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### Clinical studies on thyroid diseases

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# Chapter 2

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Selenite supplementation in euthyroid subjects with thyroid peroxidase antibodies

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## ABSTRACT

**Context:** Euthyroid TPO-Ab positive subjects are at risk for progression to subclinical and overt autoimmune hypothyroidism. Previous studies have shown a decrease of TPO-Ab and improvement of quality-of-life (QoL) in L-T4 treated hypothyroid patients upon selenium supplementation.

**Objectives:** To evaluate in euthyroid TPO-Ab positive women without thyroid medication whether selenite decreases TPO-Ab and improves QoL.

**Design:** Randomized, placebo-controlled, double-blind study.

**Patients and Methods:** Euthyroid (TSH 0.5-5.0 mU/L, FT4 10-23 pmol/L) women with TPO-Ab  $\geq 100$  kU/L were randomized to receive 200 mcg sodium selenite daily (n=30) or placebo (n=31) for 6 months. TSH, FT4, TPO-Ab, selenium (Se), selenoprotein P (SePP) and QoL were measured at baseline, 3, 6 and 9 months.

**Results:** There were no differences in baseline characteristics between the Se group and the placebo group. During selenite supplementation, serum Se and SePP did not change in the placebo group, but increased in the Se group. TPO-Ab and TSH did not change significantly in any group. TPO-Ab in the Se group were 895 (130-6800) at baseline, 1360 (60-7050) kU/l at 6 months, in the placebo group 1090 (120-9200) and 1130 (80-9900) kU/l respectively (median values with range). TSH in the Se group was 2.1 (0.5-4.3) at baseline, 1.7 (0.0-5.3) mU/L at 6 months, in the placebo group 2.4 (0.7-4.4) and 2.5 (0.2-4.3) mU/L respectively. QoL was not different between the groups.

**Conclusion:** Six months selenite supplementation increased markers of selenium status but had no effect on serum TPO-Ab, TSH or quality-of-life in euthyroid TPO-Ab positive women.

## INTRODUCTION

Selenium (Se) is an essential trace element. About 25 genes encoding for selenoproteins have been identified in humans<sup>1</sup>. The intake of Se compounds varies widely depending on the Se content of the soil, causing higher intake in e.g. the USA and lower intake in many parts of Europe. The officially recommended dietary intake for Se varies: one reasonable estimate suggests 60 mcg/day for males and 53 mcg/day for females<sup>2</sup>. In the Netherlands the average Selenium intake is in the order of 67 mcg/day<sup>3</sup>.

Various selenium compounds have a marked influence on the immune system<sup>4</sup>. Se improves expression of enzymes involved in antioxidative and redox control, thereby reducing e.g. reactive oxygen species-mediated oxidative damage. It is hypothesized that even mild nutritional Se deficiency can have adverse consequences for the initiation or progression of thyroid autoimmunity in subjects with genetic susceptibility to develop autoimmune thyroid diseases<sup>5</sup>. Low Se blood levels are associated with increased thyroid volume in females and with thyroid hypoechogenicity, a marker for lymphocytic infiltration<sup>6,7</sup>. Antibodies against thyroid peroxidase (TPO-Ab) are very prevalent in the general adult population. In euthyroid subjects, the presence of TPO-Ab is associated with a slightly higher but still normal serum TSH<sup>8</sup>. In a large community survey, TPO-Ab positive women developed hypothyroidism at an annual rate of 2.1%, compared to the annual incidence of 0.35% in the general female population. The presence of TPO-Ab thus carries a risk of impending thyroid failure<sup>9</sup>. Over the last years several prospective clinical trials were performed in patients with autoimmune thyroiditis (AIT) to examine whether Se supplementation might reduce the inflammatory activity within the thyroid and thereby diminishing TPO-Ab concentration<sup>10</sup>. A recent meta-analysis of four placebo-controlled blinded randomized clinical trials on the effectiveness of Se supplementation in patients with Hashimoto's thyroiditis under L-T4 treatment<sup>11</sup> revealed that participants assigned to Se supplementation for 3 months demonstrated significantly lower TPO-Ab (mean difference -271 kU/l, 95% CI -422 to -120) and a significantly higher chance of reporting an improvement in well-being and/or mood (RR 2.79, 95% CI 1.21-6.47) when compared with controls. Outcomes in the seven published studies so far, however, have been conflicting, with a decrease in TPO-Ab upon Se supplementation in five<sup>12,13,14,15,16</sup> and no change in two reports<sup>17,18</sup>. Except for one study<sup>16</sup>, all these studies were performed in patients using L-T4 medication. Demands in L-T4 replacement therapy were found either unaltered or underreported. Notably, all the studies published so far were conducted in Europe where Se supply is marginal. For the lack of studies involving patients with high Se status, it is not known whether the additional Se intake rather corrected a deficit (substitution) or constituted an add-on effect (supplementation)<sup>4</sup>. A thorough monitoring of the Se status changes in the patients in the autoimmune thyroid disease trials is often missing. We initiated a randomized placebo-controlled double blind clinical trial in TPO-Ab positive subjects with a normal TSH who did not use any thyroid hormone medication, to evaluate if TPO-Ab decreased and health-related quality of life (QoL) increased upon selenite supplementation. We evaluated the efficiency of our selenite supplementation regimen by assessing the Se status of the patients before, during and after study completion.

## PATIENTS AND METHODS

### Patients and study design

We conducted a randomized, placebo-controlled, double-blind, single center (Academic Medical Center in Amsterdam) clinical trial among adult women. Subjects were recruited via advertisements on websites and in information bulletins of three Dutch patient organizations and from patients with autoimmune thyroid disease visiting our outpatient clinic. We asked family members of patients with autoimmune thyroid disease to undergo blood tests for TPO-Ab and thyroid function. Inclusion criteria were female sex, TPO-Ab  $\geq 100$  kU/liter) and euthyroidism (TSH 0.5-5.0 mU/liter and FT<sub>4</sub> 10-23 pmol/liter) without thyroid medication. The rationale for selecting only women as participants was a practical one: chances to identify TPO-Ab positive but still euthyroid subjects are much higher in women than in men. Exclusion criteria were use of vitamin tablets containing selenium in the month preceding inclusion, pregnancy and absence of informed consent. A randomization list was made using blocks of 10 and the assignment was in consecutive order. Participants were randomized 1 to 1 to receive one tablet of 200 mcg sodium selenite or one placebo tablet daily for a period of 6 months. Sodium selenite or placebo was administered once daily; no specific instructions were given with regard to food intake.

After 6 months, treatment was stopped and patients were followed for another 3 months. At baseline and during follow up, participating subjects visited the outpatient clinics every 3 months, where blood samples were drawn for assessment of TSH, FT<sub>4</sub>, TPO-Ab, selenium (Se) and selenoprotein P (SePP) concentration. Furthermore, at baseline, a thyroid ultrasound was made to estimate thyroid volume using the formula width x length x thickness x 0.52 for each lobe as described previously<sup>19</sup>. We divided the thyroid volumes into two categories: not enlarged (<20 ml) and enlarged ( $\geq 20$  ml)<sup>20</sup>. Thyroid echogenicity was categorized into decreased (hypoechoogenicity) or not decreased (compared with adjacent muscles in a longitudinal scan). All ultrasound examinations were performed by one operator, who was blinded to the treatment group, using a 7.5-MHz transducer. Patient's health related QoL was evaluated at baseline, six and nine months using the MOS Short Form 36 (MOS-SF-36, Dutch version) General Health Survey<sup>21</sup>. The SF-36 is a standardized health related QoL questionnaire that measures mental and physical well-being in terms of eight health domains and two summary scores for overall physical and mental health. The overall summary measures are calculated as weighted averages of the eight domain Z scores using standard procedures. Raw scores are transformed on a 0-100 scale with higher scores associated with a better quality of life. A Z score of 50 corresponds to the average quality of life of the general Dutch population. At each outpatient visit we asked participants about complaints or possible side effects of the medication. A premature stop was made if overt hypo- or hyperthyroidism occurred. The primary endpoint of the study was change in TPO-Ab concentration at 6 months. The secondary endpoint was change in QoL. The randomized trial was approved by the Institutional Review Board of the Academic Medical Center and registered. Participants gave written informed consent.

### Laboratory analysis

TSH was initially measured by time-resolved fluoroimmunoassay (Delfia hTSH Ultra, Wallac Oy, Turku, Finland). Intra-assay variation: 1-2%; inter-assay variation 3-4%; detection limit 0.01 mU/l; reference range 0.4-4.0 mU/l. During the study, the assay for TSH was changed to an electrochemiluminiscent immunometrec assay performed on the cobas e602 analyzer (Roche Diagnostics). Intra-assay variation: 1-2%; inter-assay variation 3-4%; detection limit 0.01 mU/l; reference range 0.5-5.0 mU/l. The TSH values obtained with the Delfia method

were converted to the Roche method. FT<sub>4</sub> was measured by time-resolved fluoroimmunoassay (Delfia FT<sub>4</sub>, Wallac Oy, Turku, Finland). Intra-assay variation 4-6%; inter-assay variation 5-8%; detection limit 2 pmol/l; reference range 10-23 pmol/l. Hypothyroidism was defined as TSH > 5.0 mU/l and FT<sub>4</sub> < 10 pmol/l, hyperthyroidism as TSH < 0.5 mU/l and FT<sub>4</sub> > 23 pmol/l. TPO-Ab were determined by chemiluminescence immunoassay (LUMI-test anti-TPO, BRAHMS, Berlin, Germany), intra-assay variation 3-7%; inter-assay variation 8-12%; detection limit 30 kU/l; reference value < 60 kU/l.

Total serum selenium was quantified in triplicate using total reflection X-ray fluorescence analysis<sup>22</sup>. Briefly, 100 µl of serum were diluted with 895 µl of water and supplemented with 5 µl of a gallium standard (10 mg/l). Samples of 10 µl were placed on quartz glass sample carriers, dried and measured using a benchtop total reflection X-ray fluorescence spectrometer (S2 PICOFOX; Bruker AXS Microanalysis GmbH, Berlin, Germany) for 2000 s each. The method was validated with a Seronorm serum standard (Sero AS, Billingstad, Norway), and proved to be linear at 1:3, 1:10 or 1:20 dilutions of the standard serum; a standard sodium selenite solution was tested in addition, and signal linearity was verified by total reflection X-ray fluorescence analysis. Inter-assay variation was below 10% in the concentration range of 50-150 µg/l Se<sup>23</sup>. SePP in the serum samples was determined by an immunoluminometric sandwich assay<sup>23,24</sup>. Briefly, serum was diluted in assay buffer, and samples of 50 µl (corresponding to 0.6 µl of serum) were applied to antibody-coated tubes, incubated with tracer antibodies, and washed; chemiluminescence was measured using a luminometer (LB 953; Berthold Technologies, Oak Ridge, TN, USA). Samples were analysed in triplicate within the same assay run. Intra-assay variation was below 10% for SePP values > 1 mg/l. Analyses were performed by lab personnel blinded to the sample characteristics.

### Statistical analysis

Analyses were performed according to intention to treat. Comparison of baseline characteristics between the two randomized arms was done with the independent Student's t test for variables with normal distributions (age, FT<sub>4</sub>, BMI and selenium concentration), the Mann-Whitney test for continuous variables with skewed distributions (TSH, TPO-Ab, SePP, thyroid volume and SF-36) and the Chi-Square test for categorical variables (thyroid echogenicity). The differences between the randomization arms for lost to follow up, occurrence of subclinical hypothyroidism and subclinical hyperthyroidism were also tested with the Chi-Square test. Differences in TSH-, FT<sub>4</sub>-, selenium-, SePP- and TPO-Ab concentrations and QoL between the treatment groups over time (up to 6 months) were analyzed with repeated measurements analysis (covariance type: unstructured), differences within each treatment group during treatment with the dependent Student's t test. Statistical analyses were done using the SPSS version 16.0. In all analyses, p values < 0.05 (two sided) were considered significant.

## RESULTS

216 subjects were enrolled in the study. Excluded were 133 subjects because TPO-Ab was ≤ 100 kU/L and 15 because TSH was >5 mU/L. 68 subjects were eligible for randomization. Seven of them gave no informed consent, so 61 subjects were randomized, 30 to selenite treatment and 31 to placebo treatment (Fig 1). Table 1 shows participant's baseline characteristics. There were no significant baseline differences between the two groups. An enlarged thyroid was found on ultrasound in four subjects in the placebo group (13%) and in three in the selenite group (10%).

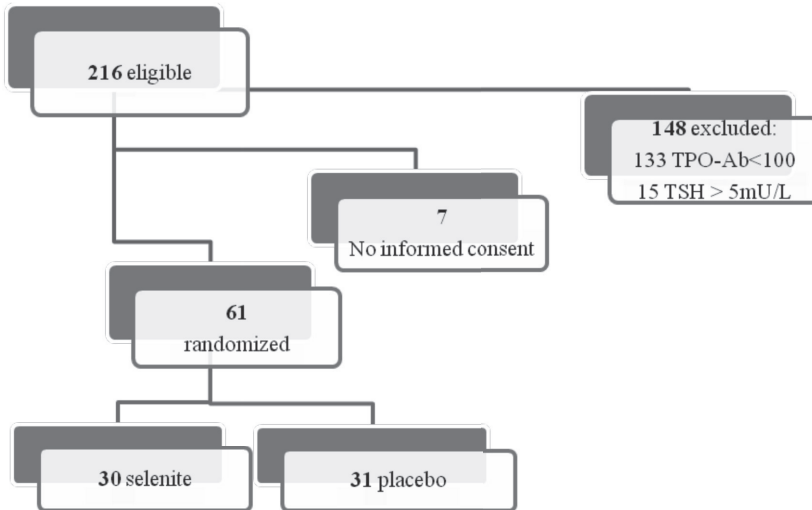


Figure 1. Flow-diagram depicting recruitment process of participants in the randomized trial on the effect of selenite supplementation in euthyroid subjects with TPO-Ab

In the subjects randomized to receive selenite there was a significant increase in serum Se and SePP concentrations ( $P < 0.001$  for both) during the six months treatment period, returning to baseline values after cessation of the treatment. Serum Se and SePP did not change in the placebo group ( $P=0.08$  and  $P=0.43$  respectively). The difference between the groups was significant ( $P<0.001$  for Se and  $P=0.001$  for SePP) (Fig 2). Lost to follow up were four patients in the placebo group (two at 6 months and two at 9 months) and one in the selenium group (at 6 months)( $P=0.17$ ). Subclinical hypothyroidism occurred in two patients in the placebo group (one at 3 months with normalization of TSH at 6 months, and one at 9 months) and in two patients in the selenite group (one was subclinical hypothyroid at 3 and 6 months, and one at 3 and 9 months)( $2/30$  vs  $2/31$ ,  $P=0.97$ ). Overt hypothyroidism occurred in neither group. Subclinical hyperthyroidism occurred in one patient in the placebo group (at 6 months) and in two patients in the selenite group; one at 9 months, the other at 3 and 6 months progressing to overt hyperthyroidism at 9 months ( $1/31$  vs  $2/30$ ,  $P=0.53$ ). The latter value at 9 months was excluded in the 9- month analyses (Table 2). There was no change in TPO-Ab concentration in any group over time ( $P=0.24$  and  $P=0.90$  for subjects randomized to selenite and to placebo respectively) and there was no difference between the two groups ( $P=0.28$ ). Also TSH did not change significantly over time ( $P=0.69$  and  $P= 0.10$  in the selenite and the placebo group resp) nor was there a difference between the groups ( $P=0.94$ ). Neither was there a change in FT4 ( $P=0.20$  and  $P= 0.67$  in the selenite and the placebo group resp) . There was also no difference between the groups in FT4 concentrations ( $P=0.14$ ) (Fig 2). The SF-36 summary measures were not different between the groups ( $P=0.72$  for mental health,  $P=0.08$  for physical health) (Table 2). Two patients, one in each group, reported hair loss as side effect.

Table 1. Baseline characteristics of euthyroid subjects with TPO-Ab

Characteristics	Selenium N=30	Placebo N=31
Age - yr	43.5 (20-68)	45.0 (21-74)
BMI	24.6 (18-33)	25.6 (19-38)
TSH – mU/L	2.1 (0.5-4.3)	2.4 (0.7-4.4)
FT <sub>4</sub> – pmol/L	14.3 (10-18)	13.3 (10-22)
TPO-Ab – kU/L	895 (130-6800)	1090 (120-9200)
Thyroid volume - ml	9.7 (4-95)	10.9 (5-34)
Thyroid hypoechogenicity	10 (33%)	5 (16%)
Selenium – µg/L	72.9 (40-101)	74.7 (40-110)
Selenoprotein P – mg/L	2.6 (1.6-6.6)	2.7 (1.4-5.0)
SF-36 Health survey – Summary measure Mental health *	49.6 (21-63)	53.8 (33-62)
SF-36 Health survey– Summary measure Physical health*	53.0 (25-64)	50.8 (16-60)

Values as median (range). \*Weighted average of Z scores transformed on a 0-100 scale. A score of 50 (0-100 scale) represents the average Dutch population.

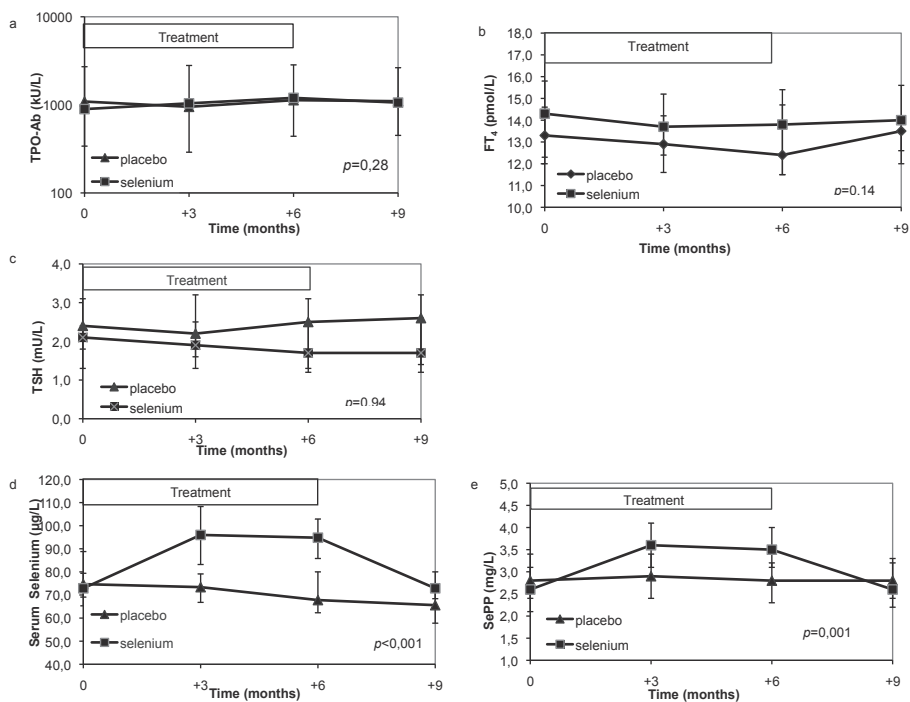


Figure 2. Concentrations of TPO-Ab (a), FT<sub>4</sub> (b), TSH (c), selenium (d) and selenoprotein P (SePP) (e) in euthyroid TPO-Ab positive subjects who were randomized to receive selenite supplementation or placebo (values as median and interquartile range; P values, difference between groups tested with repeated measurements analysis).

Table 2. Follow up of euthyroid TPO-Ab positive subjects randomized to receive selenite or placebo

	Group	0 months	3 months	6 months	9 months
TPO-Ab - kU/L	Plac	1090 (120-9200)	950 (80-8350)	1130 (80-9900)	1100 (70-7600)
	Se	895 (130-6800)	1040 (130-6600)	1360 (60-7050)	1080 (50-7000)
TSH - mU/L	Plac	2.4 (0.7-4.4)	2.2 (0.8-12.6)	2.2 (0.2-4.3)	2.6 (1.0-5.5)
	Se	2.1 (0.5-4.3)	1.9 (0.3-6.9)	1.7 (0.0-5.3)	1.6 (0.1-7.4)
FT <sub>4</sub> - pmol/L	Plac	13.3 (10-22)	12.9 (9-18)	12.8 (10-20)	13.5 (9-21)
	Se	14.3 (10-18)	13.7 (10-17)	13.8 (9-23)	14.0 (10-21)
Subclin hypo -n	Plac	0	1	0	1
	Se	0	2	1	1
Subclin hyper -n	Plac	0	0	1	0
	Se	0	1	1	1
SF-36 Mental Health*	Plac	53.8 (33-62)	NA	53.3 (18-63)	51.3 (22-59)
	Se	49.6 (21-63)	NA	50.1 (27-64)	51.7 (29-58)
SF-36 Physical Health*	Plac	50.8 (16-60)	NA	55.0 (25-62)	54.6 (33-62)
	Se	53.0 (25-64)	NA	54.1 (31-76)	53.5 (33-61)

Values as median (range). No significant differences between selenite and placebo group.

\* Weighted average of Z scores transformed on a 0-100 scale. NA, not assessed

## DISCUSSION

Intervention with 200 mcg sodium selenite daily raised serum Se concentrations from 73 µg/L at baseline to 96 µg/L at 3 months and 95 µg/L at 6 months; obviously, a plateau had been reached within three months, also for SePP. This result indicates that our subjects had not been Se replete before supplementation as one widely accepted criterion for Se sufficiency is to reach a saturating level of SePP expression. Our baseline serum Se of about 75 µg/L is at the lower end of the frequency histogram of Se concentrations in the UK, and below the lower normal limit of serum Se in the USA 2003-2004 NHANES population<sup>2</sup>. Obtained serum Se levels of about 100 µg/L during Se supplementation correspond to the limit at which selenoproteins including SePP become saturated and fully expressed. No exceedingly high levels of serum Se were observed, which is obviously also due to the selenocompound chosen for it is known that selenite in contrast to selenomethionine does not cause increasing serum Se concentrations once full expression of serum selenoproteins is reached. Accordingly, adverse effects known from continuous supplementation with selenite-containing supplements were not observed. However, clinical efficacy of the intervention on thyroid parameters was also not observed. We found no change in TPO-Ab or TSH serum concentrations after selenite supplementation in euthyroid TPO-Ab positive subjects. This is in contrast to many, but not all, previous studies in TPO-Ab positive subjects treated with various selenium forms<sup>10</sup>. In table 3 the characteristics of the other randomized clinical trials are listed. What could be the reasons for the discrepancies?

*Could it be selenium levels?* It has been suggested that supplementation with selenium compounds is more effective in decreasing TPO-Ab levels in subjects with lower baseline selenium<sup>25,11</sup>. As shown in table 3, baseline Se in our study was comparable to those in other studies. Also, serum Se levels during selenium supplementation were in the same order; 96 µg/L in the present study, and 86, 97 and 125 µg/L in the others<sup>12,13,17</sup>. Our study is the only one that measured in addition SePP, a soluble selenoprotein secreted mainly by the liver. It transports selenium and delivers it via receptor mediated uptake to peripheral organs. SePP and glutathione peroxidase 3 (GPx-3) account for the largest fraction of blood selenium. SePP and GPx-3 can both be saturated with selenium, but at different selenium intake levels. SePP responds over a larger range of selenium supply and is therefore considered to represent the best indicator of selenium status and selenium intake<sup>25,26,27</sup>. The clear increase in SePP after selenite also indicates that in our study the initial selenium status was not fully saturated. Differences in Se levels are thus unlikely to account for discrepancies between studies in terms of reduction in TPO-Ab concentration.

*Could it be the form of selenium used for supplementation?* In some studies selenomethionine or selenized yeast are used, in others sodium selenite. These compounds undergo different metabolic pathways for biosynthesis of selenoproteins and differently affect the total selenium status. Until saturation of both SePP and GPx-3 is reached in serum, it seems not to matter whether selenomethionine or selenite is administered, because all selenium is mainly used to increase selenoprotein biosynthesis. But after saturation is reached, only selenomethionine will still increase serum selenium via its unregulated incorporation into various proteins replacing methionine, whereas selenite will not be used efficiently any more for biosynthesis of selenocysteine containing selenoprotein but rather will be excreted as selenosugars or methylated seleniumforms. This means that when selenomethionine is chosen, selenium concentration in blood increases even in well-supplied individuals, whereas the effect of increased intake of anorganic seleniumforms (as selenite or selenite) is dependent on the selenium

Table 3. Randomized clinical trials on the effect of selenite supplementation on TPO-Ab concentration.

Reference	Intervention	On T <sub>4</sub> med.	No of subjects	Basal selenium concentration (µg/L)	TPO-Ab Initial (kU/L)	TPO-Ab at 3 months (kU/L)	TPO-Ab at 6 months (kU/L)	Signif.
Gärtner, 2002	200 µg Na-selenite placebo	Y	36 34	69±12 72±12	904±205 1090±277	575±46 959±267	- -	p=0.013 NS
Duntas, 2003	200 µg Selenomethionine placebo	Y	34 31	75±6 (n=10)	1875±1039 1758±917	1013±382 1389±520	884±227 1284±410	p<0.0001 p<0.001
Turker, 2006	200 µg Selenomethionine placebo	Y	48 40	- -	803±483 770±406	572±517 773±372	- -	p<0.001 NS
Karanikas, 2007	200 µg Na-selenite placebo	Y	18 18	75±11 76±12	524±452 521±349	505±464 527±354	- -	NS NS
Nacamulli, 2010	80µg Na-selenite placebo	N	46* 30*	- -	172 (95% CI 100-295) 143 (95% CI 87-232)	- -	148 (95% CI 85-259) 126 (95%CI 77-208)	NS# NS
Eskes, 2013	200 µg Na-selenite placebo	N	30 31	74±14 76±14	1508±1766 2045±2265	1681±1694 1764±2040	1792±1950 2053±2431	NS NS

Values as mean ± SD. Y, yes. N, no. \*it is not described how many of these subjects were TPO-Ab positive. # TPO-Ab were significantly decreased after 12 months selenite supplementation (p < 0.001)

status of the subject. It is currently unclear which form of selenium supplementation is more beneficial and safe<sup>27</sup> In the preceding studies a decrease in TPO-Ab is described with selenomethionine as well as with sodium selenite.

*Could it be preexisting TPO-Ab levels?* Two trials<sup>12,14</sup> reported a greater decrease in TPO-Ab concentrations in subjects with higher baseline levels. In our study the TPO-Ab concentration at baseline was comparable to that in the positive studies. In the other negative study<sup>17</sup>, the TPO-Ab concentrations were relatively low compared to most positive studies, apart from one study<sup>16</sup> in which TPO-Ab concentrations were even lower but still decreased after 12 months of selenium supplementation. This makes the level of pre-existing TPO-Ab an improbable cause of our negative results.

*Could it be treatment with thyroxine?* Probably not as in all trials except one subjects were on thyroxine medication, also in the study with negative results<sup>17</sup>. In the only other trial where subjects were not on thyroxine medication, a significant decrease of TPO-Ab concentration was found only after 12 months, not after 6 months<sup>16</sup>. However, in contrast to other studies, not all subjects participating in this study were TPO-Ab positive at baseline: they could be included if they had a high TPO-Ab and/or a high thyroglobulin-Ab concentration and/or an hypoechoogenic thyroid ultrasound pattern. The authors did not describe how many subjects were TPO-Ab positive. Selenium supplementation had no influence on thyroid function, regardless of the use of L-T4. This is in agreement with unchanged thyroid function during selenium supplementation in healthy volunteers<sup>28,29</sup>.

*Could it be sample size?* Sample size of our study is in the same order of magnitude as those in all other randomized trials published so far on this topic, irrespective whether TPO-Ab decreased or did not change (Table 3). Consequently, we think it is unlikely that sample size explains the discrepant results. None of the studies reports a formal sample size calculation.

Nevertheless, assuming a gain of median TPO of 52% in the Se group and 4% in the placebo group, at least 2x17 patients have to be included in the analysis (two sided alpha=0.05, power 80%).

*Could it be iodine intake?* Studies reporting a decrease in TPO-Ab upon selenium supplementation originate from Germany, Greece, Turkey and Italy<sup>12,13,14,15,16</sup>, countries with prevalent iodine deficiency. Studies failing to observe a decrease in TPO-Ab were done in Austria and The Netherlands, countries with sufficient iodine intake<sup>17</sup>. In the Netherlands, median urinary iodine excretion was 154 µg/L in 2001 and 165 µg/day in 2010<sup>30,31</sup>. Iodine status has not been measured in most studies, with the exception of the Italian study in which the majority of patients had an urinary iodine excretion  $\geq 100$  µg/24 hr and a modest reduction in TPO-Ab at 12 months<sup>16</sup>. No significant interactions have been observed between serum Se concentrations and iodine status in humans<sup>7</sup>. However, iodine deficiency might increase the amount of oxidative stress for the thyroid gland, and additional Se deficiency via decreased levels of selenoproteins could weaken antioxidative defense systems thereby exacerbating oxidative stress<sup>32</sup>. It is thus feasible that selenium supplementation is more likely to cause a fall in TPO-Ab concentration in iodine-deficient regions than in iodine-sufficient regions. This hypothesis needs further testing in an independent study.

QoL as assessed by SF-36 in our double-blinded RCT was not affected by selenite supplementation. Our negative results are in contrast with previous RCTs in patients with Hashimoto's thyroiditis reporting greater improvement of well-being in the Se than in the placebo groups<sup>11</sup>. QoL in these studies were assessed by SF-12<sup>12,17</sup> or not specified<sup>13</sup>. None of these studies were double-blinded, and in one of them QoL improved in the absence of a fall in TPO-Ab<sup>17</sup>. Our results are in agreement with a large RCT of selenium supplementation in healthy individuals, demonstrating no effect of selenium on quality of life<sup>33</sup>.

Strengths of our RCT are its study design and the sequential measurements of serum Se and SePP, allowing to judge compliance with study medication, verification of Se deficit at the beginning of the trial and positive effects on the Se status in the verum but not the control group. A limitation might be the duration of intervention: it cannot be excluded that treatment for a much longer period of time than 6 months may finally reduce TPO-Ab concentration. However, a decrease in TPO-Ab was already observed at 3 months<sup>12,13,14</sup> with a further fall at 6 months<sup>13,14</sup> but not at 9 months<sup>14</sup>. Also, in a RCT on Graves' ophthalmopathy, sodium selenite but not placebo resulted in a significant reduction in TPO-Ab at 6 months, which despite discontinuation of selenium medication was maintained at 9 months<sup>34</sup>, thus supporting our study design and analysis. Another limitation is that we studied only women. There are profound differences between the sexes with respect to selenium utilization, but ovariectomy and/or estradiol replacement had no significant effects on selenoproteins expression in an experimental rodent model<sup>35</sup>. It is thus unlikely that the gonadal function is an important modifier of selenite supplementation effects.

In conclusion, we found no positive effect of selenite supplementation on TPO-Ab concentration, thyroid function or quality of life in euthyroid TPO-Ab positive subjects living in an iodine-sufficient region with moderately low Se intake. Differences in outcomes between the present and previous studies remain largely unexplained, but could be related to differences in ambient iodine intake. Meaningful clinical outcomes should be demonstrated before selenium supplementation can be routinely recommended in Hashimoto's thyroiditis<sup>11</sup>.

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