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Can selenium supplementation reduce the relapse rate in Graves' Disease with remission.

Author: Oksana Madsen

Supervisor: Jesper Karmisholt

Supervisor: Inge Bülow Pedersen

Student number 20155732. Project number 39e21au5. Stud. Med., 5. Semester candidate Aalborg University.

Department of Medical Endocrinology, Aalborg University Hospital

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Contents

Abstract

Background. Graves' hyperthyroidism is an autoimmune illness causing hyperfunction of the thyroid gland with relevant clinical symptomatic picture. The absorption of selenium is high in the thyroid gland and two important groups of enzymes within the thyroid are selenoproteins, that depend on selenium. Selenium may have beneficial effects on autoimmune hypothyroidism and on Graves' orbitopathy, but the effects of selenium on Graves' hyperthyroidism is not entirely investigated. We assume that supplementation with selenium may be advantageous in the treatment of Graves' hyperthyroidism.

The objective is to investigate whether a maintenance selenium supplementation after standard treatment with anti-thyroid drugs, according to protocol entitled "Remission Induction and Sustenance in Graves' Disease (RISG1)", followed by fixed low-dose antithyroid drug treatment (FLATD), for patients in remission (RISG2), versus observation will lead to increase a prolongation of remission in patients with Graves' hyperthyroidism.

Methods. We describe two cohorts of patients with Graves' hyperthyroidism in remission. Participants from the observation subgroup in the RISG2 trial were invited to participate in the seRISG protocol, where patients received selenium supplementation for 24 months vs. continued observation. 24 participants were assigned to receive organic selenium, namely a 100 µg tablet orally once daily. 33 patients were included in the observation group.

Results. At a follow-up after 24 months and at the final protocol-defined analysis, comprehensively examining outcomes of relapse, disease-free survival was confirmed in 17 (73.9%) of 23 patients who received maintenance supplementation of selenium and in 22 (66.7%) of 33 patients in the observation group. The log-rank test does not weigh statistical significance (the actual log-rank p-value= 0.567). The Breslow test also does not assess statistical significance in our analysis (p-value= 0.525).

Conclusion. A positive but rather weak tendency for prolongation of remission was detected in the selenium supplementation group. The statistical analysis shows that the primary endpoint — relapse disease-free survival — did not differ significantly between the two investigated groups.

Introduction

The pathogenesis of Graves' Disease (GD) is complex and complicated. It is a disorder with systemic manifestations that primarily affect heart, skeletal muscle, eyes, skin, bone, and liver (1). Like all autoimmune diseases, it occurs more commonly in patients with a positive family history. It is more common in monozygotic twins than in dizygotic twins. It is precipitated by environmental factors like emotional stress, iodine excess, infections, smoking, and postpartum, as well as after highly active antiretroviral therapy (HAART) due to immune reconstitution (2). GD is the most common cause of hyperthyroidism in people under 60 years, accounting for 60% to 80% of hyperthyroid cases. Every year in Denmark there are registered around 5000 new cases of the hyperthyroidism equivalent to an incidence of 81,6/100,000(A). GD is more common in women than men. Some data suggest that lifetime risk in women and men are 10,5% and 2,4% respectively (2), 3,4).

GD is an autoimmune disorder that depends on the presence of T lymphocytes against antigens that infiltrate thyroid tissue, orbital tissue and extraocular muscles (5). In GD, a circulating autoantibody against the thyrotropin receptor provides continuous stimulation of the thyroid gland. This stimulatory immunoglobulin is diagnostic for GD and has been called TSH-receptor antibodies (TRAb) (6). TRAb bind to and activate thyrotropin receptors, causing the thyroid gland to grow and the thyroid follicles to increase synthesis of thyroid hormone independent of endogenous trophic hormones, resulting in consecutive hyperthyroidism. B lymphocytes primarily synthesize TSH-receptor antibodies within the thyroid cells, but it can also be synthesized in lymph nodes and bone marrow. B lymphocytes are stimulated by T lymphocytes which get sensitized by antigen in the thyroid gland (7).

Graves' orbitopathy is the most common extrathyroidal manifestation of Graves' Disease. Graves' orbitopathy (ophthalmopathy) is caused by inflammation, cellular proliferation and increased growth of extraocular muscles and retro-orbital connective and adipose tissues due to the actions of thyroid stimulating antibodies and cytokines released by cytotoxic T lymphocytes (killer cells) (8). These cytokines and thyroid stimulating antibodies activate periorbital fibroblasts and preadipocytes, causing synthesis of excess hydrophilic glycosaminoglycans (GAG) and retro-orbital growth. These changes give rise to proptosis, diplopia, congestion, and periorbital edema (8).

Treatment for Graves' Disease depends on its presentation. Treatment consists of rapid symptoms control and reduction of thyroid hormone secretion, using antithyroid drugs (ATD), or by reducing the amount of thyroid tissue with radioactive iodine treatment of the thyroid gland (RAI treatment) or total thyroidectomy (8).

ATD represent the predominant therapy in Europe, Asia, and in USA as well. The main ATD are thionamides, such as propylthiouracil (PTU), carbimazole (CBZ), and the active metabolite of the latter, methimazole (MMI). Common side effects of ATD are rash, urticaria, and arthralgia (1–5%). But all three options have pros and cons, and there is no consensus

on which one is the best option. It is very important to discuss all three options in detail with the patients and make an individualized decision (9).

The optimal duration of ATD therapy for the titration regimen is 12–18 months (10). Continued L-T4 treatment following initial ATD therapy does not provide any benefit in terms of the recurrence of hyperthyroidism (10). Maximum remission rates (50–55%) are achieved within 12–18 months.

Relapse is most likely within the first 6–12 months after ATD withdrawal, but may occur years later. Patients with severe hyperthyroidism, large goiters, or persistent high titers of TRAb are most likely to relapse when treatment stops, but the outcome is difficult to predict. All patients should be followed closely for relapse during the first year after treatment and at least annually thereafter (9).

A meta-analysis from 2013 has confirmed the high relapse rate following ATD therapy (52.7%) in comparison with RAI (15%, OR 6.25) or surgery (10%, OR 9.09), along with a substantial side-effect profile for these drugs (13%) (11).

In Graves' Disease, reactive oxygen species (ROS) contribute to the thyroid and peripheral tissues damage (12). The increase in ROS production, as well as the decrease in their elimination, can disrupt the balance of the cell redox state, resulting in oxidative stress and breaking of cellular homeostasis (13). A long-lasting state of oxidative stress is characterized by low levels of antioxidant markers because of exhaustion of the antioxidant cell systems (13). The ROS-induced damage of thyroid epithelial cells could lead to an increased release of autoantigens and production of TRAb, and the peripheral tissue damage might contribute the clinical manifestations of hyperthyroidism (12) It has been reported that the levels of oxidative stress markers, in the serum, plasma and erythrocytes of hyperthyroid patients were higher than in euthyroid subjects (12), (14).

Based on the role of oxidative stress in the pathogenesis of Graves' Disease and Graves' Orbitopathy, a therapy with the antioxidant agent selenium has been proposed and a number of studies have been performed(5) (12), (13).

Because of the high risk of relapse, it is essentially important to do a proper and bright analysis of risk aspects, search for prognostic factors for relapse and predictive markers for optimal therapy. It is relevant as never to do adequate research for new strategies to minimize this risk. Identifying biomarkers in GD must be an integral part of choosing treatments and understanding disease progression.

The present study is part of a multiphase study (RISG) of which the first part (RISG1) aimed to improve the knowledge on how and when GD enters remission in individual patients during ATD therapy and to study the usefulness of regular TSH-receptor antibodies (TRAb) measurements during treatment. The aim of the second part (RISG2) was to evaluate if remission can be sustained in a subgroup of patients by a more prolonged low dose ATD therapy (15,16).

The present study has yet another aim: To investigate the effectiveness of selenium supplementation versus no treatment during 24 months after cessation of ATD treatment to reduce the relapse rate in Graves' Disease with remission.

Methods

Trial Design and Oversight

We conducted an open sequential block trial in which a standardized program of ATD therapy was administrated as conventional treatment of Graves' hyperthyroidism. All patients were included after having followed the RISG1 program. The subgroup with confirmed remission were included in this study and were allocated to just observation or supplementation of selenium. The trial was managed in accordance with regulatory requirements and was approved by the local ethical committee North Denmark Region Committee on Health Research Ethics.

Recruitment was carried out in two Danish endocrine referral centers in Aalborg (Aalborg University Hospital) and Copenhagen (Herlev University Hospital) between January 2007 and June 2011.

Inclusion criteria and study protocol have been described in detail in previous publications (15,16)

In brief, standard history of illness and clinical examination was performed. A questionnaire on previous thyroid disease, intake of medication, including estrogen and nutritional supplements, smoking habits and family history of thyroid disease was filled out. Thyroid ultrasonography was performed with the measurement of thyroid volume and the eyes were evaluated for signs and symptoms of Graves' orbitopathy.

Blood samples were drawn at baseline and after 1 month, 3 months, 6 months, 9 months, 12 months and 24 months.

Inclusion of patients. Patients with newly diagnosed Grave's hyperthyroidism were recruited in the study. Grave's Hyperthyroidism had been confirmed based on measurement of TSH < 0.01 mU/L with reference intervals of (0.3 - 4.0mU/L), total T3 above 3.0 nmol/L (1.1 – 2.5nmol/L)) and TRAb $\geq$ 1.0 IU/L (< 0.1IU/L) in serum or duffuse increased uptake on a thyroid scintigram were present. The standardized ATD therapy was conducted (15,16).

Patients were excluded: age < 18 years, pregnancy within one year, moderate to severe orbithopathy with a need of medical immunosuppressive therapy, other treatment with immunomodulatory drugs, imminent or manifest thyrotoxic crisis, major co-morbidity that could prevent participants to continue protocol, intolerance to both Methimazole (MMI) and Propylthiouracile (PTU) allergy to the components in the selenium, previous surgical or radioiodine therapy or ATD therapy for Graves' Disease within two years, TRAb not measurable at time of diagnosis.

Remission was defined as negative TRAb (≤ 1.0 IU/L) with a normal TSH (> 0.4 mU/L) on a maximal MMI 5mg considered equipotent to PTU 100 mg after remission of disease for two months, and when ATD had been given for at least six months, or after a 24 months period of ATD. (15).

Relapse was defined as TSH < 0.01 mU/L, total T3 ≥ 3.0 nmol/L and TRAb ≥ 1.0 IU/L (15)

Maintenance supplementation of selenium

The compound used in trial was an organic selenium. It consists of white, round, prolonged-release tablets that contain 100 µg of selenium from more than 20 different organic yeast compounds, most of which is selenomethionine. The product is SelenoPrecise 100 µg tablets produced by Pharma Nord (www.pharmanord.dk). The daily dose is set at 200 µg, which is 2 tablets taken in the morning with meal. The tablets are manufactured in Denmark under quality control, and there is extensive documentation for bioavailability (EFSA).

Allocation

All patients who participated in RISG1 (n=208) and achieved remission (n=92) were invited to participate in the subsequent open-label study (termed RISG2) in which the patients were randomized to either continuous fixed low-dose antithyroid drug treatment (FLATD) (n=33) or no treatment control group (n=33) and followed the program for 24 additional months during the period from January 2011 to January 2013. Participants from no treatment subgroup were invited to participate in the seRISG protocol, as control group. 24 additional patients who have followed RISG 1, after completing the study underwent the same program as the no treatment group for three months, during the period of January 2013 to Marts 2013. Hereafter they received selenium supplementation for 24 months. The group with selenium supplementation and group where patients received no treatment followed the same observations procedures. Only patients from Aalborg participated in the SeRISG.

Trial appendix indicated calculated power analyze of up to 30 % effectiveness of the selenium treatment.

Figure 1: Overview of the seRISG study. There are 57 patients included in the present study.

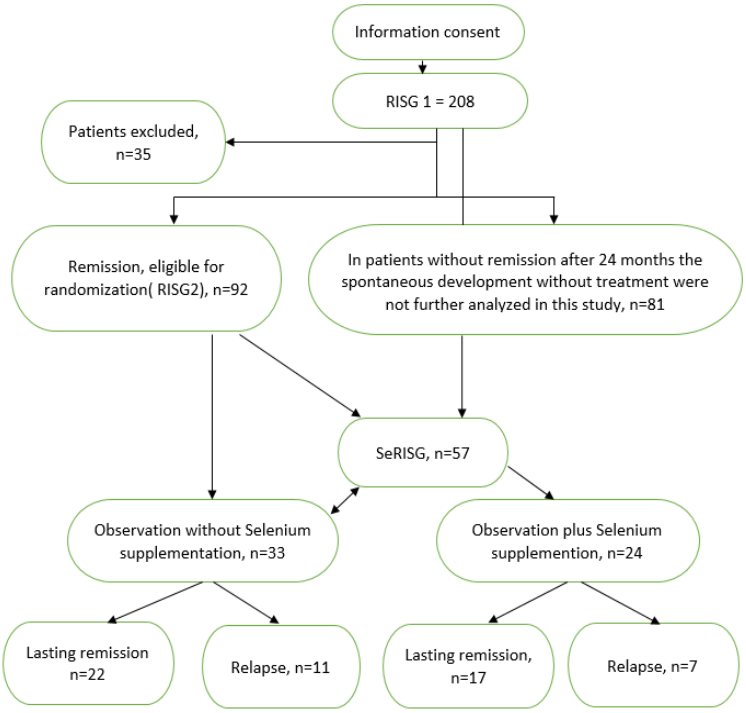


Figure: Overview of the SeRISG study. There are 57 patients included in the present study.

Statistical analysis

Data were analyzed using IBM SPSS statistics for Macintosh version 27.0 (Armonk, NY: IBM Corp. USA). There were conducted multiple descriptive analyses to report medians and interquartile ranges of the metric variables. For dichotomous variables relative frequencies were calculated. Data was checked for normality using PPlot. Mann Whitney U and Wilcoxon signed rank (metric variables) tests were used for comparisons between groups and within groups on numeric data. Chi square or Fisher's exact test (dichotomous variables) was used on categorical data. The log rank test and Breslow (Generalized Wilcoxon) was used to test for differences on the Kaplan-Meyer survival analysis. A p value < 0,05 was considered as statistically significant.

Results

The SeRISG enrolled 57 patients with GD in remission.

Table 1: shows the baseline characteristics of the patients at the time of diagnosing Graves' Disease.

	All	Se supplementation	Observation	p-value*

Participants, n (%)	57 (100)	24 (42)	33 (58)	_
Sex F/M (% F)	51/6 (90)	21/3 (88)	30/3 (91)	0.68
Age years, median (IQR)	45.00 (39.05-54.00)	50.50 (45.00-55.75)	42.00 (36.00-52.00)	**0.02**
Smoker, n (%)	17 (30)	7 (29)	10 (30)	1.00
Body height cm, median (IQR)	167.00 (162.50-173.50)	166.00 (163.50-175.25)	167.00 (160.25-172.00)	0.34
Body weight kg, median (IQR)	67.00 (58.10-78.30)	72.15 (58.55-87.53)	65.60 (56.80-73.30)	0.16
BMI, median (IQR)	24.30 (21.04-27.28)	24.50 (21.05-29.57)	23.40 (21.04-26.42)	0.48
Graves' orbitopathy, n (%)	24 (42)	10 (42)	14 (42)	1.00
Thyroid volume mL, median (IQR) R2	24.20 (14.80-31.60)	24.45 (14.30-32.10)	24.00 (15.90-32.25)	0.75
Number of month to remission in RISG1, median (IQR)	13.00 (10.00-18.00)	12.00 (8.50-18.00)	14.00 (10.00-18.00)	0.31
RISG1 S-T4 nmol/L, median (IQR)	199.00 (170.00-243.50)	192.00 (164.25-233.00)	200.00 (173.50-249.50)	0.46
RISG1 S-T3 nmol/L, median (IQR)	5.30 (4.10-7.45)	5.70 (4.08-7.60)	4.70 (4.10-6.75)	0.38
RISG1 S-TSH, mU/L, median (IQR)	0.005 (0.005-0.005)	0.005 (0.005-0.005)	0.005 (0.005-0.005)	0.45
RISG1 S-TRAb, IU/L, median (IQR)	6.50 (3.55-10.00)	6.95 (4.53-9.95)	5.80 (3.20-10.50)	0.62

*Mann -Whitney or Chi square/Fisher exact test as appropriate.
n = number.
M = male.
F = female.
IQR = interquartile range.

The patients who entered remission were subsequently invited to participate in the randomized open-label study (termed RISG2)

The baseline characteristics of patient's data from seRISG protocol is illustrated in Tabel 2.

Table 2: Clinical Characteristics of the patients at entry to RISG2.

	All	Se supplement	Observation	P value*
Participants, n (%)	57 (100)	24 (42)	33 (58)	-
Sex F/M (% F)	51/6 (90)	21/3 (88)	30/3 (91)	0.67
Age R2 years, median (IQR)	47.00 (40.50-55.50)	52.00 (46.25-56.75)	43.00 (36.50-53.00)	**0.02**
Smoker R2, n (%)	17 (30)	8(33)	9 (27)	0.77
Body height cm, median (IQR)	167.00 (162.50-173.50)	166.00 (163.50-175.25)	167.00(160.25-172.00)	0.34
Body weight R2 kg, median (IQR)	72.90 (62.35-84.15)	79.75 (61.90-94.75)	69.50 (62.35-78.65)	0.20
BMI R2, median (IQR)	26.13 (22.91-29.41)	26.54 (22.96-32.10)	26.00 (22.91-28.94)	0.73
Graves' orbitopathy R2, n (%)	17 (30)	5 (21)	12 (36)	0.25
Thyroid volume R2 mL, median (IQR)	19.50 (12.70-30.80)	19.40(13.23-30.90)	19.70 (12.40-31.35)	0.88
RISG2 S-T4 nmol/L, median (IQR)	93.00(82.50-107.50	92.00 (81.25-104.75)	97.00 (83.50-111.50)	0.33
RISG2 S-T3 nmol/L, median (IQR)	1.70 (1.45-1.90)	1.80 (1.53-2.00)	1.70 (1.40-1.80)	0.16
RISG2 S-TSH mU/L, median (IQR)	2.15(1.43-2.90)	2.35 (1.45- 3.38)	1.70 (1.43-2.54)	0.13
RISG2 S-TRAb IU/L, median (IQR)	0.50 (0.20-0.80)	0.50 (0.20-0.90)	0.50 (0.30-0.75)	0.88

*Mann -Whitney or Chi square/Fisher exact test as appropriate.
n = number.
M = male.
F = female.
IQR = interquartile range.

We evaluated 57 patients with confirmed remission, of which 33 participants in control group have followed RISG-2 study for 24 months and 24 participants with conformed remission after RISG1 who were assigned to receive 2 years of organic selenium, 200 µg tablet orally once daily. Comparing the baseline data from both groups at the point of entry of RISG1 and RISG2 there is no statistical significance in characteristics except the age with p value < 0.05, which is not unusual due to a long duration of the trials. There is, however, no statistical significance when comparing the remission rate of male and female respectively. There is also no significant difference in regard to the remission rate comparing patients under 45 years and above (data not shown).

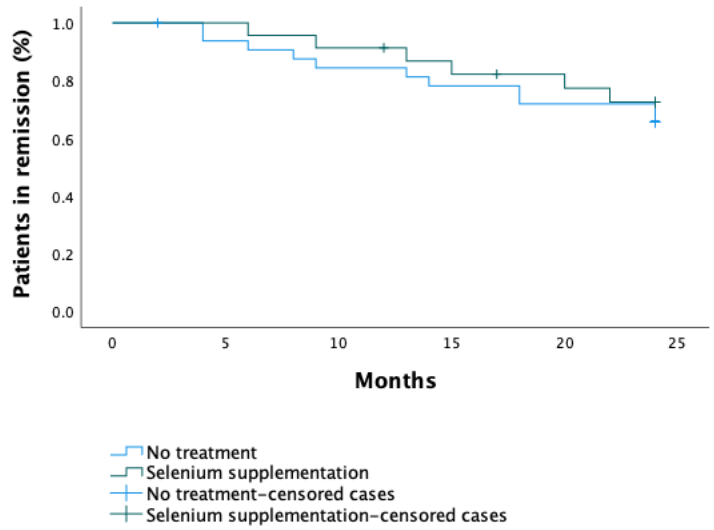
In our trial 17 patients were Smoker (30%), 8 in selenium group (33%) and 9 in the observation (27%). Statistically we are not able to detect a difference according to smoking status and relapse (p = 0.77). The rest of data were well balanced when it came to sex and smoker status, BMI, Graves' orbitopathy, Thyroid volume, T4, T3, TSH and TSH-receptor antibodies (TRAb) parameters.

At a follow-up of 24 months and at the final protocol-defined analysis, comprehensively examining outcomes of relapse, disease-free survival was confirmed in 17 (73.9%) of 23 patients who received maintenance supplementation of selenium and in 22 (66.7%) of 33 patients in the observation group, **Table 1**.

Case Processing Summary				
seRISG	All patients	Nr.of recidiv	Censored patients	
	n	n	n	Percent (%)
No treatment	33	11	22	66.7
Selenium supplementation	23	6	17	73.9
Overall	56	17	39	69.6

Table 3: Outcomes of relapse, disease-free survival in seRISG.

Figure 2: Kaplan–Meier Analysis of Response Duration and Outcomes of relapse, disease-free survival.



We used to different tests of equality of disease-free survival. These tests are based on a Chi-square distribution. We applied the log-rank test, all time points are weighted equally in this test. Compare disease-free survival given a selenium maintenance treatment with the

control group, the log-rank test does not assess statistical significance (the actual log-rank p-value= 0.567). Breslow test for comparing the equality of disease-free survival distributions helps to evaluate time points that are weighted by the number of cases at risk at each time points, also not weighs statistical significance in our analysis (p-value= 0. 525). **Table 4.**

	Chi-Square	df	Sig.
Log-Rank (Mantel-Cox)	.327	1	.567
Breslow (Generalized Wilcoxon)	.403	1	.525

Table 4: Test for comparing the equality of disease-free survival distribution.

Finally, the statistical analysis shows, that the primary endpoint — relapse disease-free survival — indicated no statistically significant differences between the two treatment groups.

Discussion

This open sequential block trial has had a goal to evaluate the effectiveness of maintenance selenium supplementation versus observation after a standardized program of ATD in patients with sustained remission. This to reduce the relapse rate further or merely stabilize the clinical status. The SeRISG study however has shown that maintenance supplementation of selenium does not prolong disease-free survival compared to observation. Our results are consistent with those of Kahaly GJ. et al. (17). They included 61 patients with untreated GH and patients were randomized to receive Methimazole plus selenium or methimazole alone for six months. Addition of selenium did not seem to improve the clinical outcome, response rate, or benefit neither clinical progression nor recurrence. The response to treatment in terms of thyroid function normalization of thyroid hormones was very similar in the two groups ATD + selenium vs. ATD + placebo at week 24. During a 12-week follow-up, GH relapsed in 48% of patients included in the selenium group and in 44% of patients of the placebo group. Serum concentrations of selenium and selenoprotein P were unrelated to response or recurrence rates. Follow-up at week 36 and 50, has found 12 of 29 patients (41%) and 15 of 33 patients (45%) were responders and still in remission in the selenium and placebo groups respectively (23). This data is well comparable with our study results, where the 17 of 23 (73.9%) and 22 of 33 (66,7%) in selenium supplementation group and control group respectively remained in remission 24 months after ATD treatment ceased.

A study by Leo M. et al (18) also showed no beneficial effect of selenium on short term control of hyperthyroidism. Administration of ATD + selenium vs. ATD lead to the normalization of FT3 and FT4 with no difference between groups. Serum levels of malondialdehyde, a marker of oxidative stress, were similarly high in the two groups and decreased significantly after therapy with no statistical difference between groups. The

results suggested that selenium had no significant effect on short-term control of hyperthyroidism.

In contrast to our study, results from a small Scandinavian study by Calissendorff J. et al. are positive, and they reported beneficial effects of using selenized yeast as a supplement (200 µgSe/d) over a course of nine months where improved biochemical control of thyroid disfunction under a block and replace regimen was observed (19). At 18 weeks, the serum levels of FT4 were lower in the selenium group compared to the placebo group (14 vs. 17 pmol/l group, p = 0.01). Similar results were also observed at 36 weeks (15 vs. 18 pmol/l, p = 0.01). In accordance, the TSH levels increased more in the selenium group at 18 weeks (0.05 vs. 0.02 mIU/l, p = 0.04) (19)

Positive effects of selenium supplementation have also been reported from a small study with a higher remission rate and improvement of laboratory parameters of hyperthyroidism in patients with recurrent GD receiving supplemental selenium for six months (20).

These conflicting results regarding the supplemental role of selenium could most likely be explained by different antithyroid drug treatment regimens, different baseline selenium status of patients, different selenium supplementation schedules, doses, period treatment time and different follow-up period.

A strategy using the antioxidant mediator selenium in patients with newly diagnosed Graves' hyperthyroidism has been evaluated in some studies. Antioxidant modulators have been used to increase the effects of antithyroid drugs in Graves' Disease patients, as well as the remodeling of orbital tissues in patients with Graves' Orbitopathy (5), (12,13),(19,21)(18) In Graves' Disease, reactive oxygen species (ROS) cause the thyroid and peripheral tissues injury, resulting in increased antigen exposure to the immune system and worsening of autoimmunity.

Selenium is a trace mineral which acts following incorporation as selenocysteine into selenoproteins, among which thioredoxin reductases (TRs), Glutathione peroxidase (GPX), and iodothyronine deiodinases (D1, D2 or D3) are the best known (12) Selenium is more densely concentrated in the thyroid gland than in any other tissue in the body. Selenoproteins have antioxidant and enzymatic capacity and, in the thyroid, where they are highly expressed, influence the balance of the cell reduction-oxidation activities (12).

Table 5: Overview (22)

Main groups of selenoproteins found in the thyroid gland and their function		
Glutathione peroxidase	GPX	• Catalyzes the reduction of H2O2 and protects against oxidative stress • Antioxidative defense • Responsible for glandular protection, since they remove the excess of oxygen free radicals produced during normal synthesis of the thyroid hormones
Iodothyronine deiodinase	DIO	Production of active thyroid hormone T3, reverse T3 (rT3), and T2 • Conversion of T4 to T3 • Local production (intracellular) of T3 from T4 • Production of rT3 from T4 and T2 from T3
Thioredoxin reductase	TXNRD	Oxidoreductase activity having NADPH as a cofactor • Main antioxidant at the cellular level • Regulates cell proliferation

Some antioxidant agents, specifically glutathione (GSH), superoxide dismutase (SOD) and glutathione peroxidase (GPX), act as ROS antagonists, consequently improving the maintenance of the cell redox state (12). Restoration of euthyroidism with antithyroid drug is associated with a reversal of the biochemical abnormalities associated with oxidative stress. The degree of cell damage in Graves' Disease has an indirect correlation with the level of oxidative stress, which depends on the efficacy of the applied therapy and capacity of the antioxidant defense of the organism.

Some studies suggest that patients with hyperthyroidism have lower level of plasma selenium than normal controls (23).

Long-term ATD treatment is beneficial for Graves' Disease and Graves' Orbitopathy due to the normalization of thyroid function and the associated decline of TSHR-Ab serum levels, which are a biomarker for Graves' Disease and Graves' Orbitopathy (24),(16). Choice of treatment should be based on the assessment of clinical activity and severity of Graves' Disease. Because of the high risk of relapse, it is essentially important to do a proper and bright analysis of risk aspects, search for prognostic factors for relapse and predictive markers for optimal therapy.

Two clinical studies were performed to investigate details of remission induction in GD patients, RISG trials (15)(16). In the first part of this trial (RISG1) was found as marker of the

clinical expression of disease that TRAb values correlated positively with serum T3 (rs = 0.54, P < 0.001) and serum T4 (rs =0.31, P < 0.001), with the presence of eye signs and symptoms of orbitopathy (r = 0.15, P = 0.036), and with thyroid volume (r = 0.25, P < 0.001). However, statistic significance of the correlation of some coefficients was rather low (15).

The RISG2 evaluated the predictors of attaining (part 1) and sustaining (part 2) remission in patients with GD treated with antithyroid drugs (ATD). Baseline TRAb was the only significant predictors otherwise prognostic factor for attaining remission in GD (17). In the part 2 of the study, the patients randomized to continue the treatment (n=33) sustained remission was achieved in 96.4%, compared to 66% in the observation group (n=33).

In RISG project we used a definition of remission as TRAb < 1.0 IU/L and TSH ≥ 0.40 mU/L on a MMI dose of 5 mg or less per day evaluated twice, two months apart. Results of the RISG are satisfying in patients randomized to 24 months of observation after entering remission as 66 % were still in remission after two years of observation (16). Unfortunately, we have a significant limitation for evaluation of T3, T4, TSH and S-TRAb and thyroid volume parameters in the observation time of 24 months, as a weighty number of values are missing.

This study has had the goal of evaluating the effectiveness of maintenance selenium supplementation versus observation after a standardized program of ATD in patients with sustained remission, to reduce the relapse rate further or merely to improve the clinical status. The trial was based on strong theoretic science and some positive clinical results. The SeRISG study has shown that maintenance supplementation of selenium does not prolong disease-free survival compared to observation. Relapse and disease-free survival did not differ in a statistically significant way when comparing the two observed groups. But there was a clinically meaningful positive tendency in the maintenance selenium supplementation group.

This study has three main limitations. First, the SeRISG has a small number of patients. However, the tendency for prolongation of disease-free survival is important to suggest that a larger confirmatory study is needed, i.e., for high-risk patients with severe manifestations of hyperthyroidism and persistently high TSH-R-Ab levels, or probably only for patients with moderate Graves' Hyperthyroidism. Second, there was a lack of data of comorbidity in both groups, which could be possible additional risk factors for relapse. Third, the lack of data of the thyroid volume and the parameters in the intensive observational period of time in both groups. It means that a substantial limitation of the study is the lack of thyroid volume data in the 12 and 24 months of observation. We have had only baseline characteristics for our disposal.

The luck of data is an essential objective limitation and could invariably lead to misinterpretation, errors and biases. Detailed baselines of the selenium status in the patients, well defined selenium supplementation schedules along with ADT or as maintenance, a longer follow-up period, and definitely a significantly larger population in stratification subgroups could give us more consistent and reliable results in the future.

A Danish trial, The GRASS (GRAves' disease Selenium Supplementation trial) trial is an investigator-initiated, randomized, blinded, multicenter clinical trial of selenium supplementation versus placebo in patients with Graves' hyperthyroidism. It is part of the ThyQoL project, a strategic project headed by Ulla Feldt-Rasmussen and funded by the Danish Agency for Science, Technology and Innovation (13). The objective of this trial was to explore if selenium supplementation plus standard treatment with anti-thyroid drugs versus standard treatment with anti-thyroid drugs will lead to a decrease in anti-thyroid drug treatment failure (that is, failure to remain euthyroid without further treatment, one year after cessation of anti-thyroid drug treatment), faster and longer lasting remission (that is, anti-thyroid drug treatment success), and improved quality of life in patients with Graves' hyperthyroidis. It was of great importance to the initiators of this trial, that the results would be directly applicable to daily clinical practice. **The results of this study are still enthusiastically awaited.**

Conclusion

This trial, as it have been mentioned before, was the part of the bigger multiphase study, that have presented two small cohorts of patients with diagnosed Graves' Disease, who entered first a big RIGS1 study aimed to improve quality of ATD therapy, followed by a fixed low-dose antithyroid drug treatment strategy (RISG2) in patients with sustained remission. In actual study one group was treated with the maintenance selenium supplementation, patients in another group continued the observation. The aim of the study was to assess an additional reduction of the relapse frequency with selenium. This pilot study was not able to confirm the expected 30% improvement in treatment results, as mentioned in the methods chapter. This analysis of the SeRISG trial did not show a statistically significant improvement in the primary endpoint of disease-free survival. The final results from a small intervention trial do not confirm any benefits of adding selenium to a maintenance strategy in patients with Graves' Hyperthyroidism, which was detected in other trials. More than anything else, this trial reminds us of the absolute necessity of putting our ideas to the test and doing appropriately powered, correctly controlled, and well-conducted randomized trials. Larger multinational studies are needed to further investigate the benefit of selenium in Graves' Hyperthyroidism within bigger subgroups.