/l by multiplying with 1.027 (the density of plasma). The slightly lower selenium status in our study population, compared with mean serum concentrations found in two previous Danish investigations, 98.7 μ g/l (10) and 94 μ g/l (22), can at least partly be explained by declining selenium status with age above 60 years (23, 24). Plasma selenium concentrations, as expected, increased dose dependently, with a large increase from baseline to 6-month measurements, and a further small increase from 6 months to 5 years. This is in accordance with a previous report of dose-dependent increases plateauing at 9–12 months of selenium supplementation (25).

While previous trials have reported conflicting results, our main findings of the effects on thyroid function are in contrast to the most readily comparable study, the UK PRECISE pilot trial: in 368 elderly individuals receiving 6 months of supplementation with 100, 200, or 300 μ g/day selenium-enriched yeast or placebo-yeast,

**Figure 2**

Dose effect diagram illustrating the dose-dependent effects of selenium supplementation on plasma selenium concentrations (A), serum TSH concentrations (B), serum FT₃ concentrations (C) and serum FT₄ concentrations (D) after 6 months and 5 years.

and using the same study protocol as this study, no effects on thyroid function or thyroid hormone conversion were found (17). In our favor, compared with the UK study, the DK PRECISE trial was slightly larger and continued for 5 years enabling assessment of trend, and both short-term and long-term supplementation, in a more powerful statistical analysis. Effects were significant in our study already after 6 months intervention. Interestingly, our short-term findings are in accordance with a recent study, in which ~6 months low-dose selenium supplementation decreased FT₄ and tended to decrease TSH, when compared with placebo (21). The comparability is however limited as study subjects were pregnant women and effects were seen only in thyroid antibody-positive individuals.

For our statistical analysis, to take the correlation between the repeated measurements into account, we first considered a mixed effects linear model with a subject-specific random effect. Estimated residuals as well as random effects did however show strong deviations from

the model assumptions of independently and identically normally distributed residuals. Moreover, neither transformations of outcome variables, such as logarithmic transformation, nor allowing for nonlinear relationships, by including higher order terms, improved the residual diagnostics. Therefore, we chose a PA-GEE model in order to weaken the distributional assumptions, combined with a sandwich estimator being robust in the case of misspecification of the variance or covariance structure (26, 27, 28).

It is well known that the thyroid gland retains selenium and selenoprotein activity even under conditions of severe deficiency (29). However, it remains unknown whether selenium modulates peripheral thyroid hormone action via less prioritized mechanisms (18). T₃:T₄ ratio is primarily dependent on the DIO1 activity (30), but DIO2 and DIO3 have been implicated in fine-tuning local intracellular T₃ concentrations tissue specifically, without changing serum T₃ concentrations (31). Previous trials may have been underpowered, due to

size or statistical analysis strategy, to detect small changes generated by altered thyroid metabolic states in selenium-sensitive target organs. Optimizing selenoprotein activity is often referred to when explaining the effects of selenium supplementation (32) and indeed, selenium repletion is followed by a rapid accumulation of selenium in endocrine organs and the brain (29). An increased activity of pituitary DIO2 leading to higher production of T₃ may explain the net effect of concurrent decreases in serum TSH and FT₄ concentrations, e.g. via altered hepatic elimination of thyroid hormone metabolites as suggested in animal experimental studies (33). In this study, however, the three active groups were all supplemented beyond optimal status threshold (13), so the dose-dependent effect of selenium supplementation on thyroid function suggests not merely a correction of deficit. In fact, DIO upregulation may be unrelated to the intracellular accumulation of selenium (34), and supra-physiological, potentially toxic, effects on thyroid hormone metabolism, beyond classical DIO and GPx3 pathways, offer alternative explanations of our findings. Direct effects of iodine and selenium on the pituitary gland, in the principle of a negative feedback loop, have recently been suggested (35). This could account for decreasing TSH concentrations in our study, warranting further investigations of relationships between the thyroid and pituitary glands at the level of trace elements.

The DK PRECISE pilot trial differs from most earlier intervention studies, and from the UK PRECISE trial in particular, in the concomitant mandatory iodization of Danish salt, offering selenium–iodine interaction as explanation of the different effects observed. In Denmark, median urinary iodine concentrations increased from 61 µg/l (1997–1998) to 101 µg/l (2004–2005), following iodine fortification (36). The PRECISE intervention period was 1998–1999 to 2004, and it is very likely that the placebo group increased their intake of iodine alone during the 5-year trial period, while the intervention groups increased their intake of selenium as well. Iodine status has been postulated to be a determinant of selenium activity in patients with thyroid autoimmunity (6), but could not be quantified in the PRECISE trial, since no urine samples were collected. The lack of iodine assessment is the single most important limitation to our study. However, no such synergistic effects were found in a randomized trial with concomitant iodine and selenium supplementation in a New Zealand population of similar selenium and iodine status (37). Furthermore, the iodine fortification program in Denmark was associated with an

increase in the TPO-Ab positivity prevalence (38). An attenuating modulation of this effect by selenium is conceivable, due to its potential to decrease thyroid antibodies in autoimmune thyroid disease (39). However, this trial was not designed or powered to investigate the effect of selenium supplementation on TPO-Ab prevalence, and TPO-Ab was measured merely to evaluate potential differences between groups at baseline.

The participants were invited via a random sample from the Danish Civil Registration System. Around 20% accepted the invitation and this constitutes a potential selection bias, as the age range (60–74 years) is the only known parameter about individuals declining or not responding to the invitation. The narrow age range of participants limits the applicability of our findings to a general population and the relatively low baseline selenium status makes it difficult to extend our findings to populations with higher baseline selenium status, e.g. most of the USA (9). At the time of the biochemical analyses, baseline samples had been stored at –80 °C for ~15 years. A study of stored samples from the Finnish Maternity Cohort concluded that TSH, FT₃, and FT₄ can be reliably analyzed in serum stored for 23 years and TPO-Ab for 14 years at –25 °C (40). It follows that our samples, stored at –80 °C, should be at least as stable.

Bearing in mind the inextricable U-shaped link between selenium intake and human health (32), our finding of dose-dependent alterations of thyroid function with minute effect sizes does not support universally increasing selenium intake for improving thyroid function under conditions of marginal selenium deficiency. However, the fact that thyroid function appears to be sensitive to selenium supplementation adds to the incentive for investigating its effects on multifactorially regulated traits, such as thyroid function (19, 41), size (42,43), and autoimmunity (44) in autoimmune thyroid diseases, where selenoprotein biosynthesis may be impaired (18). A number of trials in Hashimoto's thyroiditis have thus far not clarified the role of selenium supplementation (39). A single trial reported promising results for a subset of Graves' orbitopathy patients (45), and two Danish randomized trials are currently investigating clinical effects of selenium supplementation in Graves' disease (46) and chronic autoimmune thyroiditis (47).

In conclusion, in this double-blinded RCT in a population with no known thyroid diseases, selenium supplementation minutely and dose dependently affected thyroid function by decreasing serum TSH and FT₄ concentrations.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

S Cold designed the research; K H Winther, F Cold, and S Cold conducted the research; K H Winther, S J Bonnema, M Nybo, and L Hegedüs analyzed the data; B Debrabant performed the statistical analysis; K H Winther wrote the manuscript; S J Bonnema and L Hegedüs revised the manuscript; K H Winther had primary responsibility for final content. All authors read and approved the final manuscript.

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