12 Congresso Nazionale AME
6th Joint Meeting with AACE
Bari, 7-10 Novembre 2013

Dinner Symposium

Is there a role for selenium in thyroid diseases?

Claudio Marcocci
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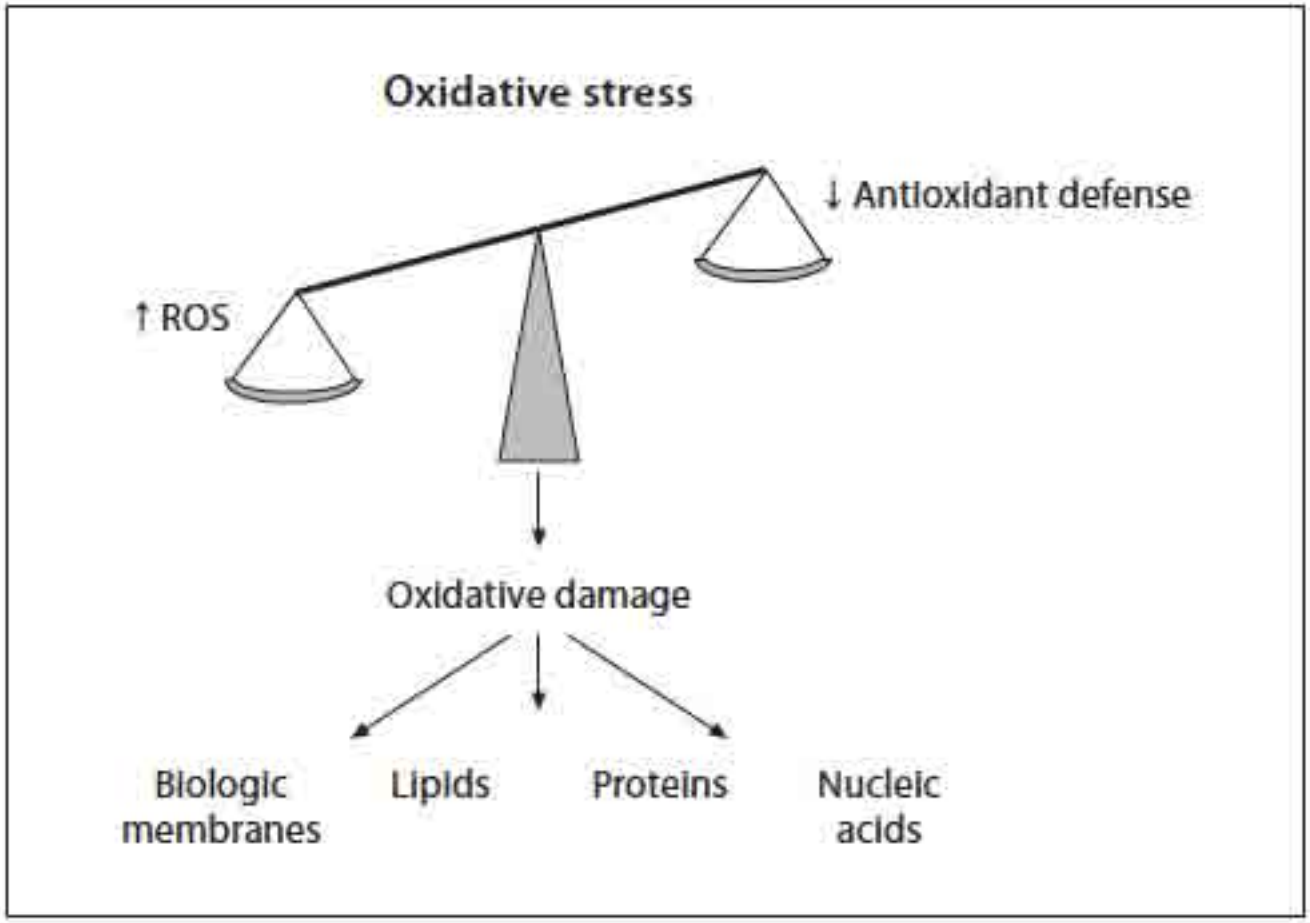
Mechanisms responsible for oxidative stress and cell damage



Importance of selenium to human health

- The trace element selenium is an essential nutrient of fundamental importance to human biology
- Selenium enters the food chain through plants, which take it up from the soil
- Selenium is incorporated as selenocysteine (21st amino acid) into several selenoproteins, some of which have important enzymatic activities
- Selenium functions as a redox center, but has additional important effects particularly in relation to the immune response and cancer prevention

Mammalian selenoproteins and their functions

Selenoprotein	Proposed function
Glutathione peroxidases (GPXs)	
GPX1	Antioxidant in cell cytosol; Selenium store?
GPX2	Antioxidant in GI tract
GPX3	Antioxidant in extracellular space and plasma
GPX4	Membrane antioxidant; structural protein in sperm; apoptosis?
GPX5	Unknown
GPX6	GPX1 homologue?
Thioredoxin reductase (TRs)	Multiple roles including dithiol-disulphide oxoreductase Detoxifies peroxides, reduces thioredoxin (control of cell growth); maintains redox state of transcription factors
TR1	Mainly cystosolic, ubiquitous
TR2	Expressed by testes
TR3	Mitochondrial, ubiquitous
Iodothyronine deiodinases	
Type D1 and D2	Converts thyroxine (T4) to bioactive 3,5,3′-tri-iodothyronine (T3)
Type D1 and D3	Converts thyroxine (T4) to bioinactive 3′, 3′, 5′ reverse T3
Selenoprotein P	Selenium-transport protein. Antioxidant on endothelium
Selenoprotein W	Antioxidant in cardiac and skeletal muscle?
Selenophosphate synthetase (SPS2)	Synthesis of selenophosphate for selenoprotein synthesis.
15 kDa Selenoprotein (Sep 15)	Protects against cancer?
H, I, K, M, N, O, R, S, T, V	Role largely unknown

GI, gastrointestinal.

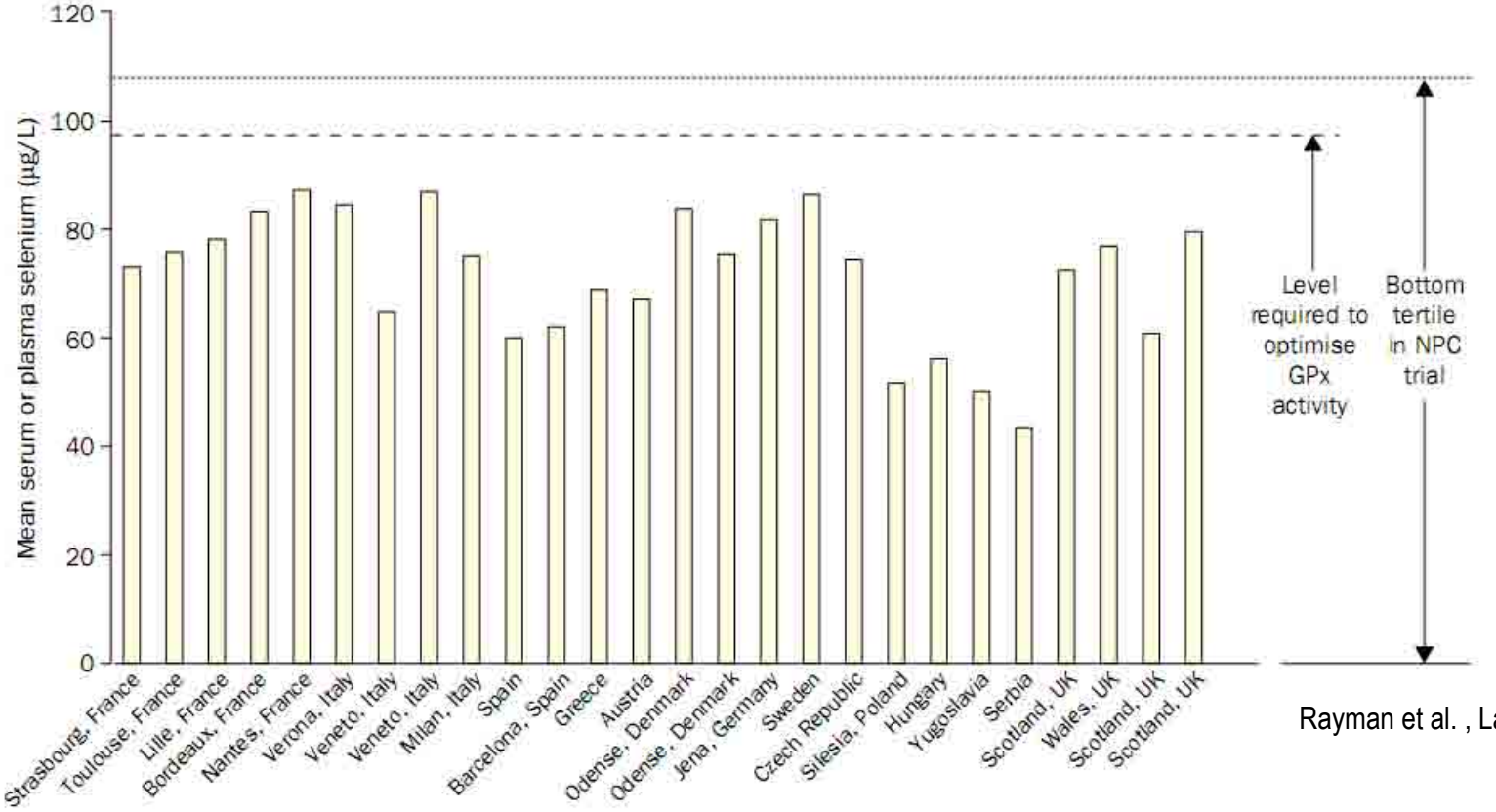
Recommended Se intake

Country	Intake (μg per day)	Information source
UK	29–39	UK Ministry of Agriculture, Fisheries, Food, 1997
Belgium	28–61	Robberecht and Deelstra, 1994
France	29–43	Lamand and colleagues, 1994
Germany (Bavaria)	35	Kumpulainen and Salonen, 1996
Netherlands	67	Kumpulainen, 1993
Denmark	38–47	Danish Government Food Agency, 1995
Sweden	38	Kumpulainen, 1993
Switzerland	70	Kumpulainen, 1993
Poland	11–24 (estimate)	Kvícala and colleagues, 1995, 1997
Slovakia	38	Kadrabova, 1998

- **UK Reference Nutrient Intake** **[maximal plasma GPx activity (serum selenium concentration of 95μg/L)]**
  - **75 μg per day for men**
  - **60 μg per day for women**

- **Office of Dietary Supplements, NIH**
  - **55 μg adult men and women**

Mean concentrations of serum or plasma Se in Europe



Rayman et al. , Lancet 2000

Nutritional Prevention of Cancer trial

Baseline plasma selenium (µg/L)	Selenium cases	Placebo cases	Relative risk	95% CI	p
<106	28	56	0·52	0·33–0·82	0·005
106–121	34	49	0·64	0·40–0·97	0·40
>121	45	41	1·00	0·65–1·54	0·99

Table 2: **Total cancers 1983–96 by plasma selenium level at baseline**

Clark et al., JAMA 1996

AGENDA

- **Selenium and autoimmune thyroid diseases**
- **Selenium and Graves' orbitopathy**

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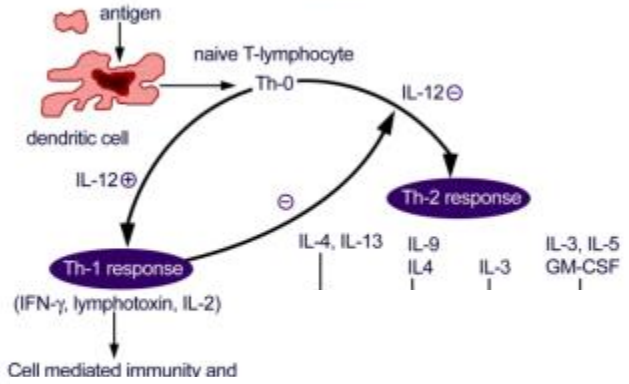
- **Selenium and Graves' orbitopathy**

Selenium and immune function

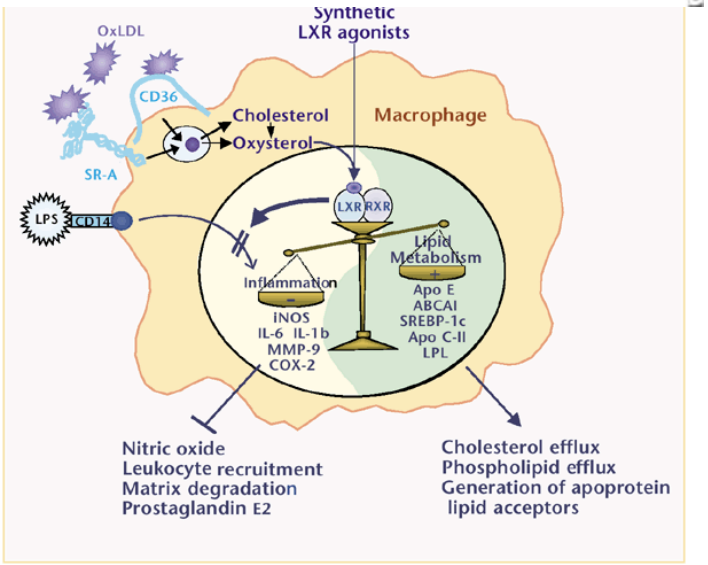
- **Selenium deficiency is accompanied by loss of immunocompetence**
  - **Impairment of humoral and cell-mediated immunity**
- **Selenium supplementation has immunostimulating effects (upregulated IL-2 receptor expression)**
  - **Proliferation of activated T cells**
  - **Increase of natural killer cell activity**
- **Immune cells have an important functional need for selenium**
  - **Activated T cells show upregulated selenophosphatase activity and increased synthesis of selenocysteine**
  - **mRNAs of several T cell-associated genes encode functional selenoproteins**

Inflammatory/immune response

Increased ROS is implicated in many immune/inflammatory pathologies



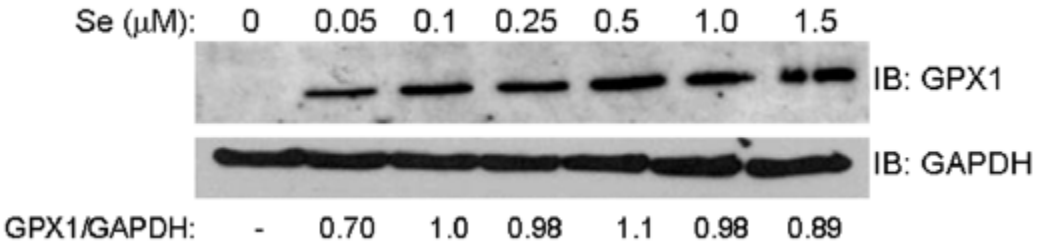
- Macrophages and dendritic cells present antigens to T lymphocytes
- Recruitment and activation of inflammatory cells
- Activated macrophages secrete pro-inflammatory enzymes, cytokines and chemochines which initiate and control the immune response

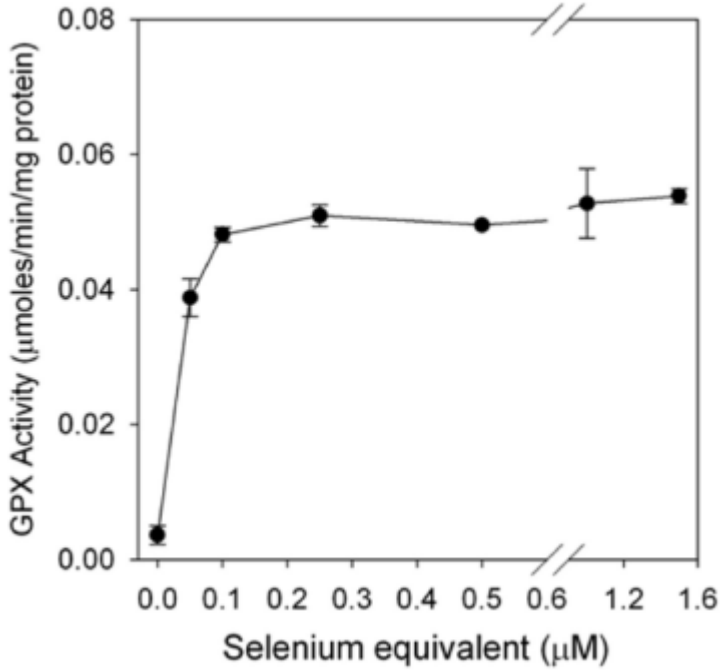


- Activated macrophages produce ROS
- Intracellular ROS represent a potential toxic insult which may led to cell death
- The antioxidant capacity of macrophages is very important for their survival
- In macrophages selenoproteins mitigate the cytotoxic effect of ROS

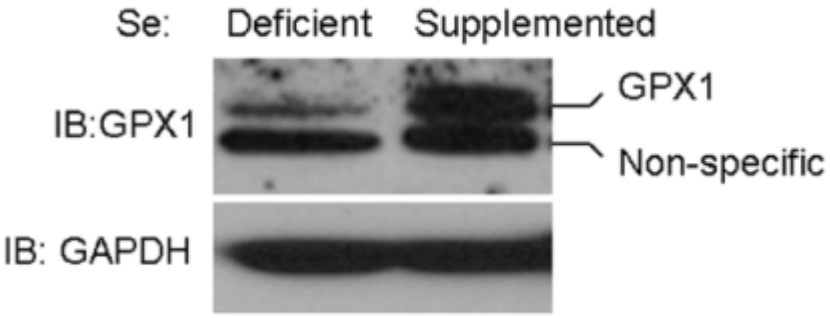
Effect of Se supplementation on GPX1 expression and activity

Murine macrophage cell line RAV264.7(ATCC)





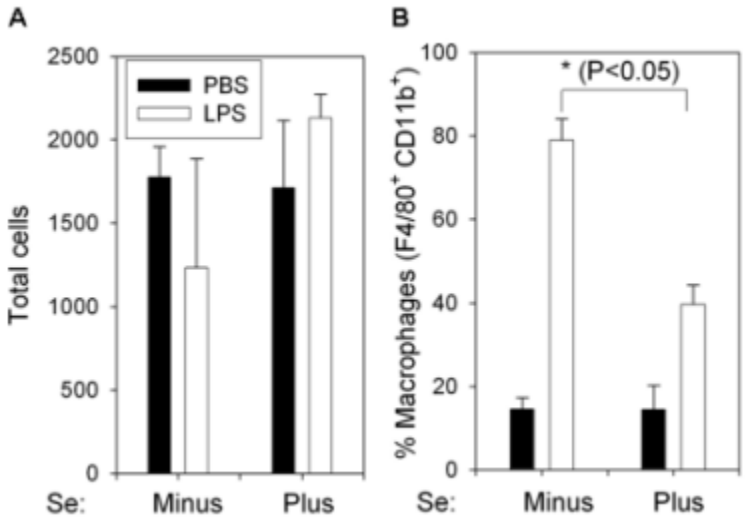
GPX1 expression in liver mouse homogenates



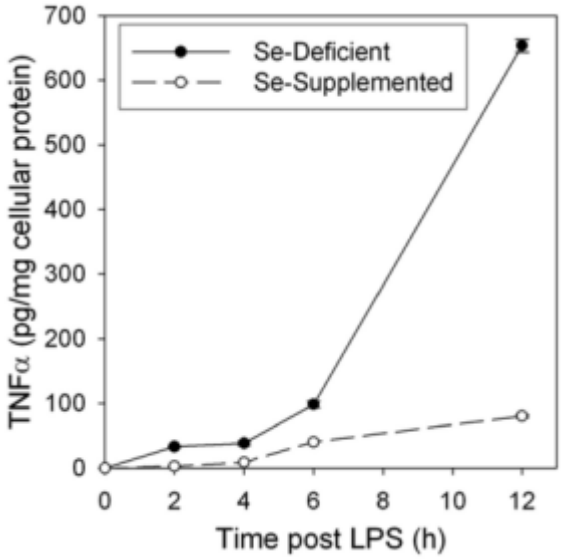
Vunta et al. Mol. Nutr. Food Res. 2008

Effect of intraperitoneal LPS injection in mice

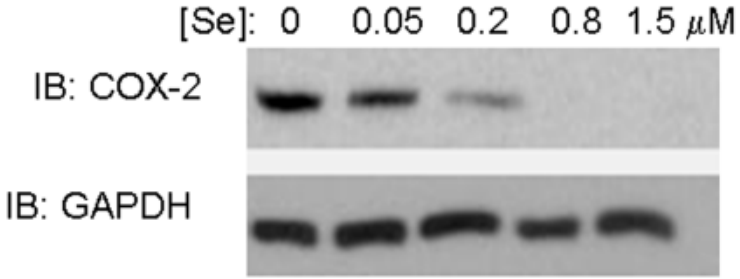
Recruitment of macrophages to the lung



Extracellular production of TNF-α by bone marrow derived macrophages

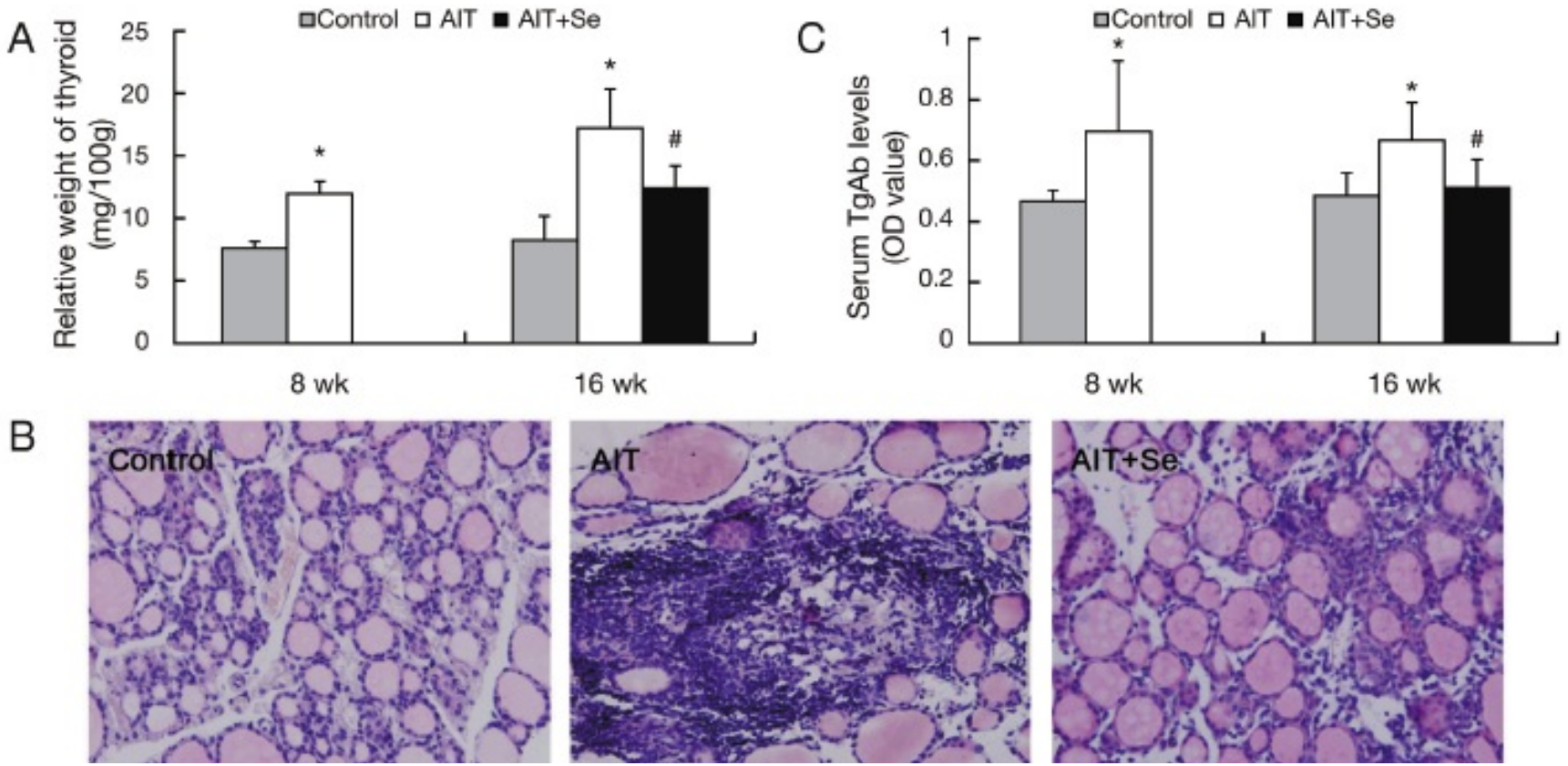


LPS-induced expression of COX-2 in RAW264.7 macrophages



Vunta et al. Mol. Nutr. Food Res. 2008

Selenium upregulates $CD4^+CD25^+$ regulatory T cells in iodine-induced autoimmune thyroiditis model of NOD.H-2^{h4} mice

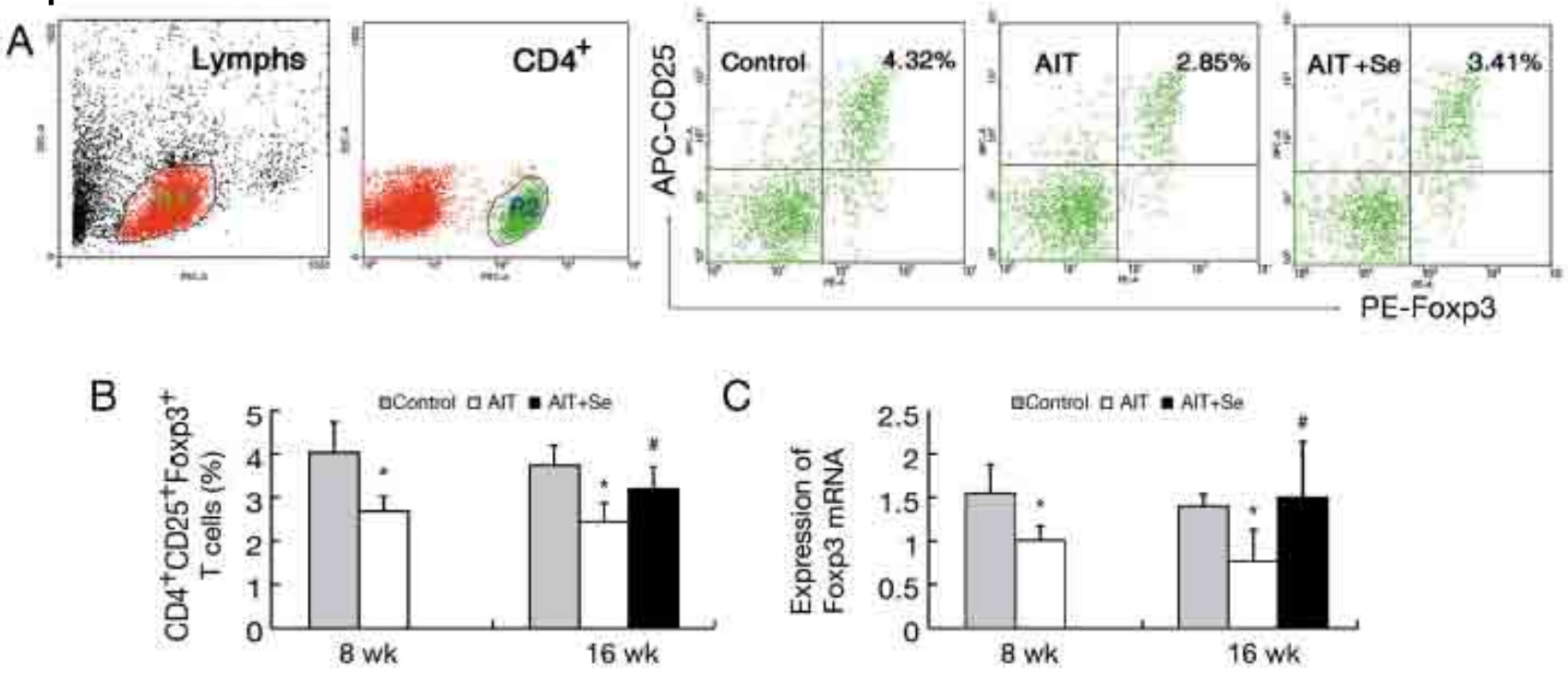


Xue et al., Endocrine Journal, 2010

Selenium upregulates $CD4^+CD25^+$ regulatory T cells in iodine-induced autoimmune thyroiditis model of NOD.H-2^{h4} mice

- **$CD4^+CD25^+$ regulatory T lymphocytes (Treg cells) contribute to the maintenance of peripheral self-tolerance and the prevention of autoimmunity**
- **Foxp3+ is a transcriptional regulator is critical for the function of Treg cells**

Splenic mononuclear cells



Xue et al., Endocrine Journal, 2010

Summary of results of randomized trials on selenium supplementation in autoimmune thyroiditis (Changes in TPO-Ab)

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

Reference	Intervention	On T_4 med.	No of subjects	Basal selenium concentration (μg/l)	TPO-Ab Initial (kU/l)	TPO-Ab at 3 months (kU/l)	TPO-Abat 6 months (kU/l)	Signif.
Gärtner *et al.*[12]	200 μg Na-selenite	Y	36	69 ± 12	904 ± 205	575 ± 46	–	$P = 0.013$
	Placebo		34	72 ± 12	1090 ± 277	959 ± 267	–	NS
Duntas *et al.*[13]	200 μg Selenomethionine	Y	34	75 ± 6	1875 ± 1039	1013 ± 382	884 ± 227	$P < 0.0001$
	Placebo		31	($n = 10$)	1758 ± 917	1389 ± 520	1284 ± 410	$P < 0.001$
Turker *et al.*[14]	200 μg Selenomethionine	Y	48	–	803 ± 483	572 ± 517	–	$P < 0.001$
	Placebo		40	–	770 ± 406	773 ± 372	–	NS
Karanikas *et al.*[17]	200 μg Na-selenite	Y	18	75 ± 11	524 ± 452	505 ± 464	–	NS
	Placebo		18	76 ± 12	521 ± 349	527 ± 354	–	NS
Nacamulli *et al.*[16]	80 μg Na-selenite	N	46*	–	172 (95% CI 100–295)	–	148 (95% CI 85–259)	NS†
	Placebo		30*	–	143 (95% CI 87–232)	–	126 (95% CI 77–208)	NS
Eskes, this article	200 μg Na-selenite	N	30	74 ± 14	1508 ± 1766	1681 ± 1694	1792 ± 1950	NS
	Placebo		31	76 ± 14	2045 ± 2265	1764 ± 2040	2053 ± 2431	NS

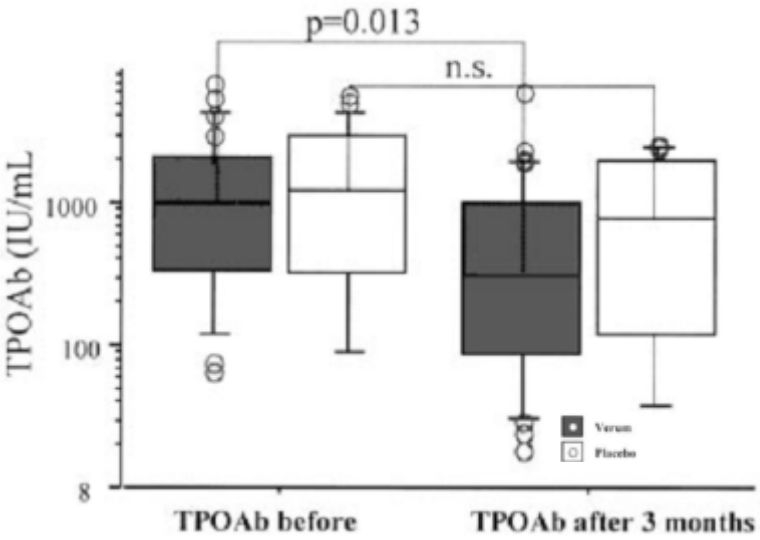
Y, yes; N, no. Values as mean ± SD.
*It is not described how many of these subjects were TPO-Ab positive.
†TPO-Ab were significantly decreased after 12 months selenite supplementation ($P < 0.001$).

Eskes et al.,Clin Endocrinol 2013

Selenium Supplementation in Patients with Autoimmune Thyroiditis Decreases Thyroid Peroxidase Antibodies Concentrations

ROLAND GÄRTNER, BARBARA C. H. GASNIER, JOHANNES W. DIETRICH, BJARNE KREBS, AND MATTHIAS W. A. ANGSTWURM

Seventy-one females with autoimmune thyroiditis and positive TPO Ab, receiving L-T_4, randomized to placebo or 200 µg selenium selenite



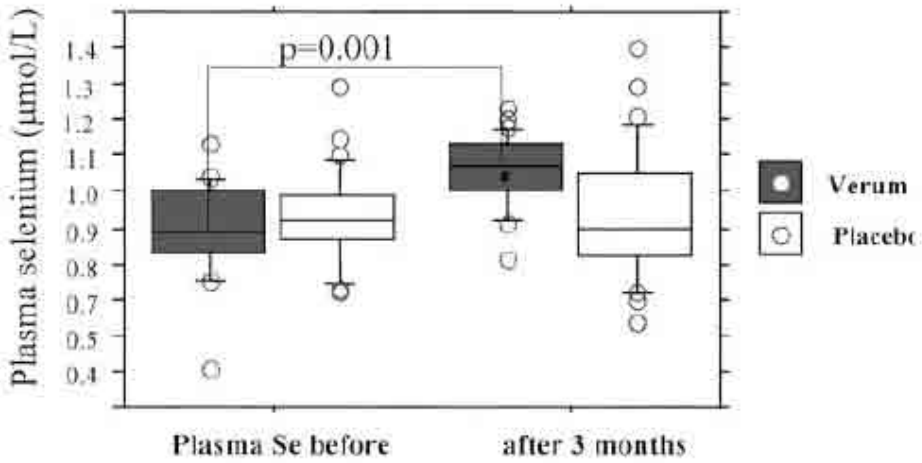
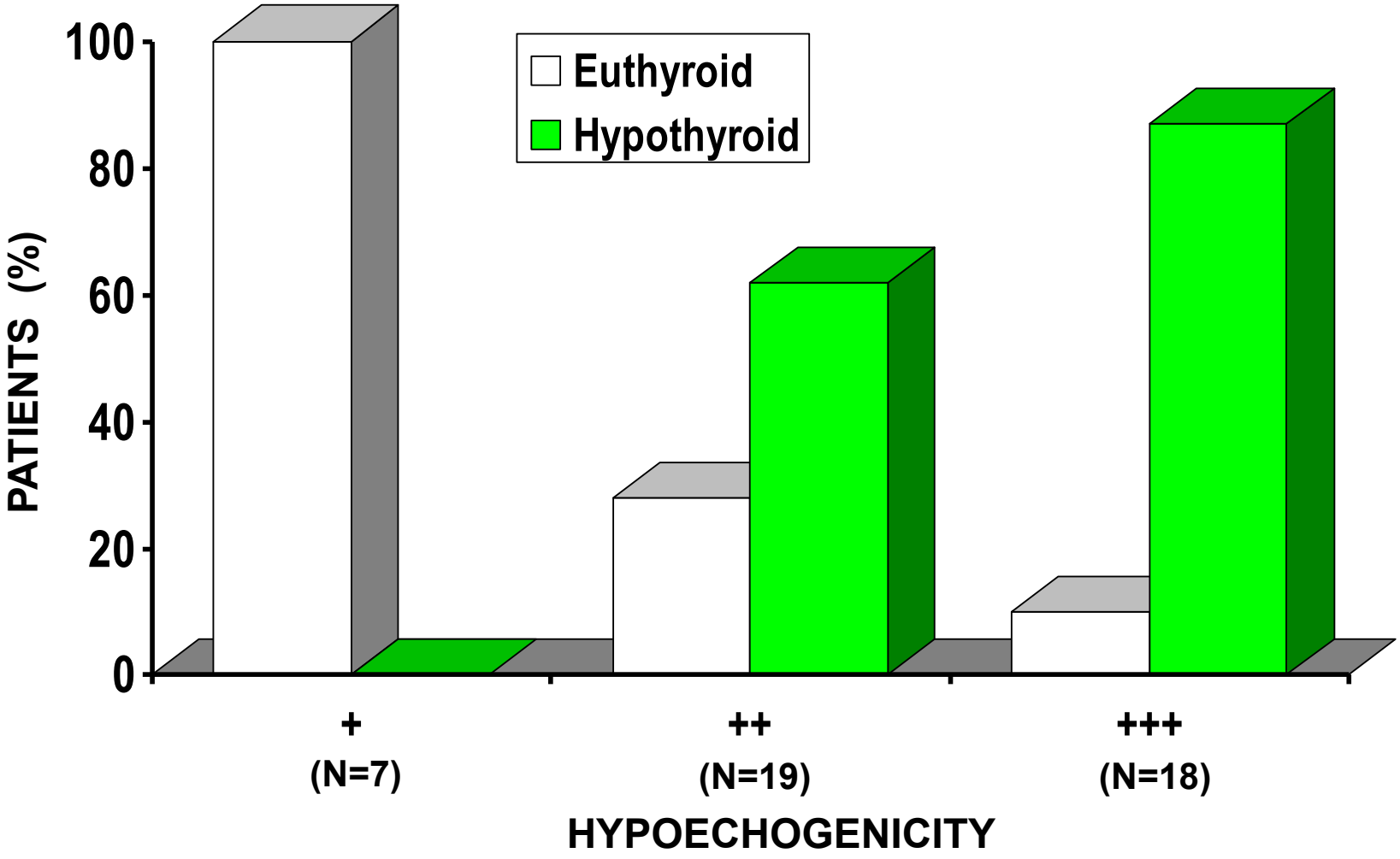


FIG. 2. Plasma selenium concentrations at study entry and 3 months after treatment with 200 μg (2.53 μmol) sodium selenite or placebo.

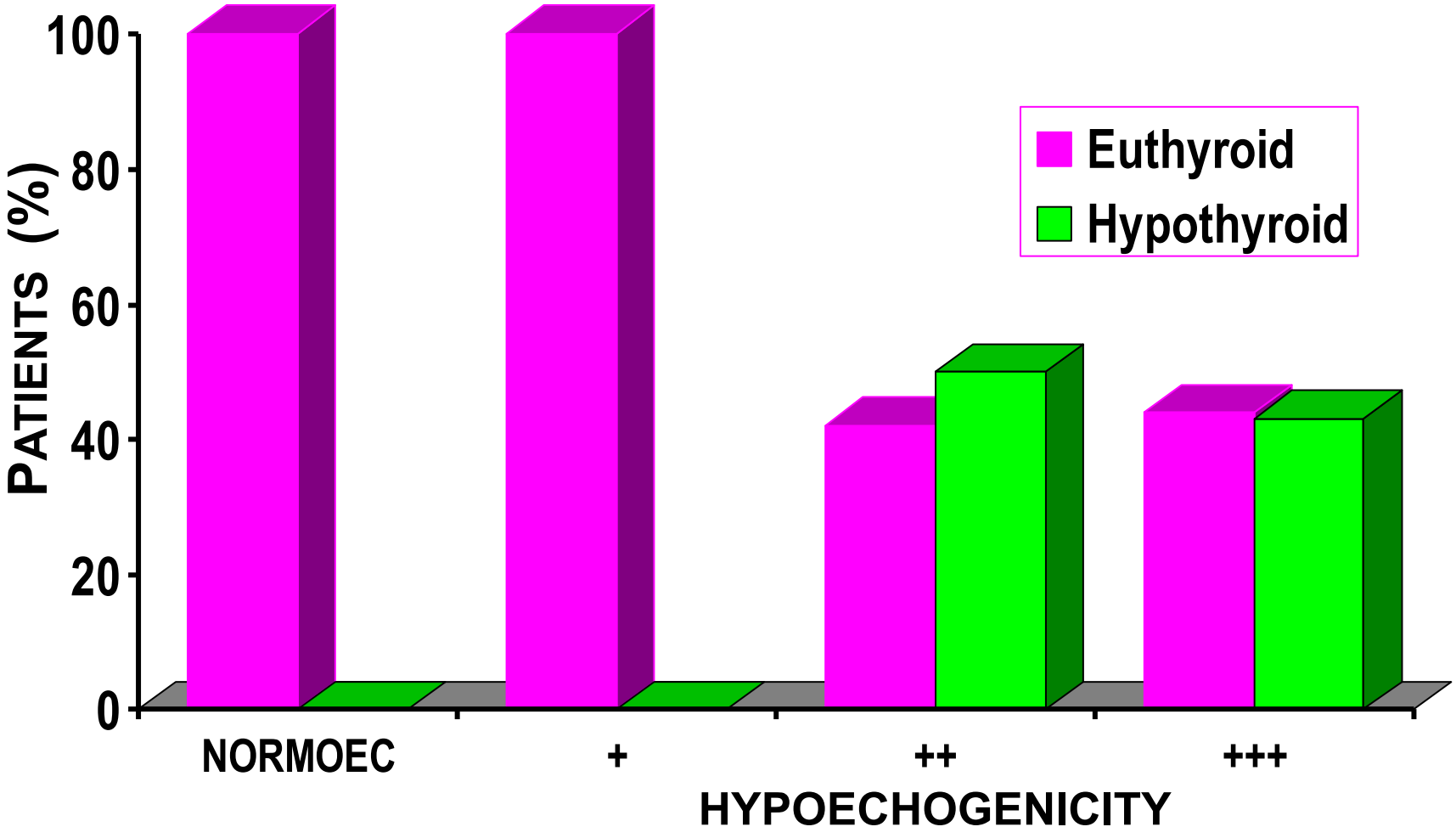
Gartner et al., JCEM 2002

Thyroid US and thyroid function in HT



Marcocci et al., JCEM 1990

Thyroid US and follow up of thyroid function in HT



Marcocci et al., JCEM 1990

Effects of a six month treatment with selenomethionine in patients with autoimmune thyroiditis

Leonidas H Duntas, Emilia Mantzou and Demetrios A Koutras

Sixty-five patients with hypothyroid AIT treated with LT_4 combined with 200 ug selenomethionine (Gr I) or LT_4 alone (Gr II) for 6 months
The study was carried out in a selenium-sufficient area

Table 2 Overall decrease in percentage of serum anti-TPO concentrations after 6 months of treatment with selenomethionine plus LT_4 (Gr I) or with LT_4 and placebo (Gr II) over a period of 6 months.

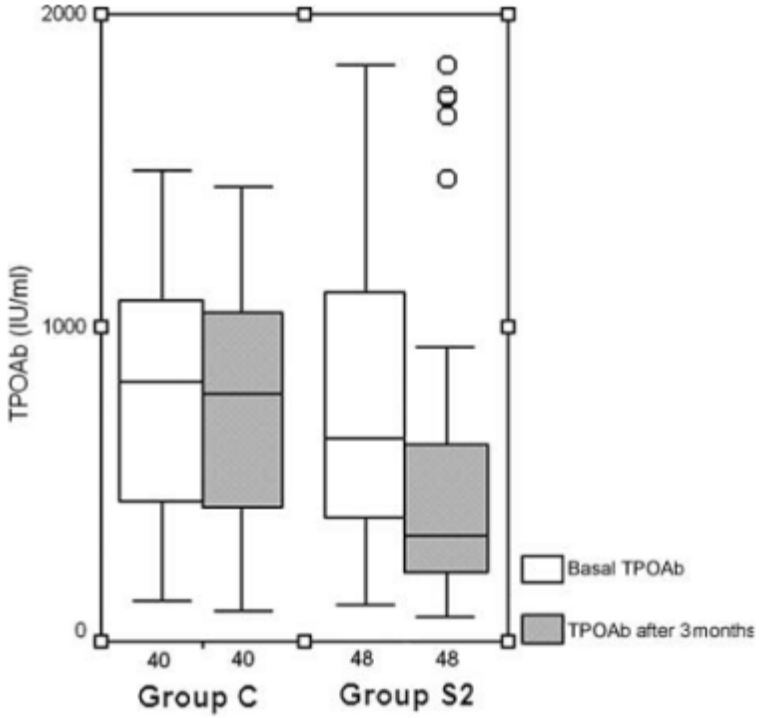
	Months					
	Gr I			*Gr II*		
	0	3	6	0	3	6
Anti-TPO (<100 U/l)	1875±1039	1013±382*	844±227**	1758±917	1389±520	1284±410***

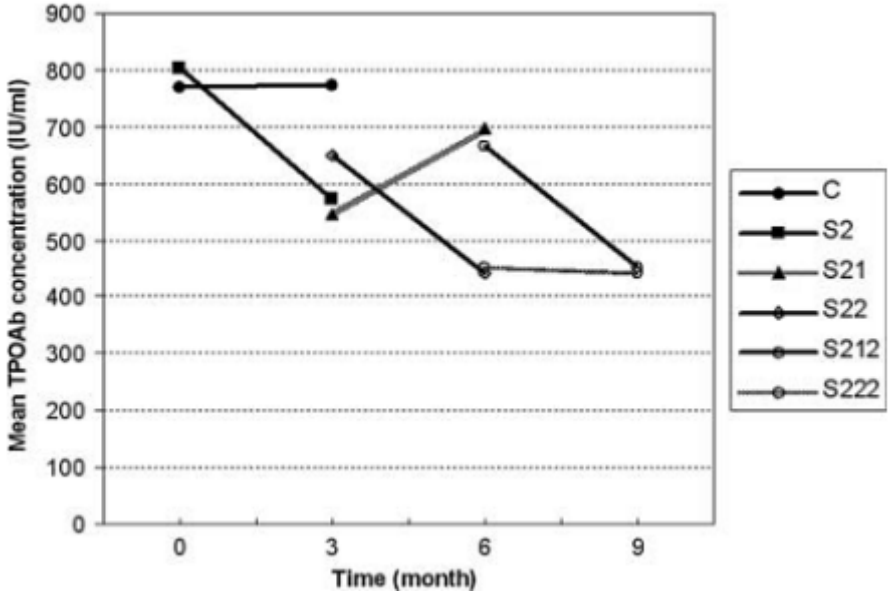
* $P < 0.0001$ vs t_0; ** $P < 0.05$ vs t_3; *** $P < 0.001$ vs t_0.

Selenium treatment in autoimmune thyroiditis: 9-month follow-up with variable doses

Omer Turker, Kamil Kumanlioglu[1], Inanc Karapolat[2] and Ismail Dogan

Eighty-eight female with autoimmune thyroiditis and elevated TPOAb, treated with LT_4, were randomly treated with placebo or 200μg L-selenomethionine for 3 months. Subsequently the dose was either maintained or reduced to 100μg





Journal of Endocrinology (2006) 190, 151–156

No Immunological Benefit of Selenium in Consecutive Patients with Autoimmune Thyroiditis

Georgios Karanikas, Matthias Schuetz, Sylvester Kontur, Heying Duan, Spyridoula Kommata, Robert Schoen, Anna Antoni, Kurt Kletter, Robert Dudczak, and Martin Willheim

36 consecutive patients with HT receiving L-T_4, randomly assigned to treatment with either placebo or sodium selenite (200µg daily) for 3 months.

TABLE 1. RESULTS OF THE LABORATORY INVESTIGATIONS[a]

	Before Se	*After Se*	*Before placebo*	*After placebo*	*Normal range*	p
TSH (μIU/mL)	2.08 ± 1.37	1.82 ± 0.75	2.19 ± 1.69	2.02 ± 0.80	0.40–4.00	NS
FT_4 (ng/dL)	1.51 ± 0.27	1.54 ± 0.40	1.53 ± 0.36	1.54 ± 0.31	0.8–1.9	NS
Se (μg/L)	75 ± 11*	125 ± 71*	76 ± 12	78 ± 12	70–130	*< 0.05
TPOAb (IU/mL)	524 ± 452	505 ± 464	521 ± 349	527 ± 354	< 34	NS

[a]TSH = thyrotropin; FT_4 = free thyroxine; TPOAb = thyroid peroxidase antibodies; * = significance before vs. after Se; NS = nonsignificant.

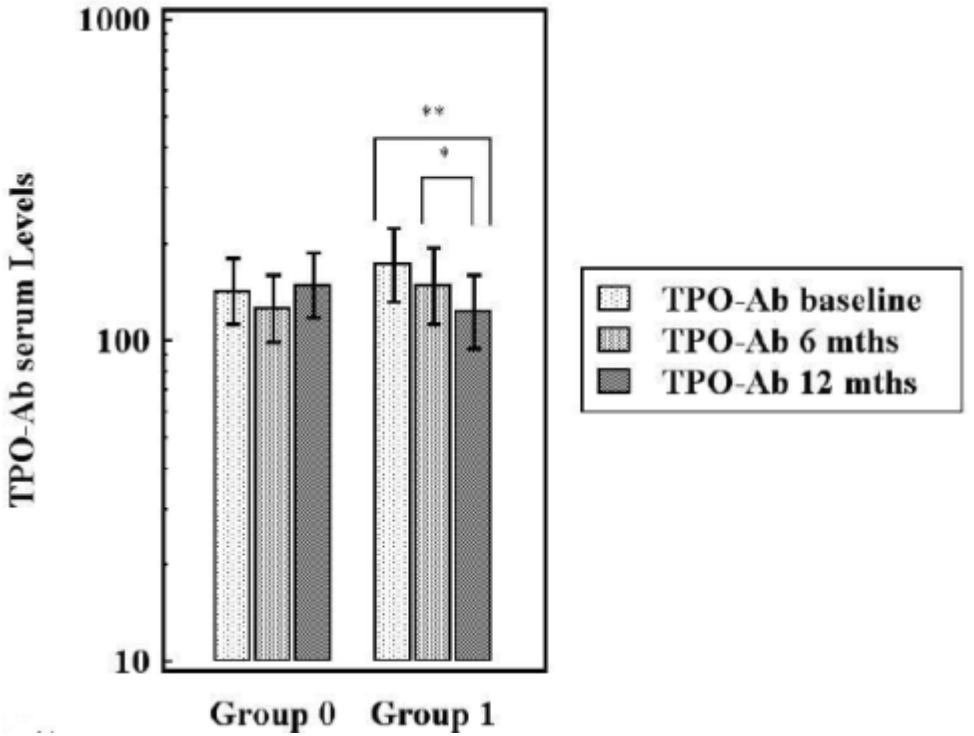
No effect of selenium supplementation on the pattern of cytokine production by $CD4^+$ and $CD8^+$ lymphocytes

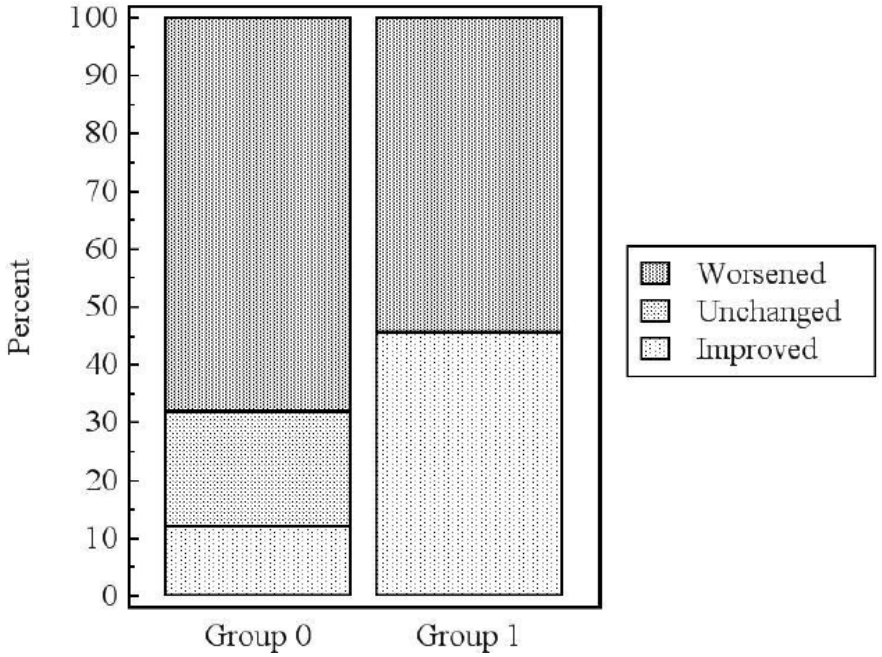
THYROID
Volume 18, Number 1, 2008

INFLUENCE OF PHYSIOLOGICAL DIETARY SELENIUM SUPPLEMENTATION ON THE NATURAL COURSE OF AUTOIMMUNE THYROIDITIS

Davide Nacamulli 1*, Caterina Mian 1*, Daniela Petricca 1, Francesca Lazzarotto 1, Susi Barollo 2, Dina Pozza 1, Stefano Masiero1, Diego Faggian 3, Mario Plebani 3, Maria Elisa Girelli 1, Franco Mantero 1, Corrado Betterle 1.

76 consecutive patients with HT and normal or slightly elevated TSH treated with either placebo or 80 µg sodium selenite daily for 1 yr





Clin Endocrinol 2010

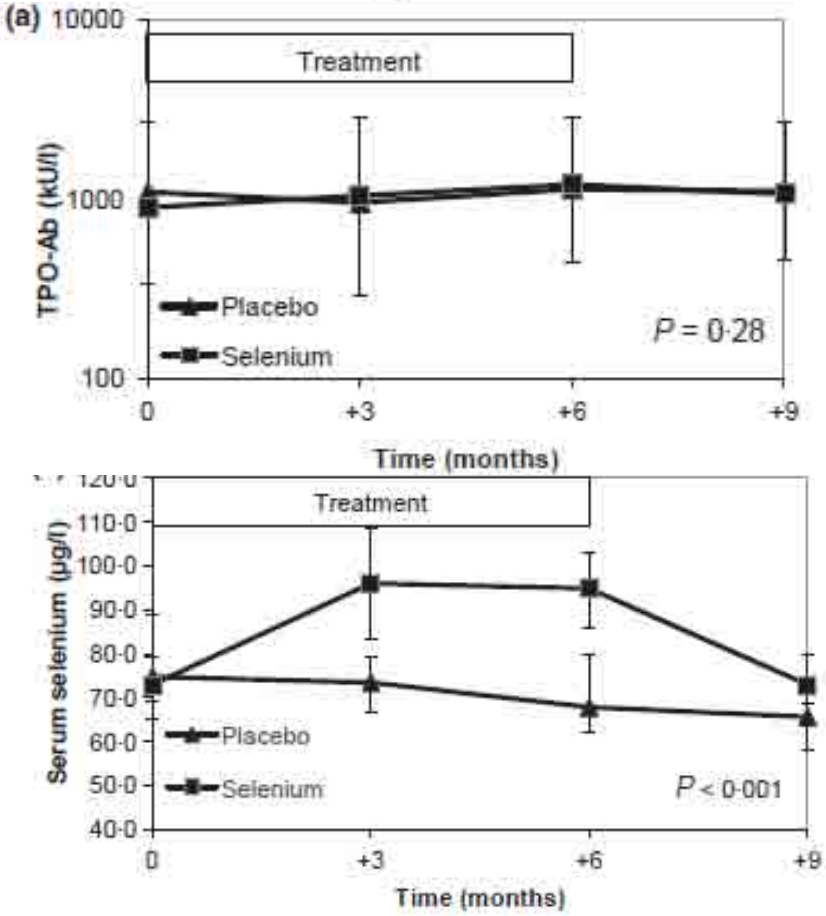
Clinical Endocrinology (2013)

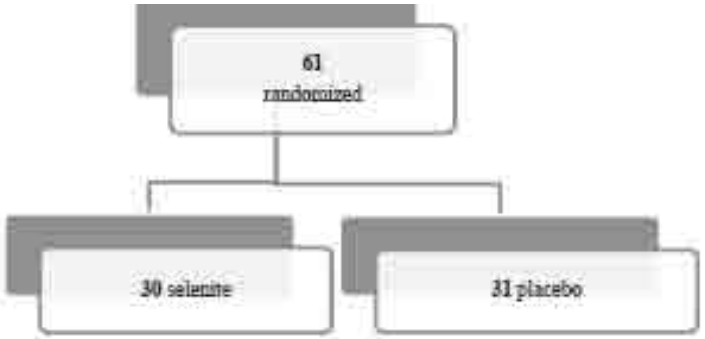
doi: 10.1111/cen.12284

ORIGINAL ARTICLE

Selenite supplementation in euthyroid subjects with thyroid peroxidase antibodies

Silvia A. Eskes*, Erik Endert*, Eric Fliers*, Erwin Birnie†‡, Birgit Hollenbach§, Lutz Schomburg§, Josef Köhrle§ and Wilmar M. Wiersinga*





Euthyroid women with TPO-Ab > 100 KU/l were randomized to receive 200 mcg sodium selenite daily or placebo for 6 months.

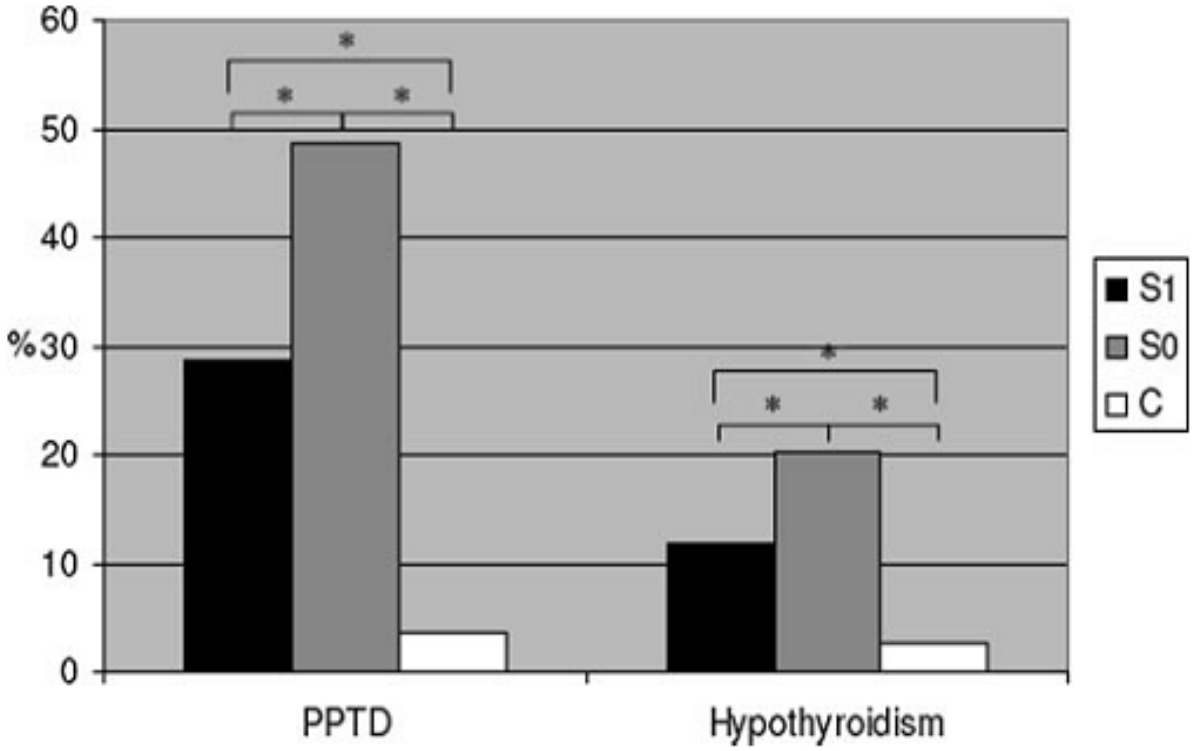
Six months selenite supplementation increased markers of selenium status but had no effect on serum TPO-Ab, TSH or quality of life in euthyroid TPO-Ab-positive women

What could be the reason of the discrepant results

1. **Could it be the selenium level?**
   Selenium levels were similar in all studies
2. **Could it be the form of selenium used for the supplementation?**
   A decrease of TPO-Ab was shown with selenomethionine as well as sodium selenite
3. **Could it be the pre-existing TPO-Ab levels?**
   Negative and positive results on TPO-Ab levels occurred independently on the pre-existing antibody titer
4. **Could it be treatment with thyroxine?**
   Most studies were in patients on thyroxine therapy. Negative and positive result were observed in both settings
5. **Could it be iodine intake?**
   Studies showing a decrease in TPO-Ab originate from Germany, Greece, Turkey and Italy (iodine deficient). Studies failing to show an effect were from Austria and the Netherlands (iodine sufficient)

Selenium reduces PPTD and the rate of hypothyroidism

169 euthyroid TPOAb positive pregnant women randomized to 200 µg selenomethionine (S1) or placebo (S0) and 85 TPOAb negative controls (C)

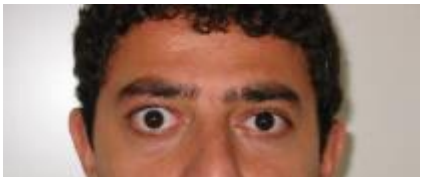


Negro et al., JCEM 2007

AGENDA

- Selenium and autoimmune thyroid diseases
- **Selenium and Graves' orbitopathy**

Management of mild GO



- Local supportive measures (lubrificans), patient reassurance and elimination of risk factors
- The "wait-and-see policy" is usually adopted in patients with mild GO, because the natural history of GO shows a tendency towards spontaneous improvement
- 20% GO patients shows a spontaneous improvement of the eye disease, 65% has no changes, and 15% actually deteriorates
- Thus, only one-fifth of the patients may actually benefit from a "wait-and-see policy"
- Patients with mild GO suffer a significant decrease in quality of life when measured using either a general health related quality-of-life (QoL) questionnaire or a disease specific QoL (GO-QoL) questionnaire
- Thus, therapy would seem justified. Treatment should be affordable, well tolerated, and widely available

Free oxygen radicals and GO

- **Increased generation of free oxygen radicals seems to play a pathogenic role in GO**

  - **Superoxide radical production stimulates retroocular fibroblast proliferation in GO (Burch et al., Exp Eye Res 1997)**
  - **Antithyroid drugs inhibit the oxygen free radical–induced expression of a 72-HSP in Graves' retroocular fibroblats (Heufelder et al. JCEM 1992)**
  - **Stress related oxygen free radicals are present in the retro-ocular tissue in GO and oxygen free radicals are involved in GAG accumulation induced by cytokine IL-1beta. (Lu et al. Thyroid 1999)**
  - **Circulating selenoprotein P concentrations are decreased in patients with Graves' disease and correlate inversely to severity of GO (Dehina et al., Acta Medica Portuguesa 2009)**

An intervention aimed at lowering free oxygen radical generation and improving the imbalance of the antioxidant/oxidant status could be of help in GO

EUGOGO
European Group on Graves' Orbitopathy

Aim of the study

- **To investigate whether selenium compared with placebo given to patients with mild GO could:**
  - **diminish the signs of GO**
  - **improve the disease-specific Quality of Life (GO-QoL)**
  - **prevent worsening of mild GO**

Six Eugogo centers participated in the study:

- **Amsterdam**
- **Olten**
- **Mainz**
- **Pisa**
- **Tessaloniki**
- **Varese**

EUGOGO
EUropean Group on Graves' Orbitopathy

Study design

- **Prospective, double-blind, multicenter, randomized clinical trial**
  - **Sodium selenite: 100 μg b.i.d. (total dose 200 μg; 91.3μg selenium*)**
  - **Placebo: one tablet b.i.d.**
- **The intervention lasted for 6 months, followed by a follow-up period of another 6 months**
- **Total duration of the study: 12 months**
- **The study was approved by the Institutional Boards of participating centers**
- **Informed consent was obtained from all patients prior to enrolment**

- The RDI of dietary selenium has been estimated at 55 or μg/d or 60-75 μg/d .
- A daily dose of approximately 90 μg selenium usualy saturates SePP and GPx-3 activity.

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Selenium and the Course of Mild Graves' Orbitopathy

Claudio Marcocci, M.D., George J. Kahaly, M.D., Gerasimos E. Krassas, M.D., Luigi Bartalena, M.D., Mark Prummel, M.D.,* Matthias Stahl, M.D., Maria Antonietta Altea, M.D., Marco Nardi, M.D., Susanne Pitz, M.D., Kostas Boboridis, M.D., Paolo Sivelli, M.D., George von Arx, M.D., Maarten P. Mourits, M.D., Lelio Baldeschi, M.D., Walter Bencivelli, Ph.D., and Wilmar Wiersinga, M.D., for the European Group on Graves' Orbitopathy

EUGOGO
European Group on Graves' Orbitopathy

Inclusion criteria

- Presence of at least one sign of GO, with a disease duration of less than 18 months (as recorded by the patient)
  - Soft tissue swelling NO SPECS class ≤2B (e.g. moderate chemosis, moderate eye lid swelling)
  - Proptosis of ≤ 22 mm
  - No diplopia in primary or reading position, and/or ocular torticollis
  - Mono-ocular duction in any direction ≥ 20 degrees
  - No optic nerve involvement
- Euthyroidism: a) after a course of ATD: b) at least 2 months since starting ATD or after surgery; for at least 6 months after 131-Iodine
- No previous treatment for GO, except for local measures (e.g., eye drops)
- Age 18-70 years

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Baseline demographic and thyroid data[1]

Parameters	Placebo (n=50)	Selenium (n=54)
Sex (F/M)	41/9	48/6
Age (yr)	44.6±10.7[2]	43.0±11.0
Thyroid disease (GD/HT/EGD)	43/3/4	51/1/1
Previous thyroid treatment (RI/Tx)	4/9	4/4
Current thyroid treatment (ATD/L-T4/none)	34/9/7	41/9/4
Duration of ophthalmopathy (mo)	6.1±4.6	7.7±5.8
TSH (mU/L)	1.3±1.4	1.3±16
TRAb +ve (%)	31 (75.6)	35(74.5)
TPOAb +ve (%)	27 (65.9)	31 (68.1)
Current smokers (%)	25(50.0)	23 (42.6)

[1] P>0.05 for all comparisons

[2] Mean±SD

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Baseline eye evaluation[1]

Parameters	Placebo (n=50)	Selenium (n=54)
CAS (7 items)	3.2±1.3[2]	3.5±1.3
Proptosis (mm)	19.8 ±2.3	19.7 ±2.7
Eyelid width (mm)	11.3 ±1.7	11.5 ±1.9
Soft tissue involvement (%)		
absent	5	5
mild	65	64
moderate	30	39
marked	0	0
Diplopia (Gorman' score)		
absent	44	43
intermittent	3	6
inconstant	3	5

Outcome measurements

Patients were evaluated at baseline, 3, 6 and 12 months
Outcomes were determined at 6 and 12 months

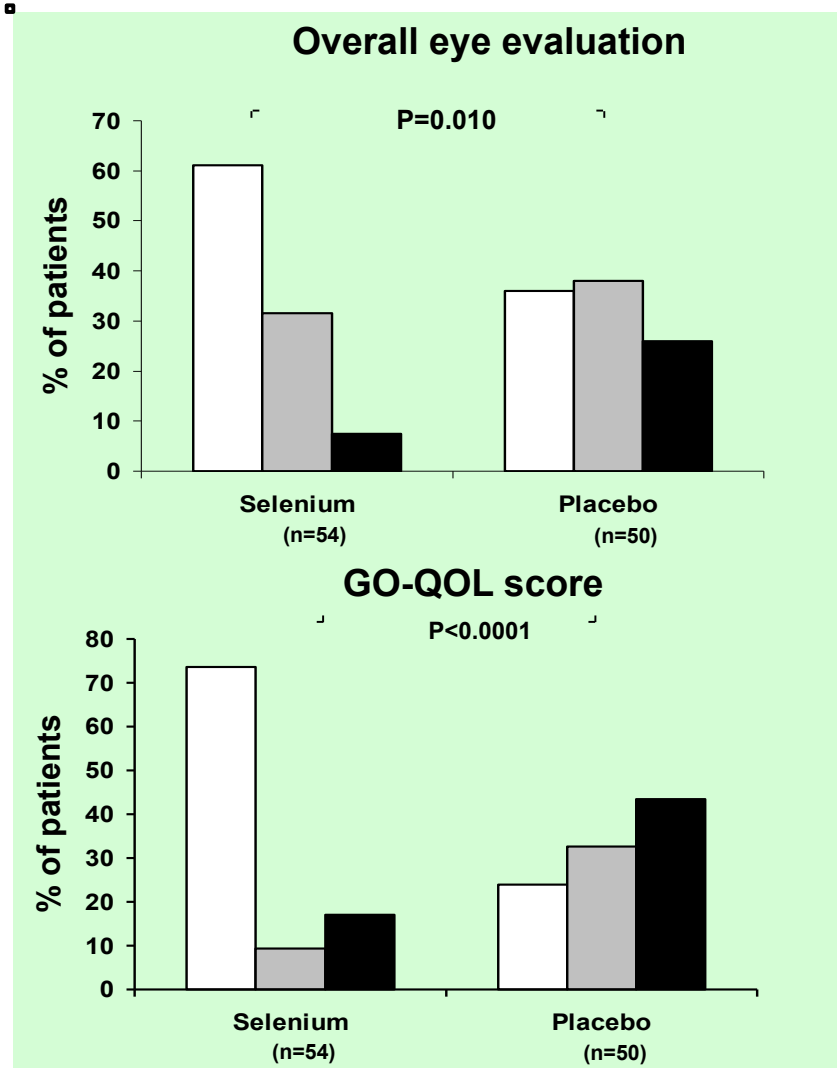
- **Primary outcome measurements (6 months):**
  - **Subjective GO-QoL questionnaire filled in by the patient**
  - **Assessment of the eye changes by a blinded ophthalmologist**

- **Secondary outcome measurements:**
  - **Clinical Activity Score (7 items)**
  - **Diplopia Gorman's score**
  - **Tolerability and safety**

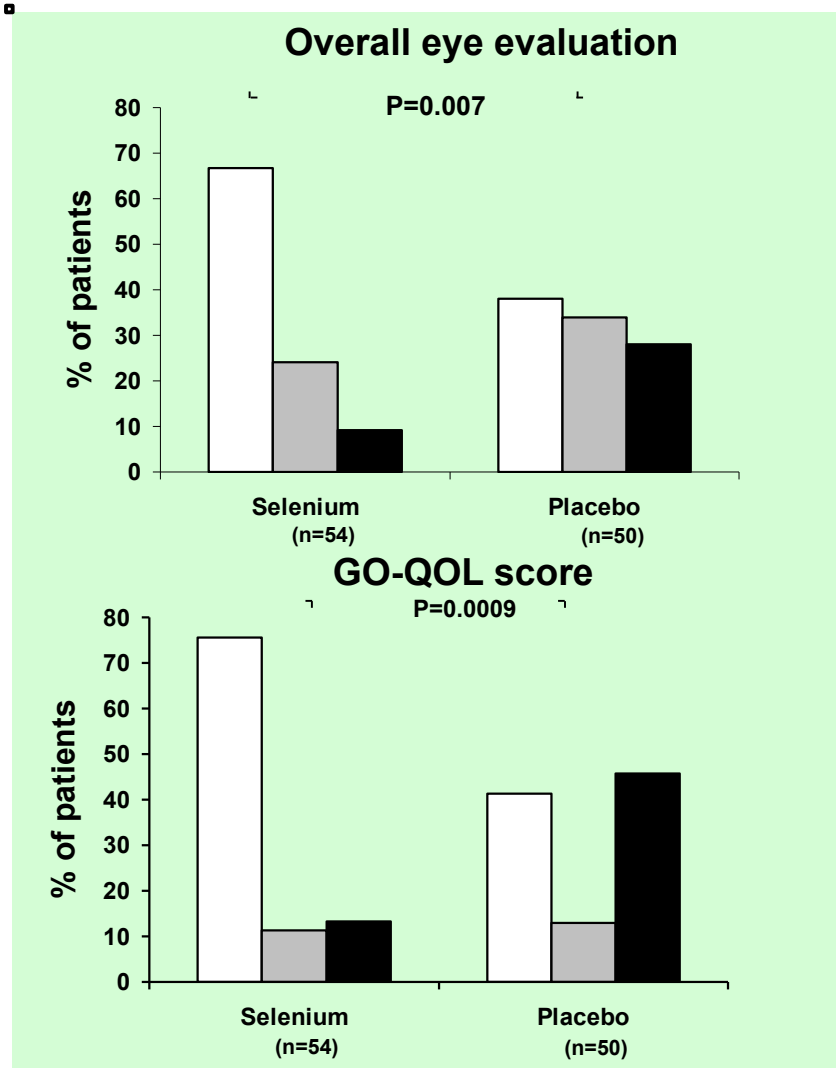
Primary end points

Improved No change Worsened

6 months



12 months



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Means (SD) GO-QoL scores (% of maximun)[1]

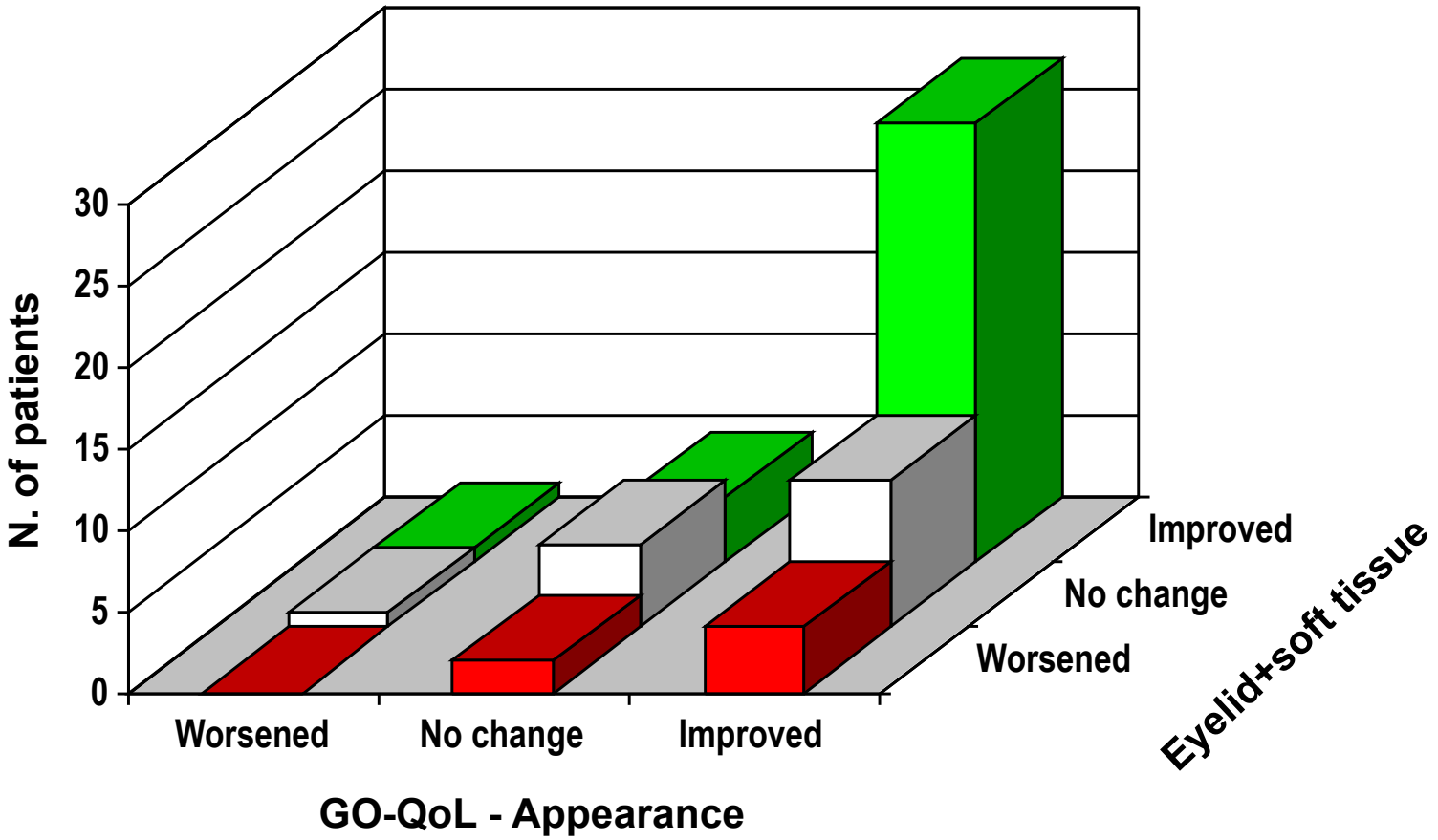
	Baseline	6 months	p*	12 months	p*
Placebo					
Visual functioning	84.0±19.5	81.2±20.2	0.26	82.3±20.9	0.55
Appearance	79.5±18.1	76.7±21.3	0.13	77.9±21.8	0.53
Selenium					
Visual functioning	80.1±11.1	88.5±17.2	0.0007	90.8±14.3	<0.0001
Appearance	74.0±19.8	84.2±18.8	<0.0001	86.2±17.3	<0.0001

[1] Healthy subjects should score 100 (no limitations)

*p vs baseline

At 6-month evaluation the visual functioning score improved in 62% and the appearance score in 75% of GO patients given selenium

Eyelid and soft tissue changes vs GO-QoL appearance in selenium treated patients at 6 months



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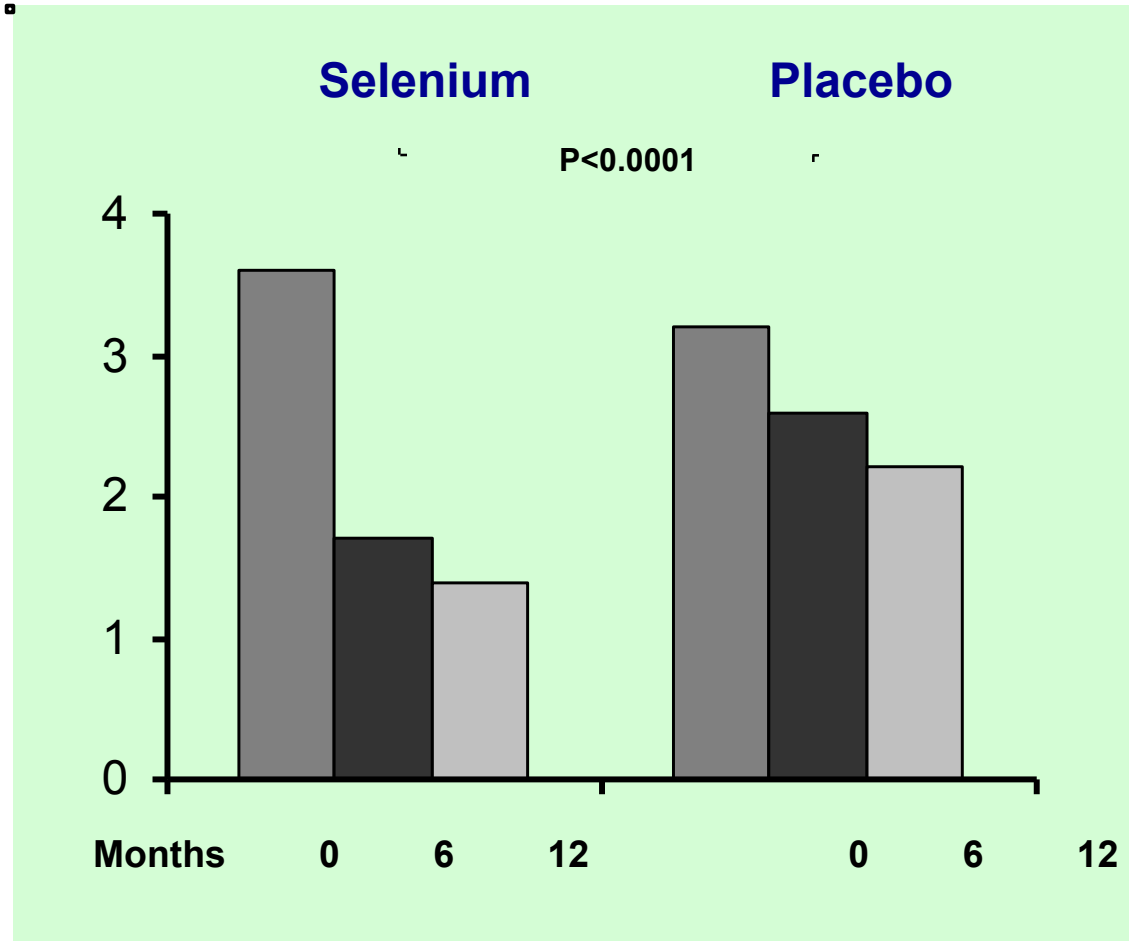
Clinical activity score



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Adverse events and premature stops

- No drug-related adverse events occurred in patients treated with selenium or placebo
- Two patients assigned to placebo required immunosuppressive therapy for deterioration of GO

Summary

Primary outcomes

- Selenium administration, compared with placebo, was associated with statistically significant beneficial effects on GO and GO-QoL at 6 months, which persisted at 12 months

Secondary outcomes

- CAS decreased in both groups but the decrease was significantly greater in the selenium group
- The Gorman's score did not changed
- Selenium was well tolerated

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Limitations of the study

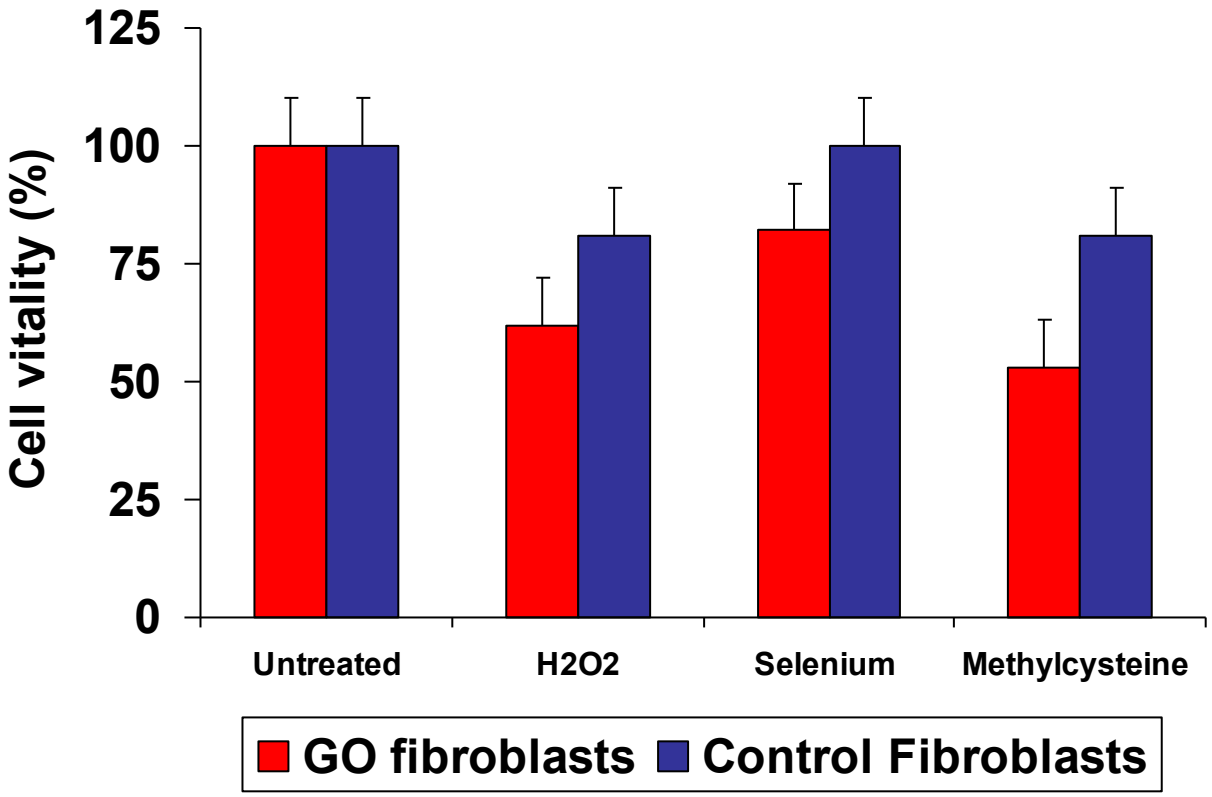
- No measurements of circulating levels of selenium nor of markers of the inflammatory/oxidative state
- Patients included in the study come from mild selenium deficient areas
- No information on the mechanism accounting for the beneficial effect of selenium on GO (anti-oxidant/anti-inflammatory or immune modulatory ?)

STUDY PROTOCOL

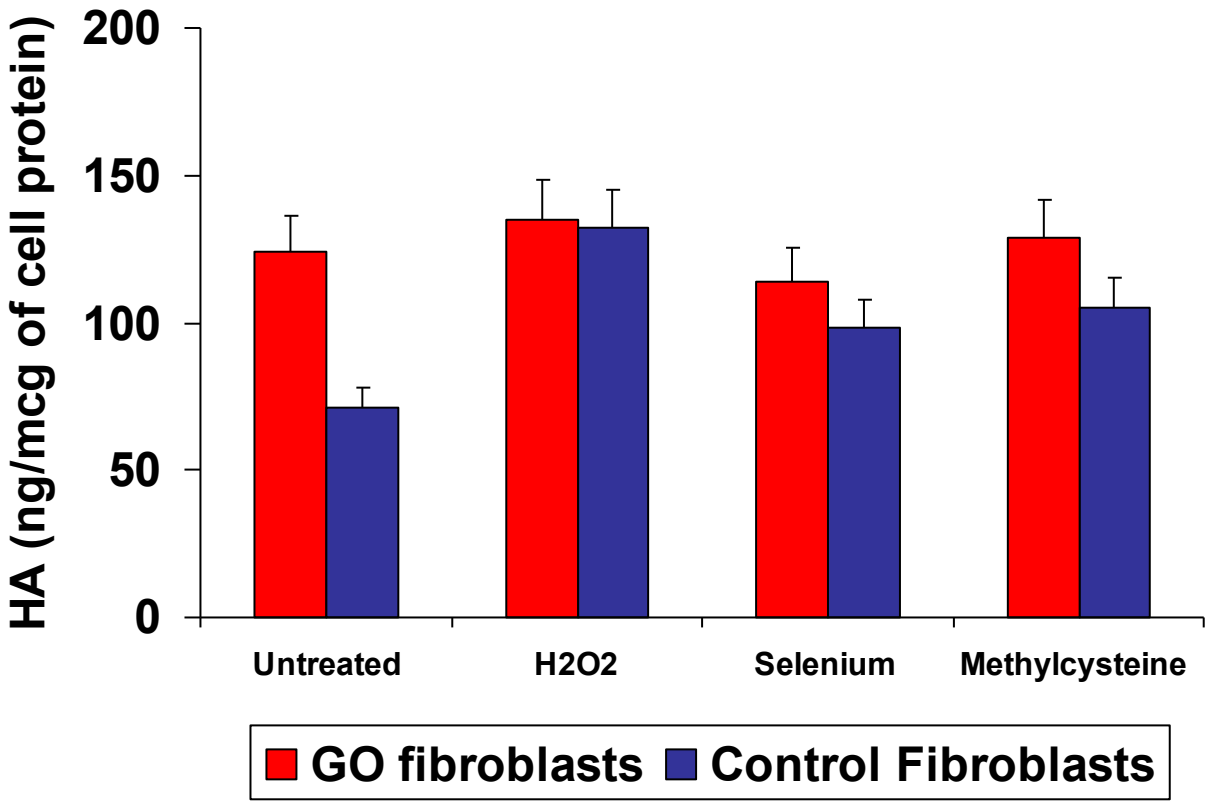
- **Primary cultures of GO fibroblasts and fibroblasts from patients without GO (control fibroblasts)**
- **Treatment with H_2O_2 to induce oxidative stress**
- **Treatment with selenium methylcysteine, or, as a control, with methylcysteine**
- **Assessment of:**
  - **Cell viability**
  - **HA release**
  - **Necrosis**
  - **Apoptosis**

Effects of selenium on cell vitality

(Alamar blue incorporation)

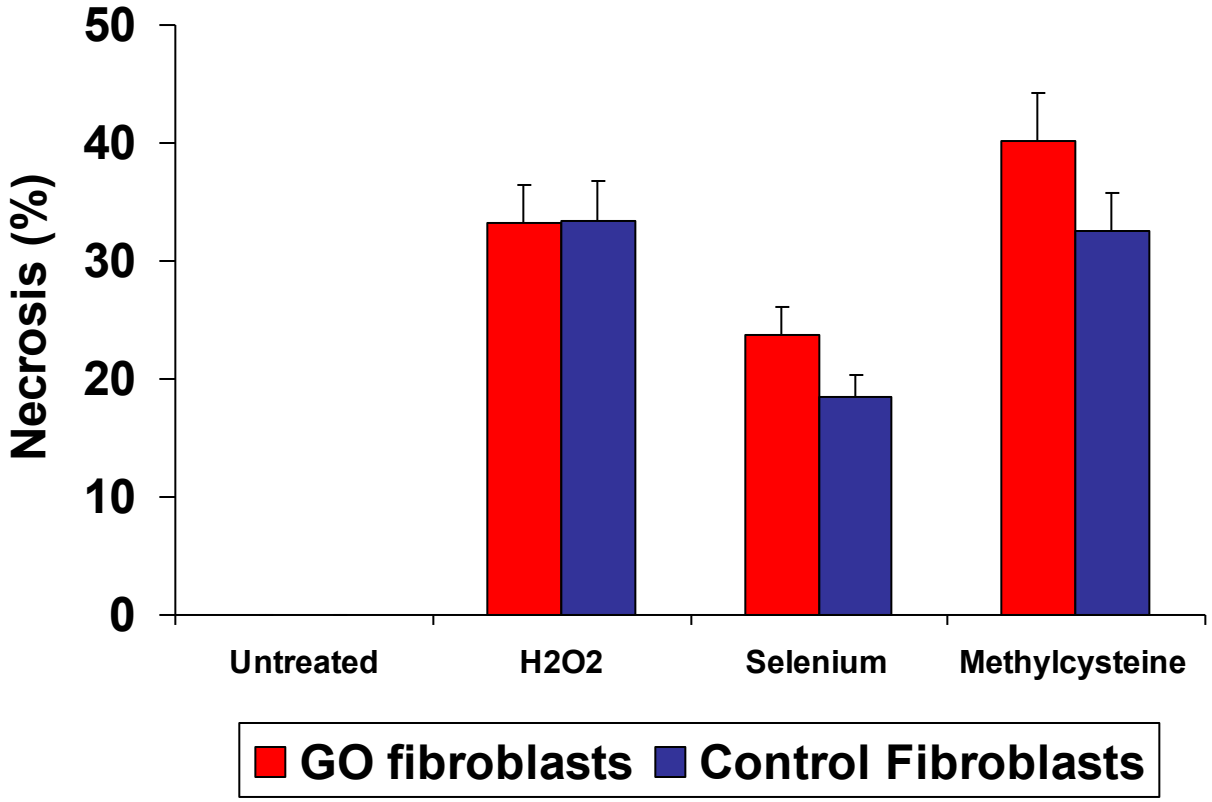


Effects of selenium on HA release



Effects of selenium on necrosis

(LDH release)



Conclusions

- **Selenium supplementation for 6 months improves the course of mild GO and related impairment of quality of life**
- **A 6-month course of selenium should be offered as initial treatment to patients with mild GO**

Thank you for your attention

Progetto "Epidemiologia dell'Iperparatiroidismo Primario in Italia"

Il progetto Epidemiologia dell' Iperparatiroidismo Primario in Italia ha lo scopo di definire le caratteristiche cliniche, biochimiche e strumentali di una delle patologie endocrinologiche più diffuse.
Il progetto si avvale di una piattaforma elettronica per la raccolta dati accessibile online e fruibile da tutti i medici sull'intero territorio nazionale

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