**Neuroendocrinology Letters** Volume 38 No. 8 2017
ISSN: 0172-780X; ISSN-L: 0172-780X; Electronic/Online ISSN: 2354-4716
*Web of Knowledge / Web of Science:* Neuroendocrinol Lett
*Pub Med / Medline:* Neuro Endocrinol Lett

# Estimation of vitamin D status in patients with secondary and primary hypothyroidism of different etiology

**Hanna Ławnicka [1], Anna Galant-Gdula [1], Ewelina Motylewska [1], Jan Komorowski [2], Jacek Świętosławski [3], Henryk Stępień [1]**

1 Department of Immunoendocrinology, Chair of Endocrinology, Medical University of Lodz, Poland
2 Department of Clinical Endocrinology, Chair of Endocrinology, Medical University of Lodz, Poland
3 Department of Neuroendocrinology, Interdepartmental Chair of Laboratory and Molecular Diagnostics, Medical University of Lodz, Poland

*Correspondence to:* Hanna Ławnicka MD., PhD.
Department of Immunoendocrinology, Chair of Endocrinology
Medical University of Lodz, Sterlinga 3, 91-425 Lodz, Poland.
TEL/FAX: +48 42 6324854; E-MAIL: hanna.lawnicka@umed.lodz.pl

*Submitted: 2017-10-06 Accepted: 2017-03-08 Published online: 2018-01-23*

*Key words:* **vitamin D; primary hypothyroidism; secondary hypothyroidism; vitamin D deficiency**

Neuroendocrinol Lett 2017; **38**(8):565–574 **PMID:** 29504734 NEL380817A07 

## Abstract

**BACKGROUND:** Though vitamin D deficiency is a global problem with wide spectrum of severe public health consequences, inadequate vitamin D status still remains one of the most common and untreated medical conditions. Thyroid diseases, including hypothyroidism, also represent the most frequent endocrinopathies in general population.

**OBJECTIVES:** To determine the vitamin D status in hypothyroid patients and to ascertain the status of thyroid hormone replacement.

**METHODS:** The 25(OH)D concentrations (ECLIA) in 71 hypothyroid patients recruited in the Outpatient Clinic of Endocrinology or Department of Clinical Endocrinology were assessed. The examined group was composed of 59 subjects diagnosed with primary hypothyroidism of different etiology and 12 patients with secondary hypothyroidism. The control group included 16 healthy individuals.

**RESULTS:** Mean serum 25(OH)D concentration in healthy volunteers was significantly lower than in hypothyroid subjects (13.09±1.63 vs. 19.92±1.37 ng/mL). Patients with a history of thyroidectomy presented with significantly higher mean 25(OH)D concentration than controls (23.25±2.75 vs. 13.09±1.63 ng/mL). Mean serum 25(OH)D concentration in effectively treated hypothyroidism was significantly higher than in controls (21.90±1.47 vs. 13.09±1.63 ng/mL) or undertreated hypothyroidism (21.90±1.47 vs. 13.52±3.39 ng/mL). Hypothyroid patients aged under 60 years presented with significantly lower mean 25(OH)D concentration than elders (16.46±1.54 vs. 24.39±1.18 ng/mL). The major 25(OH)D deficient (≤10 ng/mL) or deficient (≤20 ng/mL) hypothyroid patients were significantly younger than those with 25(OH)D concentrations exceeding 10 ng/mL or 20 ng/mL respectively.

**CONCLUSIONS:** These findings confirm the necessity for vitamin D status improvement in the general population and more effective healthcare of hypothyroid patients.

ORIGINAL ARTICLE

**Abbreaviations:**

| | |
|---|---|
| 1,25(OH)2D | - 1,25-dihydroxyvitamin D |
| 1,25(OH)2D3 | - 1,25-dihydroxycholecalciferol; 1, 25-dihydroxyvitamin D3 |
| 24,25(OH)2D3 | - 24,25-dihydroxycholecalciferol; 24,25-dihydroxyvitamin D3 |
| 24,25(OH)D | - 24,25-dihydroxyvitamin D |
| 25(OH)D | - 25-hydroxyvitamin D |
| 25(OH)D3 | - 25-hydroxycholecalciferol; 25-hydroxyvitamin D3 |
| AITD | - autoimmune thyroid disease |
| CMIA | - chemiluminescent magnetic immunoassay |
| CV | - cofficient of variation |
| ECLIA | - electro-chemiluminescence binding assay |
| fT3 | - free triiodothyronine |
| fT4 | - free thyroxine |
| HT | - chronic autoimmune Hashimoto's thyroiditis |
| LoQ | - limit of qualification |
| SEM | - standard error of mean |
| T3 | - triiodothyronine |
| T4 | - tetraiodothyronine, thyroxine |
| TGAb | - antithyroglobulin antibody |
| TPOAb | - antithyroid peroxidase antibody |
| TRH | - Thyrotropin-Releasing Hormone |
| TSH | - Thyroid-Stimulating Hormone |
| VDR | - vitamin D receptor (VDR) |

## INTRODUCTION

Vitamin D deficiency is nowadays recognized as a global problem with a wide spectrum of severe health consequences, which are both musculoskeletal and non-skeletal in nature (Holick & Chen 2008). It has been estimated that 20% to 80% of US, and European population, meaning 1 billion people worldwide, is vitamin D deficient or insufficient (Holick 2007; Hosseinnezhad & Holick 2013). Vitamin D deficiency affects 28–100% of healthy and 70–100% of hospitalized adults in Europe (Holick 2006). In adults it plays a role in the development and exacerbation of osteopenia, osteoporosis, fractures and muscle weakness. In addition, an increased risk of autoimmune diseases, as well as of some deadly cancers, cardiovascular and infectious diseases, neurocognitive dysfunction and mental illness, infertility, adverse pregnancy and birth outcomes are known non-skeletal consequences of vitamin D deficiency (Holick 2007; Pludowski *et al.* 2013). Consequently vitamin D deficiency/insufficiency is associated with an increased risk of all-cause mortality (Pludowski *et al.* 2013).

The biological functions of vitamin D in target tissues are achieved by its active metabolite, 1,25(OH)2D which is transported in the blood and binds to the nuclear high-affinity vitamin D receptor (VDR) present in most human tissues and cells throughout the body. The activated form of VDR binds to specific nucleotide sequences in the DNA known as vitamin D response elements, resulting in either up- or down-regulation of the gene (Hossein-nezhad & Holick 2013). Thus, the mechanism of vitamin D action based on the regulation of gene transcription through the specific receptor expressed in distant target tissues, such as in a classic endocrine pathway, confirms that the vitamin is a unique hormone and a distinct part of the endocrine system. Although the true biological role of vitamin D in the human body and the serious health consequences associated with its deficiency have lately become much better understood, inadequate vitamin D status still remains one of the most common, undiagnosed and untreated medical conditions in the world.

Thyroid diseases affecting 30–40% patients treated in endocrine practice, are the most common, although often undiagnosed, endocrinopathies in the general population (Garmendia Madariaga *et al.* 2014). Among them, hypothyroidism is the most prevalent dysfunction of thyroid gland commonly seen in outpatient practice. According to a recently published meta-analysis concerning the epidemiology of thyroid dysfunction in Europe, the incidence of hypothyroidism was estimated as 226.2 per 100 000 per year and the mean prevalence of total and undiagnosed disease was 3.05% and 4.94% respectively (Garmendia Madariaga *et al.* 2014).

Hypothyroidism results from the inadequate production or insufficient action of thyroid hormones, causing their deficiency in target tissues. Due to its etiology, the disease is classified thus: primary hypothyroidism (thyroid gland failure) as the most common form, with secondary (insufficient synthesis of Thyroid-Stimulating Hormone, TSH in pituitary) or tertiary disease (hypothalamic deficiency of Thyrotropin-Releasing Hormone, TRH) occurring more rarely. Chronic autoimmune Hashimoto's thyroiditis (HT), nowadays considered the most common autoimmune disease, is the main cause of primary hypothyroidism in which inflammatory cytokines and immunomodulatory agents are involved (Caturegli *et al.* 2014). Hashimoto's thyroiditis is followed by thyroidectomy/thyroid surgery, destructive radioactive iodine therapy or antithyroid medications as the next common causes of primary disease.

The possible link between thyroid function and vitamin D status arises from the fact that both vitamin D and thyroid hormones bind to similar receptors called steroid/nuclear hormone receptors (Mackawy *et al.* 2013). Moreover, a polymorphism in the VDR gene was found to be associated with the risk of autoimmune thyroid diseases (AITD) including Hashimoto's thyroiditis and Graves' disease (Feng *et al.* 2013; Yazici *et al.* 2013). In addition, VDR expression has also been observed in normal thyroid tissue as well as in benign and malignant thyroid tumors (Clinckspoor *et al.* 2012). These findings, together with the proved regulatory effect of vitamin D on inflammatory responses and autoimmunity (Pludowski *et al.* 2013), indicate the possibility of the influence of vitamin D status on thyroid function as well as on the relationship between vitamin D deficiency and AITD. However, many of the studies performed on the association of vitamin D status and AITD focus merely on autoimmune thyroiditis or hypothyroidism related just to Hashimoto's thyroiditis,

and vitamin D deficiency was usually reported in these patients (Camurdan *et al.* 2012; Mansournia *et al.* 2014; Tamer *et al.* 2011). Therefore, the aim of our study was to determine the vitamin D status in patients diagnosed with hypothyroidism related either to autoimmune thyroiditis or other common causes, considering the status of thyroid hormone replacement.

## MATERIAL AND METHODS

*Subjects*

Seventy-one patients (56 female and 15 males) aged from 24 to 90 years (54.4±17.9) (mean ± standard deviation), most of them diagnosed and treated in Outpatient Clinic of Endocrinology and some at the Department of Clinical Endocrinology, Medical University of Lodz between January 2012 and May 2013, were enrolled to the study. The examined group was composed of 59 subjects diagnosed with primary hypothyroidism and 12 patients suffering with secondary hypothyroidism caused by hypopituitarism. The primary hypothyroidism group included 22 cases of Hashimoto's thyroiditis, 24 of thyroidectomy related hypothyroidism, 3 of congenital hypothyroidism, 3 of radioactive iodine therapy followed hypothyroidism, and 7 patients of unknown etiology hypothyroidism accompanying partial hypopituitarism (meaning deficiency of pituitary hormones others than TSH).

Among 71 hypothyroid patients, 54 subjects presented normal TSH (primary hypothyroidism) or free T3/T4 (free triiodothyronine/thyroxine) (secondary hypothyroidism) levels (cases supplemented with the proper doses of levothyroxine), elevated TSH or low free T4 levels were recognized in 11 patients (too low levothyroxine doses, undertreated hypothyroidism) and 6 primary hypothyroid subjects had low TSH level (too high levothyroxine doses, overtreated hypothyroidism).

The control group included 16 healthy individuals aged from 33 to 68 years (54.4±9.3) with no history of any thyroid disease and no use of vitamin D supplements.

*Methods*

The study protocol was based on the analysis of the results of the laboratory tests routinely performed in hypothyroid patients of Outpatient Clinic of Endocrinology and Department of Clinical Endocrinology. Moreover the medical records of enrolled subjects were reviewed. The diagnosis of hypothyroidism was based on clinical evaluation (patients' history, clinical examination) and confirmed by routine laboratory tests assessing thyroid function and the presence of thyroid antibodies. The laboratory tests included measurement of serum sensitive TSH, serum free triiodothyronine (fT3) and free thyroxine (fT4) concentrations, as well as the presence of serum antithyroid peroxidase (TPOAb) and antithyroglobulin (TGAb) antibodies. Serum vitamin D concentration was analyzed in hypothyroid patients. Peripheral blood samples were collected by venipuncture after an overnight fast and centrifuged. Thyroid hormone and antithyroid antibody evaluation was performed immediately after collection, and the sera for vitamin D measurement were aliquoted and stored at –80 °C until analysis.

The serum TSH concentration was measured by automated electro-chemiluminescence binding assay (ECLIA) (COBAS e 411, Roche Diagnostics GmBH, Germany) according to the manufacturer's protocol. The assay was designed to have intermediate precision of <9% (cofficient of variation, CV), the measuring range of 0.005–100 μIU/mL; the normal range was 0.27–4.2 μIU/mL.

The serum fT3 and fT4 concentrations were evaluated by automated chemiluminescent magnetic immunoassay (CMIA) (ARCHITECT i1000SR, Abbott, Ireland) according to the manufacturer's protocols. The ARCHITECT Free T3 assay was designed to have an analytical sensitivity of ≤1.0 pg/mL and precision ≤10% (total CV); the sensitivity range was 0–30 pg/mL and the normal range was 1.71–3.71 pg/mL. The ARCHITECT Free T4 assay was designed to have the limit of qualification (LoQ) of ≤0.4 ng/dL and precision ≤10% (total CV) <10%; the sensitivity range was 0–6 ng/dL and the normal range was 0.70–1.48 ng/dL.

Vitamin D serum concentrations were measured by electro-chemiluminescence binding assay (ECLIA) for the in vitro determination of total 25-hydroxyvitamin (COBAS e 411, Roche Diagnostics GmBH, Germany) according to the manufacturer's protocol. The assay is based on the competitive protein binding test and intended for the quantitative determination of both 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2 in human serum and plasma (Vitamin D total assay). Detection limit of the assay was 3.00 ng/mL, functional sensitivity was 4.01 ng/mL (CV 18.5%), measuring range was 3.00–70.0 ng/mL, within-run precision was ≤6.5%, and intermediate precision was ≤11.5%.

According to the recommendations of the Endocrine Society, vitamin D deficiency was defined as a 25(OH) D level of 20 ng/mL or less, vitamin D insufficiency as 21 to 29 ng/mL, and vitamin D sufficiency (preferred level) as 30 ng/mL or greater (Hossein-nezhad & Holick 2013). 25(OH)D level of 10 ng/mL or less was defined as major deficiency.

*Statistical analysis*

Statistical analysis was performed using Statistica 10 Software. The Shapiro-Wilk test was applied to confirm the data distribution. Comparisons between the groups were made using the Student's paired t-test for data following a normal distribution, while a nonparametric Mann-Whitney U test was used for data not normally distributed. Comparisons of more than two different groups were performed using a one-way ANOVA followed by LSD test. A $p$-value ≤0.05 was considered

statistically significant. The values are presented as the mean ± standard error of mean (SEM). Moreover, a trial version of Graph Pad Software was used to create figures presenting each separate result.

The study protocol was in compliance with the Helsinki Convention and was approved by the Bioethics Committee of the Medical University of Lodz.

## RESULTS

The mean serum concentration of 25(OH)D in patients diagnosed with hypothyroidism and in healthy controls were both recognized as deficient levels but in healthy volunteers the obtained value was significantly lower than in hypothyroid subjects (13.09±1.63 vs. 19.92±1.37 ng/mL, $p<0.01$). Surprisingly, none of the healthy volunteers met the criteria for vitamin D sufficiency and only two of them presented with 25(OH)D values in insufficiency range (Figure 1). In contrast to secondary hypothyroid patients, the mean serum concentrations of 25(OH)D in primary hypothyroidism group were significantly higher than in controls (19.94±1.52 vs. 13.09±1.63 ng/mL, $p<0.01$) (Figure 2).

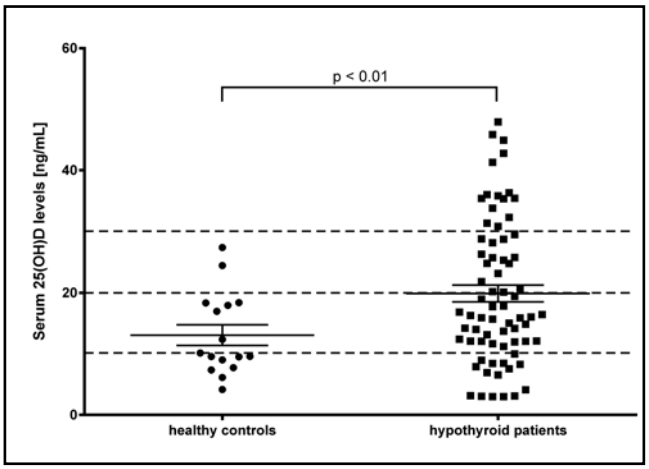


**Fig. 1.** 25(OH)D concentrations (ng/mL) in healthy volunteers (controls) and hypothyroid patients. Solid horizontal lines correspond to the means±SEM of 25(OH)D serum levels; three dashed lines (upper, middle and lower) represent the limits of 25(OH)D suficiency (30 ng/mL), deficiency (20 ng/mL) or major deficiency (10 ng/mL), respectively.

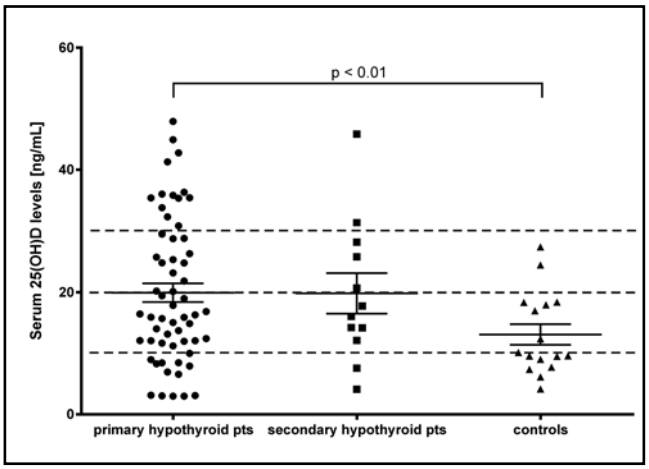


**Fig. 2.** 25(OH)D concentrations (ng/mL) in in healthy volunteers (controls) primary hypothyroid patients and secondary hypothyroid patients. Solid horizontal lines correspond to the means±SEM of 25(OH)D serum levels; three dashed lines (upper, middle and lower) represent the limits of 25(OH)D suficiency (30 ng/mL), deficiency (20 ng/mL) or major deficiency (10 ng/mL), respectively.

The analysis of vitamin D status in reference to the particular etiology of hypothyroidism revealed significantly higher mean vitamin D concentration in patients with a history of thyroidectomy (insufficiency vitamin D range) than the mean value (deficiency range) obtained in healthy controls (23.25±2.75 vs. 13.09±1.63 ng/mL, $p<0.05$) (Figure 3). Moreover in the hypothyroid group related to the thyroid surgery, 7 patients (29%) obtained preferred level of vitamin D (≥30 ng/mL) and 5 persons (21%) fulfilled slight insufficiency criteria (24.82–28.85 ng/mL), meaning 50% of subjects exceeded deficiency or sufficiency values (≥20 ng/mL) vs. only 2 healthy volunteers (12%) in control group (both presenting with insufficiency vitamin D values). The mean serum concentrations of 25(OH)D in the remaining hypothyroid groups were as follows: 19.83±3.32 ng/mL in patients diagnosed with hypopituitarism, 18.36±2.12 ng/mL in Hashimoto thyroiditis cases and 14.97±3.19 ng/mL in group of unknown etiology hypothyroidism (Figure 3).

The mean serum concentrations of 25(OH)D in hypothyroid patients effectively treated with proper doses of levothyroxine (presented with normal TSH in primary hypothyroidism or normal free T3/T4 in secondary hypothyroidism groups) were found to be vitamin D insufficient (21.90±1.47 ng/mL). This value was significantly higher than deficiency values observed in controls (21.90±1.47 vs. 13.09±1.63 ng/mL, $p<0.01$) and in patients with elevated TSH or low free T4 levels, indicating undertreated hypothyroidism due to too low levothyroxine replacement (21.90±1.47 vs. 13.52±3.39 ng/mL, $p<0.05$) (Figure 4).

The analysis of the mean vitamin D serum concentrations in hypothyroid patients aged under 60 years and aged 60 years or older revealed significantly higher 25(OH)D in elders than younger patients (24.39±1.18 vs. 16.46±1.54 ng/mL, $p<0.01$) (Figure 5), whereas serum concentrations of TSH, fT3 or fT4 did not significantly differ between those groups (data not shown). Such finding was not observed in healthy controls (data not shown).

In the group of all hypothyroid subjects, significant differences in patients' age and serum fT3 concentrations were observed between those diagnosed with major vitamin D deficiency (≤10 ng/mL) and others (>10 ng/mL). The major deficient patients (mean 25(OH)D concentration: 6.17±0.65 ng/mL) were significantly younger (45.53±4.41 vs. 59.27±2.26 yrs, $p<0.01$) and had significantly lower serum fT3 concentration

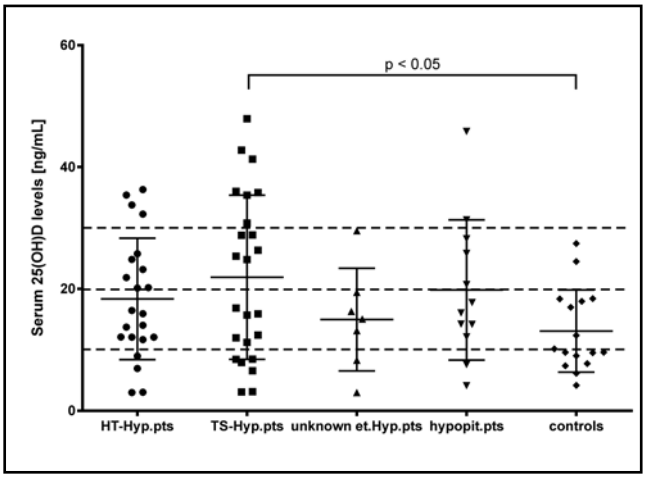


**Fig. 3.** 25(OH)D concentrations (ng/mL) in healthy volunteers (controls) and particular subgroups pf hypothyroid patients. Solid horizontal lines correspond to the mean ±SEM of 25(OH) D serum levels; three dashed lines (upper, middle and lower) represent the limits of 25(OH)D suficiency (30 ng/mL), deficiency (20 ng/mL) or major deficiency (10 ng/mL), respectively. HT-hyp – Hashimoto's thyroiditis related hypothyroidism; TS-hyp – thyroid surgery related hypothyroidism; unknown et.hyp – uknown etiology of hypothyroidism; hypopit-hyp – hypopituitarism related hypothyroidism (secondary hypothyroidism); controls – healthy volunteers.

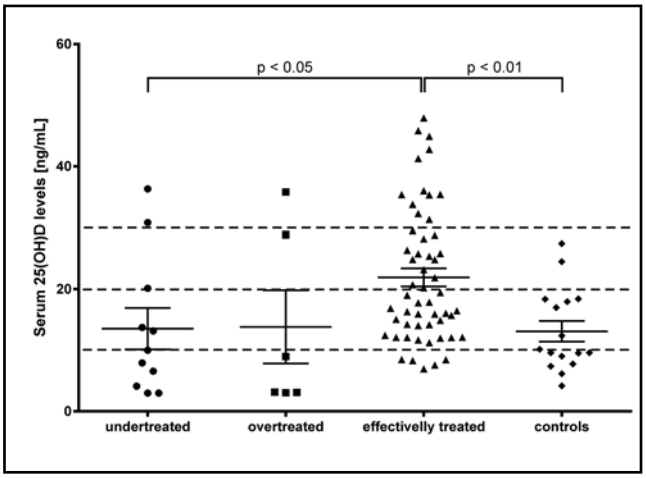


**Fig. 4.** 25(OH)D concentrations (ng/mL) in healthy volunteers (controls) and primary hypothyroid patients. Solid horizontal lines correspond to the mean ±SEM of 25(OH)D serum levels; three dashed lines (upper, middle and lower) represent the limits of 25(OH)D suficiency (30 ng/mL), deficiency (20 ng/mL) or major deficiency (10 ng/mL), respectively. Mean±SEM; undertreated – patients with too low levothyroxine replacement; overtreated - patients with too high levothyroxine replacement; effectively treated – patients with patients with proper levothyroxine replacement; controls – healthy volunteers.

(2.29±0.16 vs. 2.74±0.06 pg/mL, $p<0.05$) than those with vitamin D levels exceeding 10 ng/mL (mean 25(OH)D concentration: 23.61±1.36 ng/mL). Such observations were absent from the analysis of serum TSH or fT4 concentrations. Similarly, hypothyroid patients diagnosed with vitamin D deficiency (≤20 ng/mL, mean 25(OH) D concentration: 11.53±0.74 ng/mL) were significantly younger (51.46±2.94 vs. 63.07±2.55 yrs, $p<0.01$) than those with 25(OH)D exceeding 20 ng/mL (mean 25(OH)D concentration: 31.32±1.40 ng/mL) (Table 1).

In the primary hypothyroid group, a significant difference in patient age was also observed between subjects diagnosed with major vitamin D deficiency (≤10 ng/mL) and others (>10 ng/mL). The patients recognized as suffering from major vitamin D deficiency (mean 25(OH)D concentration: 6.22±0.73 ng/mL) were significantly younger (44,31±4.97 vs. 61.09±2.38 yrs, $p<0.01$) than others (mean 25(OH)D concentration: 23.82±1.50 ng/mL). Primary hypothyroid patients diagnosed with vitamin D deficiency (≤20 ng/mL, mean 25(OH)D concentration: 11.43±0.82 ng/mL) were significantly younger (52.68±3.38 vs. 63.80±2.54 yrs, $p<0.05$) than those with 25(OH)D exceeding 20 ng/mL (mean 25(OH)D concentration: 31.51±1.5 ng/mL) (Table 2).

In the subgroup diagnosed with thyroidectomy related hypothyroidism, a significant difference in patient age was observed between subjects classified as having a major vitamin D deficiency (≤10 ng/mL) and others (>10 ng/mL): 48.50±2.54 vs. 66.67±2.62 yrs, $p<0.01$). Such observations were absent in the analysis

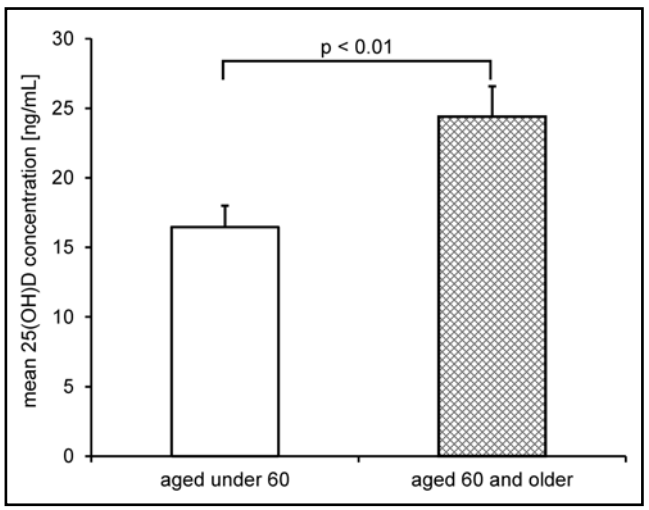


**Fig. 5.** Mean 25(OH)D concentrations (ng/mL) in hypothyroid patients aged under 60 yrs and aged 60 yrs. Mean±SEM.

of those with vitamin D deficiency (≤20 ng/mL) and remainders (>20 ng/mL) or in Hashimoto's thyroiditis related hypothyroidism subgroup (Table 3).

## DISCUSSION

### *Vitamin D deficiency in healthy controls*

The deficiencies in mean 25(OH)D serum concentration demonstrated in this study in both healthy volunteers and hypothyroid subjects might be expected since vitamin D deficiency is nowadays recognized as

**Tab. 1**. Hypothyroid patient age, serum 25(OH)D (ng/mL), TSH (μIU/mL), fT3 (pg/mL) and fT4 (ng/dL) concentrations in reference to the degree of vitamin D deficiency: major deficient hypothyroid patients (25(OH)D ≤10 ng/mL) vs. reminders (25(OH)D >10 ng/mL) and deficient hypothyroid patients (25(OH)D ≤20 ng/mL) vs. reminders (25(OH)D >20 ng/mL).

| 25(OH)D | | Age, yrs | 25(OH)D, ng/mL | TSH, μIU/mL | fT3, pg/mL | fT4, ng/dL |
|---|---|---|---|---|---|---|
| ≤ 10 ng/mL | Mean | 45.53 | 6.17 | 3.72 | 2.29 | 0.98 |
| | SD | 17.09 | 2.53 | 4.45 | 0.56 | 0.26 |
| | SEM | 4.41 | 0.65 | 1.15 | 0.16 | 0.07 |
| > 10 ng/mL | Mean | 59.27 | 23.61 | 1.66 | 2.74 | 1.06 |
| | SD | 16.95 | 10.14 | 1.69 | 0.31 | 0.16 |
| | SEM | 2.26 | 1.36 | 0.23 | 0.06 | 0.03 |
| Significance | | $p<0.01$ | $p<0.001$ | $p>0.05$ | $p<0.05$ | $p>0.05$ |
| ≤ 20 ng/mL | Mean | 51.46 | 11.53 | 2.29 | 2.60 | 1.02 |
| | SD | 18.83 | 4.76 | 3.30 | 0.54 | 0.21 |
| | SEM | 2.94 | 0.74 | 0.53 | 0.11 | 0.04 |
| > 20 ng/mL | Mean | 63.07 | 31.32 | 1.89 | 2.57 | 1.07 |
| | SD | 13.94 | 7.69 | 1.54 | 0.22 | 0.17 |
| | SEM | 2.55 | 1.40 | 0.29 | 0.06 | 0.04 |
| Significance | | $p<0.01$ | $p<0.001$ | $p>0.05$ | $p>0.05$ | $p>0.05$ |

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; fT3, free triiodothyronine; fT4, free thyroxine; TSH, Thyroid-Stimulating Hormone; SD, standard deviation; SEM, standard error of measurement; yrs, years; A *p*-value of <0.05 is considered statistically significant.

**Tab. 2.** Primary hypothyroid patient age, serum 25(OH)D (ng/mL), TSH (μIU/mL), fT3 (pg/mL) and fT4 (ng/dL) concentrations in reference to the degree of vitamin D deficiency: major deficient hypothyroid patients (25(OH)D ≤10 ng/mL) vs. reminders (25(OH)D >10 ng/mL) and deficient hypothyroid patients (25(OH)D ≤20 ng/mL) vs. reminders (25(OH)D >20 ng/mL).

| 25(OH)D | | Age, yrs | 25(OH)D, ng/mL | TSH, μIU/mL | fT3, pg/mL | fT4, ng/dL |
|---|---|---|---|---|---|---|
| ≤ 10 ng/mL | Mean | 44.31 | 6.22 | 4.25 | 2.38 | 1.02 |
| | SD | 17.92 | 2.63 | 4.55 | 0.48 | 0.22 |
| | SEM | 4.97 | 0.73 | 1.26 | 0.15 | 0.06 |
| > 10 ng/mL | Mean | 61.09 | 23.82 | 1.95 | 2.72 | 1.10 |
| | SD | 16.13 | 10.19 | 1.68 | 0.37 | 0.16 |
| | SEM | 2.38 | 1.50 | 0.25 | 0.09 | 0.03 |
| Significance | | $p<0.01$ | $p<0.001$ | $p>0.05$ | $p>0.05$ | $p>0.05$ |
| ≤ 20 ng/mL | Mean | 52.68 | 11.43 | 2.68 | 2.57 | 1.05 |
| | SD | 19.68 | 4.80 | 3.45 | 0.51 | 0.20 |
| | SEM | 3.38 | 0.82 | 0.60 | 0.11 | 0.04 |
| > 20 ng/mL | Mean | 63.80 | 31.51 | 2.17 | 2.64 | 1.11 |
| | SD | 12.72 | 7.50 | 1.47 | 0.17 | 0.15 |
| | SEM | 2.54 | 1.50 | 0.29 | 0.06 | 0.04 |
| Significance | | $p<0.05$ | $p<0.001$ | $p>0.05$ | $p>0.05$ | $p>0.05$ |

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; fT3, free triiodothyronine; fT4, free thyroxine; TSH, Thyroid-Stimulating Hormone; SD, standard deviation; SEM, standard error of measurement; yrs, years; A *p*-value of <0.05 is considered statistically significant.

a global European problem (Holick 2006). Nevertheless the mean serum 25(OH)D concentration in healthy participants was found to be in the low range of the deficiency criteria, and what is disquieting and disappointing, the level is near the severe deficiency threshold and surprisingly, even significantly lower than those observed in hypothyroid patients. On the other hand, however, this finding is comparable with the results of other European population studies including Poland (Spiro & Buttriss 2014).

In recently published data obtained from 448 adults living in northern Poland, the mean 25(OH)D concentration was 14.3 ng/mL (slightly higher than those obtained in our healthy participants), 84.4% of subjects

**Table 3.** Hashimoto's thyroiditis and thyroidectomy related hypothyroid patient age and serum 25(OH)D (ng/mL) concentration in reference to the degree of vitamin D deficiency: major deficient hypothyroid patients (25(OH)D ≤10 ng/mL) vs. reminders (25(OH)D >10 ng/mL) and deficient hypothyroid patients (25(OH)D ≤20 ng/mL) vs. reminders (25(OH)D >20 ng/mL).

| Primary hypothyroidism subgroup | | 25(OH)D | Age (yrs) | 25(OH)D (ng/mL) | 25(OH)D | Age (yrs) | 25(OH)D (ng/mL) |
|---|---|---|---|---|---|---|---|
| Hashimoto's thyroiditis | Mean | ≤ 10 ng/mL | 50.25 | 5.49 | ≤ 20 ng/mL | 51.92 | 10.83 |
| | SD | | 20.77 | 2.57 | | 19.20 | 4.31 |
| | SEM | | 10.38 | 1.29 | | 5.54 | 1.24 |
| | Mean | > 10 ng/mL | 58.78 | 21.22 | > 20 ng/mL | 63.60 | 27.38 |
| | SD | | 18.09 | 8.33 | | 16.38 | 6.08 |
| | SEM | | 4.26 | 1.96 | | 5.18 | 1.92 |
| | | Significance | $p>0.05$ | $p<0.01$ | Significance | $p>0.05$ | $p<0.001$ |
| thyroidectomy | Mean | ≤ 10 ng/mL | 48.50 | 6.28 | ≤ 20 ng/mL | 59.75 | 10.15 |
| | SD | | 15.15 | 2.32 | | 17.62 | 4.48 |
| | SEM | | 6.19 | 0.95 | | 5.09 | 1.29 |
| | Mean | > 10 ng/mL | 66.67 | 27.13 | > 20 ng/mL | 64.50 | 33.68 |
| | SD | | 11.10 | 11.03 | | 10.08 | 7.15 |
| | SEM | | 2.62 | 2.60 | | 2.91 | 2.06 |
| | | Significance | $p<0.01$ | $p<0.001$ | Significance | $p>0.05$ | $p<0.001$ |

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; SD, standard deviation; SEM, standard error of measurement; yrs, years; A *p*-value of <0.05 is considered statistically significant.

were vitamin D deficient and only 2.5% presented with sufficient vitamin D status (Kmieć *et al.* 2014). Vitamin D deficiency was also identified in the vast majority of the urban population who took part in the latest large cross sectional study, the most representative examination of the Polish population so far, covering 2687 adult volunteers from ten major Polish cities. In this examination, the mean 25(OH)D concentration was higher than those noticed in our examination and reached 17.7 ng/mL, 90.3% of subjects were found to have severe or moderate deficit of vitamin D while 8.4% were found to be adequate (Płudowski *et al.* 2014b). The observations cited above are closely consistent with those obtained from the healthy volunteers participating in our study.

The findings of this and other studies indicate an insufficient supply of vitamin D within the Polish population resulting from the ineffective vitamin D skin synthesis specific to the Central Europe latitude. Epidemiological data on vitamin D status in the large geographical region of Central Europe indicate most European population having serum 25(OH)D concentrations below the optimal values as the result of the limitation of solar ultraviolet B (UVB) synthesis. In most European regions including Poland, vitamin D solar synthesis is nearly impossible from October through to March above 40 °N latitude. The mean concentrations of vitamin D in Poland were as follows: 12 ng/mL in winter for children and adolescents, 11 ng/mL in winter or 31 ng/mL in summer for adults and 12 ng/mL in winter for seniors (Pludowski *et al.* 2014a). This is in agreement with the findings of Kmieć *et al.* (2015) who examined 25(OH)D concentration in 109 adults recruited in outpatient clinic in northern Poland twice in 2012: in winter and autumn. Mean 25(OH)D serum level in winter and in autumn were 13.3 ng/mL and 22.8 ng/mL respectively. Median 25(OH)D concentrations between the two examination periods differed significantly (11.9 vs. 22.1 ng/mL).

These findings might be the explanation for the results obtained in our studies, in which healthy volunteers presented with significantly lower vitamin D status than hypothyroid patients. This surprising finding might be related to the fact that 25(OH)D concentrations in hypothyroid subjects were measured all over the year (between January 2012 and May 2013) while all the healthy participants were examined once in March. Thus the mean vitamin D status of the hypothyroid group is the final result of both winter and summer seasons, while healthy volunteers were deprived of the effect of potentially favorable summer months. The finding concerning the poor vitamin D status in healthy volunteers in our study might be also partly related to the continued, although still unsatisfactory, ambulatory healthcare of the latter. Thus the necessity of better education, changes in nutritional habits and adequate vitamin D supplementation all over the year in supposedly healthy subjects must be recommended.

The mutual interaction of thyroid status and vitamin D supply is discussed below.

*Vitamin D deficiency in hypothyroid patients*

The vitamin D deficiency in hypothyroid patients found in our study is in agreement with that found in other studies (Muscogiuri *et al.* 2015). These are, however, mainly limited to examinations of vitamin D status in patients diagnosed with autoimmune thyroiditis and/or hypothyroidism related to Hashimoto's thyroiditis.

Kivity *et al.* (2011) report a significantly higher prevalence of vitamin D deficiency in patients with AITDs than in healthy individuals (72% vs. 30.6%), or in patients with Hashimoto's thyroiditis compared to patients with non-AITDs (79% vs. 52%), indicating the potential role of vitamin D deficiency in the development of Hashimoto's thyroiditis and/or its progression to hypothyroidism. This is supported by the results of other studies (Camurdan *et al.* 2012; Mansournia *et al.* 2014). Interestingly according to Tamer *et al.* (2011) the prevalence of vitamin D insufficiency in HT patients tended to be higher in patients with overt hypothyroidism or subclinical hypothyroidism than in those with euthyroidism. This is in agreement with our observations of significantly higher 25(OH)D concentrations in hypothyroid patients effectively treated with L-thyroxine than in those with insufficient levothyroxine replacement (21.90 vs. 13.52 ng/mL).

The causes of vitamin D deficiency in hypothyroid patients may be the same as in the general population but they may also be further combined with the effect of thyroid hormones on 25(OH)D metabolism. Barsony *et al.* (1986) were the first to differentiate the vitamin D status in hypothyroid patients based on either thyroxine-dependent effects or the seasonal changes and report the possible regulatory influence of thyroid hormones on vitamin D metabolism. Serum 25(OH)D levels measured in autumn were lower in hypothyroid subjects than in healthy individuals, and vitamin D3 supplementation resulted in a smaller increase in 25(OH)D concentration in the hypothyroid group than in controls with subclinical vitamin D deficiency. The authors propose that the elevated of 25(OH)D concentration following substitution therapy in the hypothyroid patients suggest the involvement of thyroid hormones in the regulation of steroid hormone synthesis.

The potential effect of the status of the thyroid hormones on vitamin D concentrations/metabolism might be in part an explanation for our results showing higher 25(OH)D levels in hypothyroid patients than in healthy volunteers. Already in 1982, the significant effect of the functional activity of thyroid gland on the concentrations of vitamin D metabolites in hyperthyroid patients was known: the serum concentration of 25(OH)D was normal, while 1,25(OH)D was reduced and 24,25(OH)D was increased (Jastrup *et al.* 1982). Similarly MacFarlane *et al.* (1982) demonstrated unchanged 25(OH)D3 concentrations, increased 24,25(OH)2D3 levels and decreased 1,25(OH)2D3 concentrations in patients with untreated hyperthyroidism compared with control subjects. Moreover, a correlation between serum 24,25(OH)2D3 and triiodothyronine (T3) concentrations was found. Interestingly, oral antithyroid therapy resulted in a decrease of 24,25(OH)2D3 and increase of 1,25(OH)2D3 concentrations, while 25(OH)D3 level remained unchanged. The authors propose that 1,25(OH)2D3 and 24,25(OH)2D3 concentrations in hyperthyroidism might be altered by stimulated renal 24-hydroxylase and suppressed 1-hydroxylase enzymes. Triiodothyronine (T3) was postulated to have a direct stimulatory effect on 24-hydroxylase activity (MacFarlane *et al.* 1982). This may be supported by Pantazi *et al.* (2000) who studied concentrations of vitamin D metabolites in hyperthyroid patients diagnosed with Graves' disease. They confirmed that pretreatment serum 25(OH)D was normal and remained unchanged during antithyroid therapy, whereas 1,25(OH)D was initially subnormal and increased to normal level after therapy. Recently published studies also report vitamin D deficiency in patients diagnosed with Graves' related hyperthyroidism (Yasuda *et al.* 2013) which is believed to be more than a casual association (Rotondi & Chiovato 2013).

The postulated mechanism of thyroid hormone action on vitamin D metabolism was examined in the hyperthyroid animal model. The effect of thyroid gland activity, especially of triiodothyronine, on the expression of renal hydroxylases responsible for the synthesis of active or inactive vitamin D metabolites was postulated (Kozai *et al.* 2013). Thus the thyroid hormone excess in hyperthyroidism might promote the synthesis of inactive 24,25(OH)D and/or intensify the catabolism of active 1,25(OH)2D, whereas euthyroidism or hypothyroidism might favor renal synthesis of calcitriol or inhibit its catabolism.

The possible influence of thyroid hormones on metabolism of vitamin D and its derivatives might explain our findings showing higher 25(OH)D levels in hypothyroid patients than in healthy volunteers. Similarly, the significantly lower 25(OH)D concentrations identified in the present study in younger hypothyroid subjects than in those aged 60 years or more, and the fact that all hypothyroid patients diagnosed with major vitamin D deficiency (≤10 ng/mL) were significantly younger than those with 25(OH)D concentration exceeding 10 ng/mL also indicates that body metabolism may play a role in vitamin D supply. The same was observed for vitamin D deficient subjects (≤20 ng/mL) and remainders. Although this observation is contradictory to the general opinion that older age is the risk factor for vitamin D deficiency, some recently published results indicate better vitamin D supply among the older population than in young adults (Kmieć *et al.* 2014; Płudowski *et al.* 2014b).

On the other hand, our observations of higher 25(OH)D concentration in effectively treated hypothyroid subjects than in undertreated ones, as well as those indicating a significantly lower serum fT3 level in primary hypothyroid patients with major vitamin D deficiency than in those with vitamin D levels exceeding

10 ng/mL are difficult to explain with regard to thyroid related metabolism. Nevertheless it should be stressed that the present study assesses the general vitamin D status in hypothyroid patients but not the concentrations of particular vitamin D active/inactive metabolites. Secondly, although the serum concentrations of fT3 were lower in vitamin D major deficiency group than in remaining primary hypothyroid patients, they still obtained the reference ranges. Thirdly, the mutual complex interactions of thyroid hormones and vitamin D metabolites are possible since VDR expression is present in the thyroid (Clinckspoor *et al.* 2012) and thyroid hormone receptors are present in the kidney (Kozai *et al.* 2013).

Finally, our hypothyroid patients were under ambulatory healthcare with supposed awareness of the vitamin D deficiency and the necessity of its supplementation. This might be supported by the highest 25(OH)D concentrations being noted in patients with hypothyroidism related to thyroid surgery (in whom usually parathyroid glands activity is examined) and those diagnosed with hypopituitarism-related hypothyroidism (in whom usually the bone mass is examined). Nevertheless it must be stressed that all the hypothyroid patients, independent of etiology, age, sex or adequate/inadequate thyroid hormone replacement, were vitamin D deficient and the healthcare was unsatisfactory.

## CONCLUSIONS

The findings of our study confirm the necessity for vitamin D status improvement in general population and more effective healthcare of hypothyroid patients in that field. The strategies for vitamin D deficiency prevention in hypothyroidism might be of special importance since impaired vitamin D(3) metabolism was suggested to play significant role in thyroid follicular cell oncogenesis (Stepien *et al.* 2010) and vitamin D deficiency was supposed to be the first modifiable risk factor for thyroid cancer (Roskies *et al.* 2012).

## ACKNOWLEDGMENTS

This work has been supported with the contribution of the Foundation of Diagnostics and Therapy Development in Warsaw (Warsaw, Poland) and Medical University of Lodz, Grant No. 503/1-153-03/503-11-003.

There are no financial disclosures from any author.

REFERENCES

1 Bársony J, Lakatos P, Földes J, Fehér T (1986). Effect of vitamin D3 loading and thyroid hormone replacement therapy on the decreased serum 25-hydroxyvitamin D level in patients with hypothyroidism. Acta Endocrinol (Copenh). **113**: 329–334.

2 Camurdan OM, Döğer E, Bideci A, Celik N, Cinaz P (2012). Vitamin D status in children with Hashimoto thyroiditis. J Pediatr Endocrinol Metab. **25**: 467–470.

3 Caturegli P, De Remigis A, Rose NR (2014). Hashimoto thyroiditis: clinical and diagnostic criteria. Autoimmun Rev. **13**: 391–397.

4 Clinckspoor I, Hauben E, Verlinden L, Van den Bruel A, Vanwalleghem L, Vander Poorten V, et al (2012). Altered expression of key players in vitamin D metabolism and signaling in malignant and benign thyroid tumors. J Histochem Cytochem. **60**: 502–511. doi: 10.1369/0022155412447296.

5 Feng M, Li H, Chen SF, Li WF, Zhang FB (2013). Polymorphisms in the vitamin D receptor gene and risk of autoimmune thyroid diseases: a meta-analysis. Endocrine. **43**: 318–326. doi: 10.1007/s12020-012-9812-y.

6 Garmendia Madariaga A, Santos Palacios S, Guillén-Grima F, Galofré JC (2014). The incidence and prevalence of thyroid dysfunction in Europe: a meta-analysis. J Clin Endocrinol Metab. **99**: 923–931.

7 Holick MF (2006). High prevalence of vitamin D inadequacy and implications for health. Mayo Clin Proc. **81**: 353–373.

8 Holick MF (2007). Vitamin D deficiency. N Engl J Med. **357**: 266–281.

9 Holick MF, Chen TC (2008). Vitamin D deficiency: a worldwide problem with health consequences. Am J Clin Nutr. **87**: 1080S–6S.

10 Hossein-nezhad A, Holick MF (2013). Vitamin D for health: a global perspective. Mayo Clin Proc. **88**: 720–755. doi: 10.1016/j.mayocp.2013.05.011.

11 Jastrup B, Mosekilde L, Melsen F, Lund B, Lund B, Sørensen OH (1982). Serum levels of vitamin D metabolites and bone remodelling in hyperthyroidism. Metabolism. **31**: 126–132.

12 Kivity S, Agmon-Levin N, Zisappl M, Shapira Y, Nagy EV, Dankó K, et al (2011). Vitamin D and autoimmune thyroid diseases. Cell Mol Immunol. **8**: 243–247.

13 Kmieć P, Żmijewski M, Lizakowska-Kmieć M, Sworczak K (2015). Widespread vitamin D deficiency among adults from northern Poland (54°N) after months of low and high natural UVB radiation. Endokrynol Pol. **66**: 30–38. doi: 10.5603/EP.2015.0006.

14 Kmieć P, Żmijewski M, Waszak P, Sworczak K, Lizakowska-Kmieć M (2014). Vitamin D deficiency during winter months among an adult, predominantly urban, population in Northern Poland. Endokrynol Pol. **65**: 105–113.

15 Kozai M, Yamamoto H, Ishiguro M, Harada N, Masuda M, Kagawa T, et al (2013). Thyroid hormones decrease plasma 1α,25-dihydroxyvitamin D levels through transcriptional repression of the renal 25-hydroxyvitamin D3 1α-hydroxylase gene (CYP27B1). Endocrinology. **154**: 609–622.

16 MacFarlane IA, Mawer EB, Berry J, Hann J (1982). Vitamin D metabolism in hyperthyroidism. Clin Endocrinol (Oxf). **17**: 51–59.

17 Mackawy AM, Al-Ayed BM, Al-Rashidi BM (2013). Vitamin d deficiency and its association with thyroid disease. Int J Health Sci (Qassim). **7**: 267–275.

18 Mansournia N, Mansournia MA, Saeedi S, Dehghan J (2014). The association between serum 25OHD levels and hypothyroid Hashimoto's thyroiditis. J Endocrinol Invest. **37**: 473–476. doi: 10.1007/s40618-014-0064-y.

19 Muscogiuri G, Tirabassi G, Bizzaro G, Orio F, Paschou SA, Vryonidou A, et al (2015). Vitamin D and thyroid disease: to D or not to D? Eur J Clin Nutr. **69**: 291–296. doi: 10.1038/ejcn.2014.265

20 Pantazi H, Papapetrou PD (2000). Changes in parameters of bone and mineral metabolism during therapy for hyperthyroidism. J Clin Endocrinol Metab. **85**: 1099–1106.

21 Pludowski P, Grant WB, Bhattoa HP, Bayer M, Povoroznyuk V, Rudenka E, et al (2014a). Vitamin D status in Central Europe. Int J Endocrinol. **2014**: 589587. doi: 10.1155/2014/589587

22 Pludowski P, Holick MF, Pilz S, Wagner CL, Hollis BW, Grant WB, et al (2013). Vitamin D effects on musculoskeletal health, immunity, autoimmunity, cardiovascular disease, cancer, fertility, pregnancy, dementia and mortality-a review of recent evidence. Autoimmun. Rev. **10**: 976–989. doi: 10.1016/j.autrev.2013.02.004.

23 Płudowski P, Konstantynowicz J, Jaworski M, Abramowicz P, Ducki C (2014b). Ocena stanu zaopatrzenia w witaminę D w populacji osób dorosłych w Polsce. [Assesment of vitamin D status in Polish adult population]. Standardy Medyczne. **11**: 609–617.
24 Roskies M, Dolev Y, Caglar D, Hier MP, Mlynarek A, Majdan A, et al (2012). Vitamin D deficiency as a potentially modifiable risk factor for thyroid cancer. J Otolaryngol Head Neck Surg. **41**: 160–163.
25 Rotondi M, Chiovato L (2013). Vitamin D deficiency in patients with Graves' disease: probably something more than a casual association. Endocrine. **43**: 3–5.
26 Spiro A, Buttriss JL (2014). Vitamin D: An overview of vitamin D status and intake in Europe. Nutr Bull. **39**: 322–350.
27 Stepien T, Krupinski R, Sopinski J, Kuzdak K, Komorowski J, Lawnicka H, et al (2010). Decreased 1-25 dihydroxyvitamin D3 concentration in peripheral blood serum of patients with thyroid cancer. Arch Med Res. **41**: 190–194.
28 Tamer G, Arik S, Tamer I, Coksert D (2011). Relative vitamin D insufficiency in Hashimoto's thyroiditis. Thyroid. **21**: 891–896. doi: 10.1089/thy.2009.0200.
29 Yasuda T, Okamoto Y, Hamada N, Miyashita K, Takahara M, Sakamoto F, et al (2013). Serum vitamin D levels are decreased in patients without remission of Graves' disease. Endocrine. **43**: 230–232.
30 Yazici D, Yavuz D, Tarcin O, Sancak S, Deyneli O, Akalin S (2013). Vitamin D receptor gene ApaI, TaqI, FokI and BsmI polymorphisms in a group of Turkish patients with Hashimoto's thyroiditis. Minerva Endocrinol. **38**: 195–201.