Pharmacological Reports 71 (2019) 367–373

Contents lists available at ScienceDirect

Pharmacological Reports

journal homepage: www.elsevier.com/locate/pharep

Original article

Selenomethionine potentiates the impact of vitamin D on thyroid autoimmunity in euthyroid women with Hashimoto's thyroiditis and low vitamin D status

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ARTICLE INFO

Article history:
Received 30 August 2018
Received in revised form 14 November 2018
Accepted 14 December 2018
Available online 14 December 2018

Keywords:
25-hydroxyvitamin D
Selenium
Thyroid autoimmunity
Vitamin D status

ABSTRACT

Background: Both exogenous vitamin D and selenium reduce thyroid antibody titers. The aim of the study was to investigate whether the impact of vitamin D on thyroid autoimmunity is affected by selenium intake.
Methods: The study included 47 euthyroid women with Hashimoto's thyroiditis and low vitamin D status, 23 of whom had been treated with selenomethionine (200 μg daily) for at least 12 months before the beginning of the study. During the study, all patients were treated with vitamin D preparations (4000 IU daily). Serum titers of thyroid peroxidase and thyroglobulin antibodies, as well as circulating levels of thyrotropin, free thyroid hormones and 25-hydroxyvitamin D were measured before vitamin D supplementation and 6 months later. Moreover, at the beginning and at the end of the study, we calculated Jostel's thyrotropin index, the SPINA-GT index and the SPINA-GD index.
Results: With the exception of the free triiodothyronine/free thyroxine ratio and the SPINA-GD index, there were no differences between the study groups. In both groups, vitamin D increased 25-hydroxyvitamin D levels, reduced thyroid peroxidase and thyroglobulin antibody titers, as well as increased the SPINA-GT index. The effects on antibody titers and the SPINA-GT index were more pronounced in women receiving selenomethionine. Neither in selenomethionine-treated nor in selenomethionine-naïve women vitamin D affected serum hormone levels, Jostel's index and the SPINA-GD index.
Conclusions: The results of the study suggest that selenium intake enhances the effect of vitamin D on thyroid autoimmunity.

Introduction

Autoimmune thyroiditis (Hashimoto's thyroiditis) is the most common organ-specific autoimmune disorder, as well as the most common cause of thyroid hypofunction in developed countries [1,2]. The disease, caused by replacement of follicular cells by lymphocytic infiltrate, is characterized by the occurrence of thyroid peroxidase (TPOAb) and thyroglobulin (TgAb) antibodies [3,4].

It seems that autoimmune thyroiditis is associated with low vitamin D status [5,6]. The presence of Hashimoto's thyroiditis was accompanied by reduced serum levels of 25-hydroxyvitamin D and this association remained significant after adjustment for age, sex and body mass index [7]. Circulating levels of 25-hydroxyvitamin D correlated with titers of TPOAb, duration of thyroiditis and thyroid volume [8]. Hypovitaminosis D was an independent factor determining the presence of TPOAb [9]. Finally, some vitamin D receptor gene polymorphisms (TaqI and BsmI) increased the risk of developing autoimmune thyroiditis [10,11]. It remains unclear, however, whether low vitamin D status lies at the bottom of autoimmune thyroiditis or is a consequence of this disorder. Interestingly, exogenous vitamin D preparations reduced thyroid antibody titers (mainly TPOAb) in women with thyroid autoimmunity and this effect was observed in patients with low, as well as with normal vitamin D status [12,13]. A significantly greater

Abbreviations: CI, confidence interval; IU, international unit; SD, standard deviation; SPINA, structure parameter inference approach; TgAb, thyroglobulin antibodies; TPOAb, thyroid peroxidase antibodies.

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https://doi.org/10.1016/j.pharep.2018.12.006
1734-1140/

reduction in titers of TPOAb was observed in patients with Hashimoto's thyroiditis and thyrotropin levels not exceeding 10 mIU/L than in patients with thyrotropin levels above this value [14].

The recent meta-analyses based on the results of interventional studies have shown that also selenium supplementation reduced titers of thyroid antibodies [15–17]. This effect was particularly pronounced in subjects receiving 200 μg of selenomethionine daily, but not in subjects receiving sodium selenite, as well as was observed mainly in patients with autoimmune thyroiditis receiving levothyroxine treatment [15–17]. Moreover, selenium was found to reverse Hashimoto's thyroiditis-induced changes in the echostructure of the thyroid gland [18]. These beneficial effects seem to be, at least in part, attributed to anti-inflammatory and immunosuppressive effects of selenium [19].

To the best of our knowledge, no previous study has investigated whether there is any relationship between vitamin D action on thyroid autoimmunity and selenium intake. Therefore, it the present study, we have compared the impact of vitamin D/selenomethionine combination therapy and vitamin D monotherapy on thyroid antibody titers, hypothalamic-pituitary-thyroid axis activity and estimated structure parameters of thyroid feedback control in women with Hashimoto's thyroiditis.

Materials and methods

Patients

The participants of the study (n = 47) were enrolled between January and August 2016. They were selected among 75 euthyroid women with Hashimoto's thyroiditis (aged between 20 and 45 years), supervised by the investigators at the Department of Internal Medicine and Clinical Pharmacology and the Gyncentrum Fertility Clinic (Katowice, Poland). To limit the impact of seasonal fluctuations in vitamin D status, 24 women were recruited in January and February, while the remaining ones in July and August. Women were considered eligible for enrollment if they had: (a) serum TPOAb titers above 100 U/mL; (b) reduced echogenicity of the thyroid parenchyma on thyroid ultrasonography; (c) serum levels of thyrotropin in the range between 0.4 and 4.5 mU/L; (d) serum levels of free thyroid hormones within the reference range and (e) serum levels of 25-hydroxyvitamin D below 30 ng/mL. Only patients fulfilling all criteria were included in the study. All potential participants were asked about using vitamin D-containing supplements. Women who had used any vitamin D preparations within 12 months before the beginning of the study were excluded. In order to eliminate women with euthyroid Graves' disease, in whom TPOAb and TgAb may be present [20], subjects with positive thyrotropin receptor antibodies were also not enrolled. The remaining exclusion criteria were as follows: other autoimmune or endocrine disorders, BMI $\geq 35\,kg/m^2$, chronic inflammatory processes, the presence of symptoms suggestive of hypothyroidism, myocardial infarction or stroke within 3 months preceding the study, impaired renal or hepatic function, pregnancy or lactation, and non-compliance with the study protocol. Moreover, we excluded women treated within 6 months before the beginning of the study with glucocorticoids or other immunosuppressive agents, nonsteroidal anti-inflammatory drugs, drugs affecting hypothalamic-pituitary-thyroid axis activity (including levothyroxine) or with agents known to interact with vitamin D. A sample size analysis showed that, assuming a power of 80% and a significance level of 0.05, at least 19 individuals had to be included in each group to detect a 20% difference in titers of thyroid antibodies. The study was approved by the local institutional review board and all subjects included in the study signed informed consent after careful explanation of the study protocol.

Study design

Some participants (n = 23) had been treated with selenomethionine (200 μg daily) for at least 12 months before the beginning of the study onset, while the remaining ones (n = 24) had not received any selenium preparations. The second group was chosen among 52 selenium-naïve women fulfilling the inclusion criteria. The purpose of this selection, based on a computer algorithm, was to obtain two study groups well-matched for age, weight, titers of thyroid antibodies and levels of thyrotropin and free thyroid hormones. Both groups were then treated with vitamin D, administered at a dose of 4000 IU once-daily for 6 months. No changes in dosage were allowed. Throughout the study, selenomethionine-treated patients received this agent at the same dose as before the beginning of the study. Vitamin D was administered between 8.00 and 9.00 a.m., while selenomethionine between 8.00 and 9.00 p.m. In selenomethionine-naïve women, vitamin D was administered in the morning. Compliance with medication usage was assessed at each visit by interrogation and pill count.

Laboratory assays

Venous blood samples were drawn from antecubital vein, after a 12-h overnight fast, in a quiet temperature-controlled room (24–25 °C) between 8.00 and 9.00 a.m. Serum levels of thyrotropin, free thyroxine and free triiodothyronine, as well as serum titers of TPOAb and TgAb were determined in duplicate by direct chemiluminescence using acridinium ester technology (ADVIA Centaur XP Immunoassay System, Siemens Healthcare Diagnostics, Munich, Germany). Serum 25-hydroxyvitamin D levels were detected by competitive immunoassay using Roche Diagnostic commercial kits and a multichannel automatic analyzer (Roche Cobas e 411, Mannheim, Germany). The intra- and inter-assay coefficients of variation for 25-hydroxyvitamin D were 5.5% and 7.8%, respectively.

Structure parameters of thyroid homeostasis

A structure parameter inference approach (SPINA) is a method that allows for calculating constant structure parameters of hypothalamic-pituitary-thyroid axis activity based on circulating hormone levels. Jostel's thyrotropin index is as a quantitative marker for pituitary thyrotropic function [21,22]. Pituitary hypofunction and euthyroid sick syndrome are associated with decreased values of this index, while thyrotropinomas and resistance to thyroid hormone are characterized by Jostel's index above the upper limit of the reference range [23]. The SPINA-GT index provides an estimate of thyroid secretory capacity and is below the lower limit of the reference range or low-normal in patients with autoimmune thyroiditis. Elevated values of this index characterize patients with Graves' disease and toxic adenoma, as well as some subjects with euthyroid nodular goiter [21]. The SPINA-GD index estimates total step-up deiodinase activity. The SPINA-GD index is abnormally low in patients with impaired conversion of thyroxine to triiodothyronine, while increased in rare states of hyperdeiodination [22]. All three parameters were calculated using SPINA-Thyr 4.0.1 for Windows software. Jostel's index was calculated using the following formula: ln [thyrotropin] + 0.1345 x free thyroxine. The SPINA-GT index was calculated as follows: β_T x (D_T + thyrotropin) x (1 + K_{41} x standard concentration of thyroxine-binding globulin + K_{42} x standard concentration of transthyretin x free thyroxine) / (α_T x thyrotropin). The SPINA-GD index was calculated using the formula: β_{31} x (K_{M1} + free thyroxine) (1 + K_{30} x standard

Table 1
Baseline characteristics of patients.

Variable	Selenomethionine-treated women	Selenomethionine-naive women	Difference [95% CI]
Number of patients	23	24	
Age [years; mean (SD)]	32 (6)	31 (6)	−1 [-5, 3]
Smokers [%]	22	25	
Body mass index [kg/m^2; mean (SD)]	26.2 (2.9)	25.9 (3.0)	−0.3 [-2.0; 1.4]
Underweight [n (%)]	1 (4)	2 (8)	
Normal weight [n (%)]	10 (43)	10 (42)	
Overweight [n (%)]	10 (43)	7 (37)	
Class I obesity [n (%)][1]	2 (9)	3 (13)	
TPOAb [U/mL; mean (SD)]	896 (295)	975 (324)	79 [-103; 261]
TgAb [U/mL; mean (SD)]	829 (302)	867 (368)	38 [-160; 236]
Thyrotropin [mIU/L; mean (SD)]	2.1 (0.9)	2.3 (1.0)	0.2 [-0.4, 0.8]
Free thyroxine [pmol/L; mean (SD)]	14.8 (2.3)	16.2 (2.9)	1.4 [-0.1, 2.9]
Free triiodothyronine [pmol/L; mean (SD)]	3.9 (0.8)	3.5 (0.7)	−0.4 [-0.1, 0.9]
Free triiodothyronine/free thyroxine ratio [mean (SD)]	0.264 (0.074)	0.216 (0.053)	−0.048 [-0.086, 0.010]*
Jostel's thyrotropin index [mean (SD)][2]	2.7 (0.7)	3.0 (0.6)	0.3 [-0.1, 0.7]
SPINA-GT index [pmol/s; mean (SD)][3]	2.59 (0.48)	2.70 (0.56)	0.11 [-0.20, 0.42]
SPINA-GD index [nmol/s; mean (SD)][4]	24.37 (4.88)	19.98 (5.02)	−4·39 [-7.30, -1.48]*
25-hydroxyvitamin D [ng/mL; mean (SD)]	20.2 (6.0)	21.0 (5.1)	0.8 [-2.5, 4.1]

CI: confidence interval; IU: international unit; SD: standard deviation; SPINA: structure parameter inference approach; TgAb: thyroglobulin antibodies; TPOAb: thyroid peroxidase antibodies.
* Statistically significant difference between both groups.
[1] Patients with BMI ≥ 35 kg/m^2 were excluded.
[2] Reference range:1.3–4.1.
[3] Reference range: 1.4–8.7 pmol/s.
[4] Reference range: 20–60 nmol/s.

concentration of thyroxine-binding globulin) x free triiodothyronine/ (α_{31} x free thyroxine). Constants in both equations were as follows: $\beta_T = 1.1 \times 10^{-6}$/s, $D_T = 2.75$ mU/L, $K_{41} = 2 \times 10^{10}$ L/mol, standard concentration of thyroxine-binding globulin = 300 nmol/L, $K_{42} = 2 \times 10^{8}$ L/mol, standard concentration of transthyretin = 4.5 mmol/L, $\alpha_T = 0.1$/L, $\beta_{31} = 8 \times 10^{-6}$/s, $K_{M1} = 5 \times 10^{-7}$ mol/L, $K_{30} = 2 \times 10^{9}$ L/mol and $\alpha_{31} = 0.026$/L [21,22].

Statistical analysis

Owing to the skewed distributions, values for hormones, thyroid antibodies and indices were natural-log transformed to achieve normality and homogeneity of variance. Both groups were compared by Student's *t*-tests for independent samples. The differences between post-treatment and baseline values within the same treatment group were compared with the Student's paired *t*-test. Categorical variables were analyzed by χ^2test. Both study populations were divided into three groups stratified by baseline levels of 25-hydroxyvitamin D. Cut-off points for tertiles of 25-hydroxyvitamin D were as follows: selenomethionine-treated women, ≤15.3, 15.2–22.6, and ≥22.6 ng/mL; selenomethionine-naive women, ≤15.8, 15.8–23.0, and ≥23.0 ng/mL. Percent changes in thyroid antibody titers from baseline after adjustment for baseline values (reflecting the strength of vitamin D action) were compared between tertiles using one-way analysis of covariance followed by the post hoc Bonferroni test. The clinical importance of the result was assessed based on the 95% confidence interval. A *t* statistic and two sample means were used to generate an interval estimate of the difference between two population means. Correlations were determined using Pearson's correlation coefficient (r). The results were regarded as statistically significant if 95% confidence intervals did not include the null value and/or two-tailed *p* values were below 0.05.

Results

There were no significant differences between the study groups in age, smoking habits, body mass index, serum titers of TPOAb and TgAb, as well as in serum levels of thyrotropin and free thyroid hormones. At entry, the SPINA-GD index and the free triiodothyronine/free thyroxine ratio, but not Jostel's thyrotropin index and the SPINA-GT index, was higher in selenomethionine-treated than selenomethionine-naïve women (Table 1). No significant adverse effects were reported throughout the study and no patient was withdrawn from the study.

In both study groups, vitamin D treatment increased serum levels of 25-hydroxyvitamin D, decreased titers of TPOAb and TgAb, as well as increased the SPINA-GT index (Table 2). The effect of treatment on TPOAb, TgAb and the SPINA-GT index was stronger in selenomethionine-treated than selenomethionine-naïve women. The drug produced a neutral effect on serum levels of thyrotropin, free thyroxine and free triiodothyronine. Vitamin D did not also affect the free triiodothyronine/free thyroxine ratio, Jostel's thyrotropin index and the SPINA-GD index. At the end of the study, there were differences between the study groups in titers of TPOAb and TgAb, the free triiodothyronine/free thyroxine ratio, as well as in values of the SPINA-GD index (Table 2). There were significant differences in percent changes of thyroid antibody titers between tertiles of 25-hydroxyvitamin D levels (Table 3)

At the beginning of the study, there were correlations between titers of TPOAb and TgAb, titers of thyroid antibodies and levels of 25-hydroxyvitamin D, as well as between titers of TPOAb and the SPINA-GT index. Treatment-induced changes in antibody titers correlated with baseline levels of 25-hydroxyvitamin, with the changes in 25-hydroxyvitamin D levels, as well with the effect of treatment on the SPINA-GT index Table 4.

Discussion

This study has shown for the first time that the effect of exogenous vitamin D on thyroid autoimmunity is more pronounced in selenomethionine-treated than selenomethionine-naive young euthyroid women with Hashimoto's thyroiditis. The obtained results indicate that euthyroid women with thyroid autoimmunity may benefit more from vitamin D/selenomethionine combination therapy than from treatment with only one of these agents.

Table 2
Effect of exogenous vitamin D on thyroid antibody titers, hormones, thyroid function tests and 25-hydroxyvitamin D levels in women with Hashimoto's thyroiditis.

Variable	Selenomethionine-treated women	Selenomethionine-naive women	Difference [95% CI]
TPOAb [U/mL; mean (SD)]			
Baseline	896 (295)	975 (324)	79 [-103; 261]
After 6 months	551 (206)#	765 (287)#	214 [67, 361]*
Change	−345 (165)	−210 (123)	135 [50, 220]&
TgAb [U/mL; mean (SD)]			
Baseline	829 (302)	867 (368)	38 [-160; 236]
After 6 months	549 (184)#	671 (211)#	122 [5, 239]*
Change	−280 (128)	−196 (107)	84 [15, 153]&
Thyrotropin [mIU/L; mean (SD)]			
Baseline	2.1 (0.9)	2.3 (1.0)	0.2 [-0.4, 0.8]
After 6 months	1.8 (0.8)	2.1 (0.9)	0.3 [-0.2, 0.8]
Change	−0.3 (0.2)	−0.2 (0.3)	0.1 [-0.1, 0.3]
Free thyroxine [pmol/L; mean (SD)]			
Baseline	14.8 (2.3)	16.2 (2.9)	1.4 [-0.1, 2.9]
After 6 months	15.8 (2.9)	17.1 (3.2)	1.3 [-0.5, 3.1]
Change	1.0 (0.8)	0.9 (0.6)	−0.1 [-0.5, 0.3]
Free triiodothyronine [pmol/L; mean (SD)]			
Baseline	3.9 (0.8)	3.5 (0.7)	−0.4 [-0·1, 0.9]
After 6 months	4.3 (0.9)	3.8 (0.9)	⁻0.5 [-1.1, 0.1]
Change	0.4 (0.3)	0.3 (0.2)	−0.1 [0.3, 0.1]
Free triiodothyronine/free thyroxine ratio [mean (SD)]			
Baseline	0.264 (0.074)	0.216 (0.053)	−0.048 [-0.086, 0.010]*
After 6 months	0.272 (0.083)	0.222 (0.064)	−0.050 [-0.093, -0.007]*
Change	0.008 (0.011)	0.006 (0.010)	−0.002 [-0.008, 0.004]
Jostel's thyrotropin index [mean (SD)][1]			
Baseline	2.7 (0.7)	3.0 (0.6)	0.3 [-0.1, 0.7]
After 6 months	2.7 (0.6)	3.0 (0.5)	0.3 [-0.1, 0.7]
Change	0.0 (0.5)	0.0 (0.6)	0.0 [-0.3, 0.3]
SPINA-GT index [pmol/s; mean (SD)][2]			
Baseline	2.59 (0.48)	2.70 (0.56)	0.11 [-0.20, 0.42]
After 6 months	3.04 (0.57)#	3.00 (0.46)#	−0.04 [-0.34, 0.26]
Change	0.45 (0.26)	0.30 (0.24)	−0.15 [-0.3, -0.1]&
SPINA-GD index [nmol/s; mean (SD)][3]			
Baseline	24.37 (4.88)	19.98 (5.02)	−4.39 [-7.30, -1.48]*
After 6 months	25.16 (5.15)	20.55 (4.68)	−4.61 [-7.50, -1.72]*
Change	0.79 (0.62)	0.57 (0.69)	−0.22 [-0.61, 0.17]
25-hydroxyvitamin D [ng/mL; mean (SD)]			
Baseline	20.2 (6.0)	21.0 (5.1)	0.8 [-2.5, 4.1]
After 6 months	43.2 (7.3)#	41.1 (6.4)#	−2·1 [-6.1, 1.9]
Change	23.0 (11.8)	20.1 (11.3)	−2.9 [-9.7, 3.9]

CI: confidence interval; IU: international unit; SD: standard deviation; SPINA: structure parameter inference approach; TgAb: thyroglobulin antibodies; TPOAb: thyroid peroxidase antibodies.
* Statistically significant difference between both groups.
# Statistically significant difference between post-treatment and baseline values in the same group.
& Statistically significant difference between the effect of vitamin D in both groups.
[1] Reference range:1.3–4.1.
[2] Reference range: 1.4–8.7 pmol/s.
[3] Reference range: 20–60 nmol/s.

The strength of our study was including two relatively homogenous groups of patients. Because the participants were not treated with exogenous thyroid hormones, the study protocol enabled us to exclude the hypothesis that the impact of both micronutrients on antibody titers in modulated by levothyroxine treatment. Such a possibility was suggested by some observational studies showing that levothyroxine decreased thyroid antibody titers [19,24], the effect of selenium on thyroid autoimmunity was stronger in patients receiving levothyroxine [19], as well as that cholecalciferol reduced thyroid antibody titers in levothyroxine-treated patients, even if baseline 25-hydroxyvitamin D levels were within the reference

Table 3
Treatment-induced changes in thyroid antibody titers in different tertiles of 25-hydroxyvitamin D levels.

	Selenomethionine-treated women			Selenomethionine-naïve women		
	Lowest tertile	Intermediate tertile	Highest tertile	Lowest tertile	Intermediatetertile	Highest tertile
ΔTPOAb [%; mean (SD)]	−50.6 (15.5)*	−44.0 (12.4)*	−20.9 (9.8)	−32.0 (9.1)*,#	−22.3 (7.4)*	−10.2 (7.1)
ΔTGAb [%; mean (SD)]	−42.1 (12.0)*	−39.5 (9.5)*	−20.4 (8.2)	−32.9 (9.7)*,#	−23.4 (6.9)*	−11.5 (6.4)

Data represent percent changes in thyroid antibody titers from baseline after adjustment for baseline values.
TgAb: thyroglobulin antibodies; TPOAb: thyroid peroxidase antibodies.
* Statistically significant *vs.* the highest tertile.
# Statistically significant *vs.* the intermediate tertile.

Table 4
Correlations between the assessed variables.

Correlated variables		Selenomethionine-treated women	Selenomethionine-naive women
TPOAb	TgAb	0.52*	0.55*
TPOAb	25-hydroxyvitamin D	−0.46*	−0.37*
TgAb	25-hydroxyvitamin D	−0.34*	−0.29*
TPOAb	SPINA-GT index	−0.34*	−0.31*
TgAb	SPINA-GT index	−0.23	−0.24
ΔTPOAb	25-hydroxyvitamin D	−0.37*	−0.49*
ΔTgAb	25-hydroxyvitamin D	−0.32*	−0.39*
ΔTPOAb	Δ25-hydroxyvitamin D	0.34*	0.41*
ΔTgAb	Δ25-hydroxyvitamin D	0.29*	0.35*
ΔTPOAb	ΔSPINA-GT index	0.43*	0.35*
ΔTgAb	ΔSPINA-GT index	0.39*	0.29*

Data represent the correlation coefficients (r values).
TgAb: thyroglobulin antibodies; TPOAb: thyroid peroxidase antibodies.
* Statistically significant.

range [13]. Similar baseline antibody titers in both treatment arms resulted from a selection procedure, used in the study, the aim of which was to obtain two groups of women with Hashimoto's thyroiditis of similar severity. In turn, excluding women with BMI $\geq$ 35 kg/m^2 decreased sequestration of large amounts of vitamin D in the adipose tissue and therefore the daily dose of cholecalciferol used in the study was enough to normalize 25-hydroxyvitamin D levels in both groups.

The Upper Silesia, where our study was conducted, is an area with inadequate selenium supply, particularly in women [25]. Although the study of Kłapcińska et al. [25] was published in 2005, severe selenium deficits have been noticed recently in the population of young Polish women with and without thyroid autoimmune disease [26]. Both study groups differed in baseline values of the free triiodothyronine/free thyroxine ratio and the SPINA-GD index, reflecting activity of all peripheral deiodinases and conversion efficiency [21], and these differences persisted after vitamin D treatment. Taking into account that deiodinases (enzymes converting thyroxine to triiodothyronine) are selenoproteins [27], higher values of free triiodothyronine/free thyroxine ratio and the SPINA-GD index in selenomethionine-treated than selenomethionine-naïve women seem to reflect differences in baseline selenium status resulting from its supplementation in one treatment arm. We did not measure circulating (plasma, serum or whole-blood) levels of selenium or selenoproteins, not correlating well with dietary consumption of this micronutrient in adults. Non-specific incorporation into albumin and other proteins causes that selenomethionine leads a higher increase in circulating levels of selenium than intake of inorganic forms of this micronutrient, such as selenite or selenite [28]. Moreover, owing to treatment-induced saturation of several selenium-containing enzymes, the relationship between selenium consumption and circulating selenium status is not linear and therefore, selenium/selenoproteins levels better reflect baseline status of this micronutrient than treatment-induced improvement in selenium homeostasis [29]. Interestingly, the lack of correlations between baseline and treatment-induced changes in the free triiodothyronine/free thyroxine ratio and in the SPINA-GD index, being indirect markers of selenium homeostasis, and thyroid antibody titers may indicate that the effect of vitamin D/selenomethionine combination therapy on thyroid autoimmunity is more likely to depend on the immunological effects of selenium than on attained levels of this micronutrient.

No difference between both study arms in cholecalciferol-induced increase in 25-hydroxyvitamin D levels may imply that the effect of cholecalciferol on thyroid autoimmunity is independent of vitamin D status. However, other results of the study contradict this interpretation. In both study groups, treatment-induced changes in thyroid antibody titers correlated with both baseline and treatment-induced changes in serum levels of 25-hydroxyvitamin D. Moreover, in selenomethionine-naïve women, the impact of vitamin D on TPOAb and TgAb was strongest in patients with 25-hydroxyvitamin D levels in the lowest tertile. Finally, between-group differences in the SPINA-GD index were still present at the end of the study period. All these findings suggest that there were not pharmacokinetic interactions between selenomethionine and vitamin D, exogenous cholecalciferol preparations did not modulate selenomethionine action on thyroid hormone metabolism and that selenium intake, by enhancing vitamin D action, may make patients with Hashimoto's thyroiditis more susceptible to the beneficial effect of cholecalciferol.

Although neither the combination therapy nor vitamin D alone affected plasma levels of thyrotropin and free thyroid hormones, both treatment options (but stronger vitamin D/selenomethionine combination therapy) increased the SPINA-GT index, being a sensitive marker of secretory function of the thyroid gland [21,22]. Moreover, the effect of the combination therapy and, to a lesser extent, also of vitamin D alone on the SPINA-GT index correlated with both baseline and treatment-induced changes in thyroid antibodies. Therefore, it seems that patients with the highest titers of thyroid antibodies are probably the best candidates for the combination therapy. Because titers of thyroid antibodies, particularly of TPOAb, have a predictive value in determining the risk of future hypothyroidism [30, 31], vitamin D/selenomethionine combination therapy should be considered as a treatment of choice particularly in euthyroid women at high risk of permanent thyroid hypofunction. Interestingly, in selenomethionine-naïve women, the effect of vitamin D depended mainly on baseline levels of 25-hydroxyvitamin D. Taking into consideration that the relationships between the effect on antibody titers and a tertile of 25-hydroxyvitamin D were more pronounced, while correlations between treatment-induced changes in titers of TPOAb and TgAb and 25-hydroxyvitamin vitamin D levels were stronger in selenomethionine-naïve than selenomethionine-treated women, vitamin D monotherapy should be used mainly in women with severe forms of hypovitaminosis D and only moderately increased antibody titers.

Because of paucity of data, it is difficult to explain the obtained results on a molecular level. According to the protocol, during the study period, no treatment arm received selenomethionine alone. However, its inhibitory effect on thyroid autoimmunity does not seem to have increased with time because the strongest impact on antibody titers was observed between 6 and 12 months since the initiation of selenomethionine therapy, but no later [Krysiak et al., unpublished]. This was the main reason why the current study included only patients who had been treated with selenomethionine for at least 12 months. Although our study did not include a control group of vitamin D- and selenomethionine-naïve women (which is undoubtedly its limitation), the obtained results do not seem to reflect a time-dependent spontaneous improvement in antibody titers. In line with this assumption, six-month placebo treatment did not affect circulating levels of thyrotropin and free thyroid hormones, as well as titers of thyroid antibodies in a similar population of euthyroid women with Hashimoto's thyroiditis inhabiting the Upper Silesia [19]. Similarly, no differences in Jostel's thyrotropin index between both treatment arms at study entry and in the last day indicate that neither vitamin D/selenomethionine combination therapy nor vitamin D alone affect pituitary thyrotropic function [23]. The most probable mechanism is an additive effect of selenium and vitamin D either on enzymes

playing a role in the regulation of redox processes or inflammation, or on inflammatory cells. In line this explanation, sodium selenite increased calcitriol-induced expression of selenoprotein thioredoxin reductase [32], an enzyme protecting against oxidant injury, cell growth and transformation [33]. Alternatively, taking into account its inhibitory action on T cells [34], vitamin D may potentiate lymphocyte-suppressive effects of selenomethionine, acting similarly to exogenous levothyroxine [19]. Another possible explanation is a stimulatory effect of selenium on vitamin D activation because organoselenium was found to increase 1α-hydroxylation of one of vitamin D analogs [35]. Finally, calcitriol may enhance cellular selenium uptake by self-reactive cells, the effect observed in the brush border membrane vesicles isolated from duodena [36].

Some other study limitations should be acknowledged. Firstly, a small number of patients, as well as a short period of treatment limit the statistical significance of the obtained results. Secondly, Poland is a country with low selenium status [25] and adequate iodine intake [37]. Therefore, it is not certain whether the effect of vitamin D/selenomethionine treatment is the same in selenium-sufficient and iodine-depleted areas. Moreover, the results may differ in men, not included in the study. Finally, the question whether the effect of vitamin D and selenomethionine is similar in hypothyroid patients receiving levothyroxine treatment requires further research.

In conclusion, vitamin D/selenomethionine combination therapy and, to a lesser extent also vitamin D monotherapy affected serum titers of thyroid antibodies in young euthyroid women with Hashimoto's thyroiditis. The effect on thyroid autoimmunity depended on baseline antibody titers and vitamin D status. The obtained results suggest that optimal candidates for vitamin D/selenomethionine combination are women with very high thyroid antibody titers and that cholecalciferol monotherapy may bring particular benefits to women with Hashimoto's thyroiditis with severe vitamin D deficiency and moderately increased antibody titers. Because of study limitations, large randomized, double blind, placebo-controlled studies are required to support the obtained results.

Author Contributions

R. Krysiak conceived of the study, participated in its design, performed the statistical analysis, as well as drafted and edited the manuscript. K. Kowalcze conducted the literature search, carried out the assays and performed the statistical analysis. B. Okopień participated in its design and coordination, and provided critical input during manuscript preparations. All authors read and approved the final manuscript.

Disclosure Statement

The authors declare no conflicts of interest

Acknowledgements

The study was supported by the statutory grant of the Medical University of Silesia (KNW-1-062/N/7/0). The experiments comply with the current law of Poland.

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