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# Vitamin D status including 3-epi-25(OH)$D_3$ among adult patients with thyroid disorders during summer months

## Status witaminy D w organizmie, w tym stężenie 3-epi-25(OH)D3, u osób dorosłych z chorobami tarczycy w miesiącach letnich

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## Abstract

**Introduction**: In the context of pleiotropic vitamin D effects, its role has also been investigated in thyroid pathology, in particular autoimmune thyroid diseases (AITD). However, available data concerning vitamin D status in Polish patients with thyroid disorders are inconclusive. In the study we investigated vitamin D status and adequacy of supplementation, as well as sunlight exposure during summer months among adult patients with thyroid diseases.

**Material and methods:** Adults with diagnosed or suspected thyroid disease were recruited almost entirely in an ambulatory setting between June and September in Northern Poland. Questionnaire examinations were performed, and serum concentrations of 25(OH)$D_2$, 25(OH)$D_3$, 3-epi-25(OH)$D_3$, and 24,25$(OH)_2D_3$ were determined by LC-MS/MS.

**Results**: Thirty men and 194 women participated in the study, mean age ± standard deviation (SD): 42 ± 15 years, mean ± SD body mass index (BMI) 26 ± 6 kg/m$^2$. Among the participants, 133 declared L-thyroxine treatment, 44 — Hashimoto's thyroiditis, 40 — nodular goitre, and 20 — hyperthyroidism and/or Graves' disease.

Mean ± SD 25(OH)D level was 26.9 ± 8.2 ng/ml, and deficiency (< 20 ng/ml) was stated in 12%, insufficiency (20 ≤ 25(OH)D < 30 ng/ml) in 50.4% of study participants. Calcidiol was significantly higher in subjects who declared supplementation, mean ± SD: 29.4 ± 7.5 *vs*. 25.2 ± 8 ng/ml. Among participants without vitamin D supplementation sunlight exposure correlated with 25(OH)D.

The C3 epimer of 25(OH)$D_3$ was detected in all subjects; its concentration correlated strongly with that of 25(OH)$D_3$. 24,25$(OH)_2D_3$ levels also strongly correlated with those of 25(OH)$D_3$.

**Conclusions**: To our knowledge, the current study is the first in Poland to analyse vitamin D status in summer months among patients with thyroid diseases, as well as serum 3-epi-25(OH)$D_3$ and 24,25$(OH)_2D_3$ concentrations. The data presented here indicate that vitamin D sufficiency is not attained even in summer months in patients with thyroid diseases. **(Endokrynol Pol 2018; 69 (6): 653–660)**

**Key words:** *vitamin D deficiency; calcifediol; sunlight; ultraviolet rays; thyroid disease*

## Streszczenie

**Wstęp:** W badaniach nad witaminą D w kontekście jej plejotropowego działania analizowano między innymi rolę tej witaminy w rozwoju chorób tarczycy, szczególnie tych o podłożu autoimmunologicznym (AITD). Jednak dostępne dane dotyczące stanu zaopatrzenia w witaminę D u polskich pacjentów z zaburzeniami tarczycy są niejednoznaczne.

W przedstawionym badaniu oceniono stan zaopatrzenia w witaminę D oraz jej suplementację, a także ekspozycję na światło słoneczne w miesiącach letnich u osób dorosłych z chorobami tarczycy.

**Materiał i metody:** Niemal wszystkie osoby dorosłe z rozpoznaną lub podejrzewaną chorobą tarczycy włączone do badania rekrutowano w warunkach ambulatoryjnych w okresie od czerwca do września w Północnej Polsce. Przeprowadzono badanie ankietowe oraz oznaczono stężenia w surowicy 25(OH)$D_2$, 25(OH)$D_3$, 3-epi-25(OH)$D_3$ i 24,25$(OH)_2D_3$ za pomocą techniki LC-MS/MS.

**Wyniki:** W badaniu uczestniczyło 30 mężczyzn i 194 kobiety, średnia wieku ± odchylenie standardowe (SD): 42 ± 15 lat, średnia ± SD wskaźnika masy ciała (BMI) 26 ± 6 kg/m$^2$. Jak wskazują dane uzyskane w badaniu ankietowym, 133 uczestników badania przyjmowało L-tyroksynę, 44 miało chorobę Hashimoto, 40 — wole guzkowe, a 20 — nadczynność tarczycy i/lub chorobę Gravesa-Basedowa.

Średnie ± SD stężenie 25(OH)D wyniosło 26,9 ± 8,2 ng/ml. Niedobór witaminy D (< 20 ng/ml) stwierdzono u 12%, a hipowitaminozę (20 ≤ 25(OH)D < 30 ng/ml) u 50,4% uczestników badania. Stężenie kalcydiolu było istotnie wyższe u osób, które deklarowały suplementację witaminy D, średnia ± SD: 29,4 ± 7,5 *vs.* 25,2 ± 8 ng/ml. W grupie uczestników, którzy nie stosowali suplementacji witaminy D, ekspozycja na światło słoneczne korelowała ze stężeniem 25(OH)$D_3$.

U wszystkich uczestników badania wykryto epimer C3 25(OH)$D_3$, a jego stężenie silnie korelowało ze stężeniem 25(OH)$D_3$. Stwierdzono również silną korelację między stężeniami 24,25$(OH)_2D_3$ i 25(OH)$D_3$.

**Wnioski:** Zgodnie z wiedzą autorów jest to pierwsze w Polsce badanie oceniające stężenie witaminy D w miesiącach letnich u osób z chorobami tarczycy, a także stężenia surowicze 3-epi-25(OH)$D_3$ i 24,25$(OH)_2D_3$. Przedstawione w niniejszej pracy dane wskazują, że u osób z chorobami tarczycy stężenie witaminy D nawet w miesiącach letnich nie jest prawidłowe. **(Endokrynol Pol 2018; 69 (6): 653–660)**

**Słowa kluczowe:** *niedobór witaminy D; kalcyfediol; światło słoneczne; promieniowanie ultrafioletowe; choroby tarczycy*

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## Introduction

In recent years a plethora of data has been accumulated that demonstrate widespread vitamin D deficiency. Multiple associations have also been made between vitamin D deficiency and deleterious health effects, including autoimmune, cardiovascular, and neoplastic diseases. The role of vitamin D has been investigated also in the context of autoimmune thyroid disorders (AITD) and to a lesser extent thyroid neoplasms [1, 2].

While the term "vitamin D" encompasses several chemical compounds, it most commonly refers to two of them: cholecalciferol (vitamin D3) and ergocalciferol (vitamin D2), which are synthesised by animals and plants (and fungi), respectively. In humans cholecalciferol is the main form of vitamin D; synthesised from 7-dehydrocholesterol (also a precursor for cholesterol) in skin exposed to solar ultraviolet B radiation, but also ingested in food products and acquired through supplementation [3].

Vitamin D status in humans is reflected by serum 25-hydroxyvitamin D (25(OH)D, calcidiol) concentration. Calcidiol is formed by hydroxylation of vitamin D (D3 and D2) in the liver. In accordance with the 2013 Central European guidelines, vitamin D deficiency, insufficiency, adequate status and high vitamin D supply was defined here by respective calcidiol concentrations of: less than 20 ng/ml, 20 up to 30 ng/ml, 30 up to 50 ng/ml, and 50 up to 100 ng/ml [4]. Calcitriol, 1,25$(OH)_2$D, the fully active form of vitamin D, is acquired through hydroxylation of 25(OH)D by 1-alpha-hydroxylase (CYP27B1) in the kidneys. Furthermore, it is now well established that vitamin D may also be activated in multiple organs, which are involved in autocrine and paracrine regulation of local homeostasis [5].

Vitamin D deficiency has been shown to be prevalent in Northern Poland [6], and summer sun exposure was not sufficient to fully eliminate it [7, 8]. Furthermore, vitamin D deficiency was stated in up to 90% of Poland's adult population [9]. The effects of vitamin D on the immune system have been suggested as the link between decreased vitamin D levels and AITD [1, 2, 10]. The aim of the presented study was to assess vitamin D status among patients with thyroid disorders in Poland during summer months.

## Material and methods

The study was approved by the Independent Bioethics Commission for Research of the Medical University of Gdańsk, Poland.

Study participants were recruited during summer months (June 6 to September 13, 2017) in an ambulatory medical centre in Gdańsk (Endomed Medical Diagnostic Centre) and at the Department of Endocrinology and Internal Medicine of the Medical University of Gdańsk, i.e. hospitalised patients. All subjects received an information sheet about the study and gave informed consent to participate. The only inclusion criterion was a diagnosed or suspected thyroid disorder (which included hypo- and hyperthyroidism, thyroid nodule, and/or cancer). There were no exclusion criteria.

A questionnaire was used to acquire the following information about the participants: age, body weight, and height; thyroid disorder and other diseases; vitamin D supplementation and intake of other medications; physical activity (outdoor and indoor); presence of muscle weakness and bone pain; skin phototype according to Fitzpatrick's classification (i.e. questions about eye, hair, and skin colour, tanning ability); date of most recent tanning; attitude toward sun exposure (on a five-point scale ranging from "always avoid" to "always expose"); duration of sun exposure ("less than 10 minutes", "between 10 and 30 minutes", and "more than 30 minutes") per day; and parts of body (head and palms, arms, legs, or bathing suit) exposed to the sun in the preceding 14 days between 10:00 and 15:00. Number of days with a given duration of sunlight exposure (categorised on a 1–20 scale) and body surface area exposed were used to calculate a sun exposure score (weighted arithmetic means of body surface on given days and points for duration of exposure).

Venous blood drawn from study participants was centrifuged for 10 minutes at 3500 g, then serum was transferred to separate tubes and frozen at –80°C. Serum tubes were transported to the Masdiag laboratory (Masdiag, Warsaw, Poland), where 25-hydroxyvitamin $D_2$, 25-hydroxyvitamin $D_3$, 24,25$(OH)_2D_3$ — inactivated vitamin D form, and 25-hydroxy-3-epi-vitamin D3 (3-epi-25$(OH)D_3$) were determined. The sample preparation method was based on liquid-liquid extraction of 100 $\mu$l of serum using hexane as an extractant, followed by DAPTAD derivatisation (4-(4'-dimethylaminophenyl)-1,2,4-triazoline-3,5-dione). Such a solution provided the desired sensitivity. The analyses were performed using liquid chromatography coupled with tandem mass spectrometry (QTRAP4500, SCIEX). Chromatographic conditions were optimised to achieve sufficient separation of 3-epi-25$(OH)D_3$ and 25$(OH)D_3$. The limit of detection was 0.1 ng/ml, linear range: 0.1–10 ng/ml. The stationary phase was Kinetex F5 1.7 $\mu$m (50 × 2.1 mm) (Phenomenex, Torrance, CA, USA). The analysis was performed at a flow rate of 0.45 ml/min. The temperature of the column oven was 40°C. The mobile phase consisted of water and acetonitrile with 0.1% formic acid as an additive.

The sum of 25$(OH)D_2$ and 25$(OH)D_3$ is further referred to as 25(OH)D (calcidiol) (measuring both

25-hydroxy-vitamin D forms to reflect vitamin D status is recommended [4]).

Statistical analysis was performed using Graphpad Prism 5 (GraphPad Software). In most analyses non-parametric tests were used because 25(OH)D, age, and BMI values did not follow a Gaussian distribution (as verified with the Shapiro-Wilk test). Spearman rank correlations were calculated. The significance level was set at 0.05.

## Results

### *Demographic characteristics*

In our study 224 participants were enrolled; 30 men (13.4%) and 194 women. Ambulatory patients comprised 200 participants, while 24 persons were recruited in the hospital (three men). Only two hospitalised patients were admitted to our Department acutely due to thyroid pathology, i.e. uncontrolled hyperthyroidism, while others had scheduled admissions for diagnostic workup.

Among the enrolled study participants 198 (88%) were less than 65 years old. There were 58 (25.9%) overweight participants (i.e. with a body mass index, BMI, calculated by dividing the weight in kilograms by the square of height in meters, ≥ 25 and < 30 kg/m$^2$) and 41 (18.3%) obese (BMI ≥ 30 kg/m$^2$) participants, while eight (3.6%) were underweight (BMI < 18.5 kg/m$^2$) (Table I). Study subjects were inhabitants of the Pomerania Province in northern Poland (with few exceptions), and 137 lived or worked in Gdańsk.

### *Thyroid disorders*

Questionnaire data concerning thyroid disorders and their treatment are presented in Table II. Hypothyroidism and/or levothyroxine treatment was stated by 62.5% of study participants, Hashimoto disease by 19.6%, nodular goitre by 17.6%, and hyperthyroidism by 7.1%. Among patients treated with thyroxine 78 provided the dose of the hormone, which ranged between 12.5 $\mu$g and 167.5 $\mu$g/day [median 1 $\mu$g/kg body weight, interquartile range (IQR) 0.6].

### *Vitamin D metabolites and status*

Table III shows basic descriptive statistics of measured vitamin D metabolites. Because vitamin $D_2$ is not used for supplementation in Poland, the sole source of 25(OH)$D_2$ in this study was alimentation, which explains the low concentrations of this metabolite.

Vitamin D deficiency (i.e. 25(OH)D concentration lower than 20 ng/ml) was found in 27 study participants, insufficiency in 113, and sufficiency in 76 study participants (Table III). The mean total calcidiol concentration was 27.3 ng/ml (standard deviation, SD ± 8.1), median: 27.2 ng/ml (IQR, 9.1) (Table III and IV). In three hospital patients 25(OH)D concentration was lower than 10 ng/ml.

There were no significant correlations between 25(OH)D and BMI or age for all study subjects. Calcidiol concentrations were not significantly different in ambulatory and hospitalised patients, men and women, or in hyperthyroid and hypothyroid/thyroxine-treated subjects.

The 3-epimer form of 25(OH)$D_3$ was detected in all study participants. There was a highly significant

**Table I.** *Demographic characteristics of study participants*

**Tabela I.** *Charakterystyka demograficzna uczestników badania*

| | | Total | Hospitalised |
|---|---|---|---|
| Sex | Male | 30 | 3 |
| | Female | 194 | 21 |
| Age [years] | N | 224 | 24 |
| | Mean | 42.3 | 46 |
| | SD (±) | 14.7 | 16.1 |
| | Median | 40 | 40.5 |
| | IQR | 22 | 30.3 |
| BMI [kg/m$^2$] | N | 202 | 24 |
| | Mean | 25.9 | 26.1 |
| | SD (±) | 5.6 | 7.8 |
| | Median | 24.9 | 23.4 |
| | IQR | 7.7 | 8.6 |

BMI — body mass index; IQR — interquartile range; SD — standard deviation

**Table II.** *Questionnaire data on thyroid disorders provided by study participants*

**Tabela II.** *Dane na temat zaburzeń czynności tarczycy podane przez uczestników w badaniu ankietowym*

| | All subjects n (men), % of total | Hospitalised subjects n (% of total) |
|---|---|---|
| Hashimoto's thyroiditis | 44 (1), 19.6% | 6 (2.7%) |
| Hypothyroidism | 10 (4), 4.5% | 0 |
| Treatment with L-thyroxine (total) | 133 (10), 59.4% | 10 (4.5%) |
| participants, who provided the dose | 78 (3), 34,8% | 10 (4.5%) |
| Nodular goitre | 40 (9), 17.9% | 2 (0.9%) |
| Thyroid cancer | 3 (0), 1.3% | 1 (0.4%) |
| Status post thyroid surgery | 3 (0), 1.3% | 0 |
| Hyperthyroidism / / Graves' disease | 20 (4), 8.9% | 6 (2.7%) |
| Antithyroid medication | 14 (4), 6.3% | 5 (2.2%) |
| Unknown thyroid disorder | 16 (3), 7.1% | 0 |

**Table III.** *Basic statistics of measured vitamin D metabolites*
**Tabela III.** *Podstawowe statystyki oznaczonych metabolitów witaminy D*

| | Mean | SD (±) | Median | Q1 | Q3 | Min. | Max. |
|---|---|---|---|---|---|---|---|
| $25(OH)D_3$ [ng/ml] | 26.87 | 8.18 | 26.65 | 22.02 | 31.77 | 4.12 | 53.82 |
| $25(OH)D_2$ [ng/ml] | 0.44 | 0.24 | 0.39 | 0.28 | 0.52 | 0.08 | 1.59 |
| $25(OH)D = 25(OH)D_3 + 25(OH)D_2$ [ng/ml] | 27.31 | 8.12 | 27.15 | 22.49 | 32.22 | 4.62 | 53.98 |
| 3-epi-$25(OH)D_3$ [ng/ml] | 1.63 | 0.89 | 1.41 | 1.05 | 1.95 | 0.24 | 5.66 |
| 3-epi-$25(OH)D_3/25(OH)D_3$ | 0.06 | 0.02 | 0.05 | 0.05 | 0.07 | 0.02 | 0.14 |
| $24,25(OH)_2D_3$ [ng/ml] | 2.81 | 1.37 | 2.54 | 1.86 | 3.66 | 0.28 | 8.57 |
| $25(OH)D_3/24,25(OH)_2D_3$ | 11.06 | 3.73 | 10.17 | 8.61 | 12.58 | 5.22 | 33.75 |

SD — standard deviation

**Table IV.** *Vitamin D status of study participants*
**Tabela IV.** *Status witaminy D u uczestników badania*

| | Serum 25(OH)D [ng/ml] | n (%) |
|---|---|---|
| Deficiency | < 10 | 3 (1.3%) |
| | 10–20 | 24 (10.7%) |
| Insufficiency | 20–30 | 113 (50.4%) |
| Sufficiency | 30–40 | 62 (23.3%) |
| | ≥ 40 | 14 (6.3%) |

strong positive correlation between concentrations of 3-epi-$25(OH)D_3$ and $25(OH)D_3$ (Fig. 1). Relative concentration of 3-epi-$25(OH)D_3$ to $25(OH)D_3$ ranged between 2.2 and 14.4% (Table IV).

Also, a highly significant strong positive correlation was found between $24,25(OH)_2D_3$ and $25(OH)D_3$ (Fig. 2). The relative concentration of $25(OH)D_3$ to $24,25(OH)_2D_3$ ranged between 5.2 and 33.8, mean ± SD: 11.1 ± 3.7, median (IQR): 10.2 (3.9). A negative correlation was observed between $25(OH)D_3$ to $24,25(OH)_2D_3$ ratio and $25(OH)D_3$, as depicted in Figure 3. There was a weak negative correlation between $24,25(OH)_2D_3$ concentration and BMI ($r = -0.17$, $p < 0.05$).

### *Effects of supplementation and sun exposure on vitamin D status*

Supplementation of vitamin D was declared by 46.9% of participants (n = 105); their 25(OH)D concentrations were significantly higher than those of patients who did not supplement vitamin D: 28.2 *vs.* 25.3 ng/ml (IQR 9.8 and 9.4, respectively), mean ± SD: 29.4 ± 7.5 *vs.* 25.2 ± 8 ng/ml (Table V). Only 31 subjects reported the daily cholecalciferol dose, which ranged between 200 and 5000 IU.

Subjects who declared vitamin D supplementation compared to those who did not had significantly higher

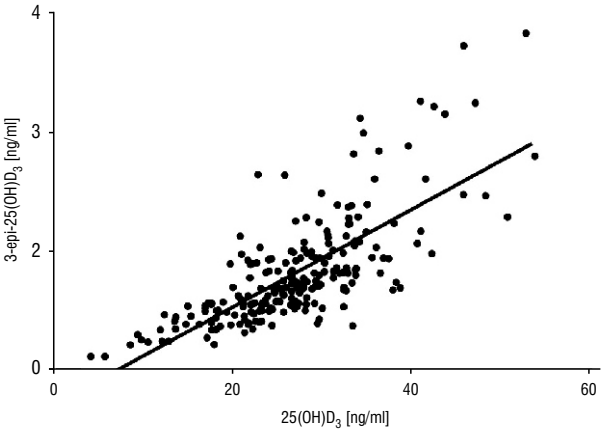


**Figure 1.** *Relationship between 3-epi-$25(OH)D_3$ and $25(OH)D_3$; Spearman $r = 0.75$, $p < 0.0001$, 95% confidence interval: 0.69 to 0.81*

**Rycina 1.** *Zależność między stężeniami 3-epi-$25(OH)D_3$ i $25(OH)D_3$*

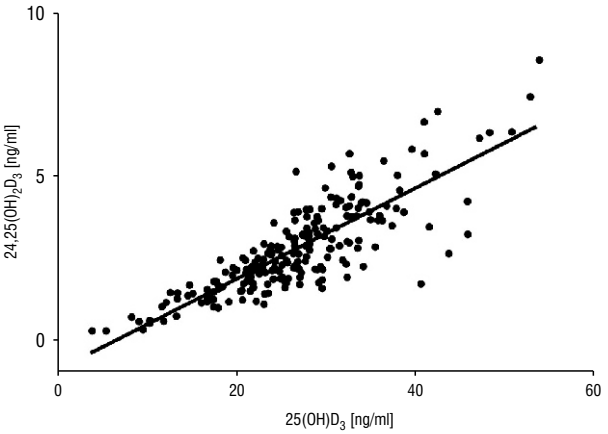


**Figure 2.** *Relationship between $24,25(OH)_2D_3$ and $25(OH)D_3$; Spearman $r = 0.83$, $p < 0.0001$, 95% confidence interval: 0.78 to 0.87*

**Rycina 2.** *Zależność między stężeniami $24,25(OH)_2D_3$ i $25(OH)D_3$*

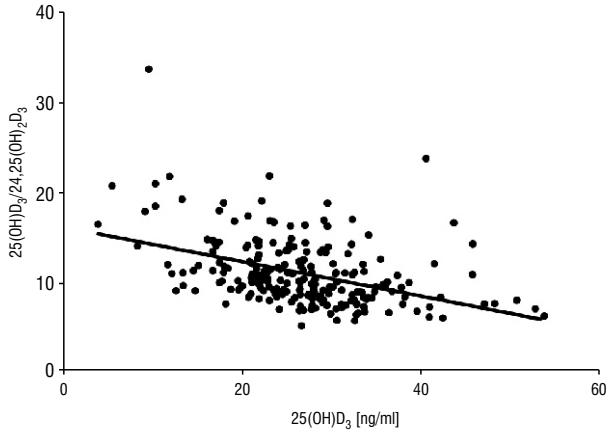


**Figure 3.** *Relationship between 25(OH)$D_3$/24,25(OH)$_2D_3$ ratio and 25(OH)$D_3$; Spearman r = –0.46, p < 0.0001, 95% confidence interval: –0.56 to –0.34*

**Rycina 3.** *Zależność między stosunkiem stężeń 25(OH)$D_3$/ / 24,25(OH)$_2D_3$ a stężeniem 25(OH)$D_3$*

median 3-epi-25(OH)$D_3$ concentrations, and median ratios of 25(OH)$D_3$ to 24,25(OH)$_2D_3$ (Table V).

Data for calculating sun exposure scores were available for 174 study participants of the study; among these subjects:

— significantly different median 25(OH)D concentrations were found between those who did and did not supplement vitamin D: 28.2 (IQR = 8.6, n = 77) versus 25.4 ng/ml (IQR = 7.9, n = 97), respectively (Mann Whitney test, p < 0.005);
— significant Spearman rank correlations were found between 25(OH)D concentrations and skin phototype, r = 0.18 (p < 0.05), date of most recent tanning, r = 0.17 (p < 0.05), and attitude toward sun exposure, r = 0.17 (p < 0.05).

Among 77 participants of the study who declared vitamin D supplementation and whose sun exposure data were provided, no significant differences in 25(OH)D concentration were found between sexes, and no correlations were found between 25(OH)D level and BMI, skin phototype, date of tanning, attitude toward sunlight exposure, and sun exposure score.

There were 97 study subjects who provided information for calculating sun exposure scores and reported no vitamin D supplementation. Among these subjects calcidiol concentrations significantly correlated with:

— sun exposure score, r = 0.24, p < 0.05;
— skin phototype, r = 0.25, p < 0.05;
— date of most recent tanning, r = 0.26, p < 0.05;
— attitude toward sun exposure, r = 0.31, p < 0.005 (Spearman rank test) (Fig. 4).

For 127 study participants we were able to provide TSH concentrations, and for 97 free thyroxine

**Table V.** *Vitamin D status and supplementation*
**Tabela V.** *Status witaminy D a suplementacja*

| | Cholecalciferol supplementation | |
|---|---|---|
| | **No** | **Yes** |
| **25(OH)D [ng/ml]** | | |
| n | 115 | 105 |
| Mean | 25.2 | 29.4 (*) |
| SD | 8 | 7.5 |
| Median | 25.3 | 28.2 (*) |
| Q1 | 20.6 | 24.1 |
| Q3 | 30 | 33.9 |
| Min. | 4.6 | 10.8 |
| Max. | 53 | 51.3 |
| **3-epi-25(OH)$D_3$ [ng/ml]** | | |
| Median (IQR) | 1.25 (0.88) | 1.65 (0.99) (*) |
| **25(OH)$D_3$ to 24,25(OH)2$D_3$ ratio** | | |
| Median (IQR) | 9.8 (3.7) | 10.9 (5) (*) |

IQR — interquartile range; SD — standard deviation; 25(OH)D: p = 0.0003 in Mann-Whitney test, p = 0.0001 in t test, 3-epi-25(OH)D3: p = 0.001, 25(OH)$D_3$ to 24,25(OH)$_2D_3$ ratio: p = 0.028

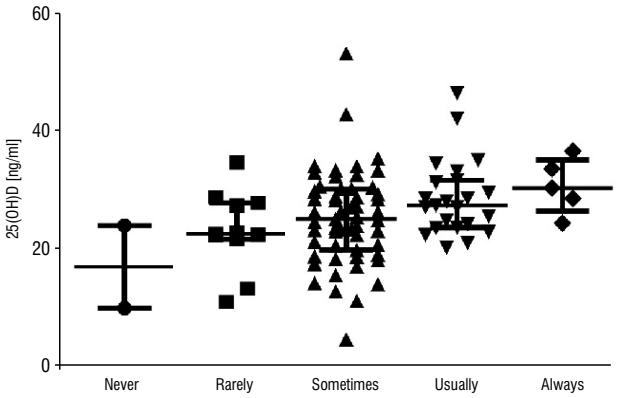


**Figure 4.** *Vitamin D and declared attitude toward sun exposure in subjects without vitamin D supplementation; lines and bars denote medians and interquartile ranges; n = 97*

**Rycina 4.** *Status witaminy D a deklarowana ekspozycja na słońce u osób stosujących suplementację witaminy D*

concentrations, which were measured either during hospitalisation in our Department or in ambulatory setting preceding a doctor's appointment due to thyroid disease. Study participants were categorised as hypothyroid based on TSH level above 3.5 mIU/ml (11 patients), hyperthyroid based on TSH < 0.1 mIU/ml (15 patients), and euthyroid (101 patients). Hypothyroid patients had significantly lower 25(OH)D and 3epi-25(OH)$D_3$ levels than euthyroid subjects (Table VI). There were no significant differences in BMI, age, sun exposure parameters, and supplementation

**Table VI.** *Vitamin D status and thyroid function*
**Tabela VI.** *Status witaminy D a czynność tarczycy*

| | Hypothyroid | Euthyroid | Hyperthyroid |
|---|---|---|---|
| **TSH [mIU/]** | | | |
| n | 11 | 101 | 15 |
| Mean (SD) | 8.26 (12.43) | 1.52 (0.73) | 0.0009 (0.012) |
| Median (IQR) | 4.6 (1.78) | 1.49 (0.98) | 0.005 (0.017) |
| Range | 3.55–45.65 | 0.11–3.16 | 0–0.034 |
| **fT4 [pmol/l]** | | | |
| n | 8 | 74 | 15 |
| Mean (SD) | 14.2 (3.91) | 16.07 (2.66) | 24.15 (16.38) |
| Median (IQR) | 13.2 (6.9) | 15.93 (4.47) | 17.2 (21.25) |
| Range | 9.03–20.4 | 10.6–21.7 | 9.17–61.2 |
| **25(OH)D [ng/ml]** | | | |
| n | 11 | 101 | 15 |
| Mean (SD) | 21.38 (7.5) | 27.91 (7.85)* | 24.34 (9.5) |
| Median (IQR) | 22.56 (10.68) | 27.69 (10.13)# | 25.41 (15.59) |
| Range | 5.82–30.10 | 9.85–51.26 | 4.62–41.78 |
| **$25(OH)D_3$/$24,25(OH)_2D_3$** | | | |
| n | 11 | 101 | 15 |
| Mean (SD) | 11.82 (3.61) | 11.31 (4.04) | 11.31 (3.48) |
| Median (IQR) | 11.09 (3.73) | 10.53 (4.83) | 11.03 (5.72) |
| Range | 7.18–20.79 | 5.23–33.75 | 7.33–18.89 |
| **3-epi-$25(OH)D_3$ [ng/ml]** | | | |
| n | 11 | 101 | 15 |
| Mean (SD) | 1.03 (0.39) | 1.7 (0.94)* | 1.3 (0.6) |
| Median (IQR) | 1.12 (0.71) | 1.46 (0.91)# | 1.11 (1.02) |
| Range | 0.24–1.49 | 0.43–5.45 | 0.42–2.34 |

IQR — interquartile range; SD — standard deviation; 25(OH)D and 3-epi-25(OH)$D_3$ concentrations were significantly different between hypothyroid versus euthyroid patients, respectively: ANOVA with Tukey's post hoc test: $p = 0.0179$ and $p = 0.0223$, respectively, Kruskall-Walis test $p = 0.0309$ and $p = 0.0133$, respectively

between hypo-, eu-, and hyperthyroid patients. There were no statistically significant correlations between TSH (as well as free thyroxine) and vitamin D metabolites, age, or BMI.

## Discussion and conclusions

Vitamin D status of participants recruited in this study corresponds well with findings by other authors in Poland. Bartoszewicz et al. recorded a mean calcidiol level of 25.5 ng/ml in the April–September period in 57 healthy pregnant women, 42 of whom supplemented vitamin D [11]. Krzywański et al. reported mean summer 25(OH)D levels in 409 elite Polish outdoor and indoor athletes of 36 and 27 ng/ml, respectively [12]. In our previous study, mean 25(OH)D among 304 adult volunteers (59% female, mean age 46 years) in autumn was 21.1 ng/ml, and less than 20% of participants declared vitamin D supplementation [7].

In the current study, the C3 epimer form of 25(OH)$D_3$ was also measured. Vitamin D metabolism via the C3 epimerisation pathway has been discovered recently, but its significance has not been investigated sufficiently [13]. It has been observed that all major vitamin D metabolites can be epimerised and later metabolised analogously to standard ones; however, the C3 epimer form of active vitamin D (C3-epi-1,25$(OH)_2$D) does not exhibit all effects of the classic isomer [13]. In the current study, in only 13 participants (5.8%) vitamin D status would change, if concentrations of 3-epi-25(OH)$D_3$ were taken into account (i.e. if vitamin D status was reflected by the sum of 25(OH)$D_2$, 25(OH)$D_3$, as well as 3-epi-25(OH)$D_3$). This result is comparable to reports by other authors; also, as previously reported, 25(OH)$D_3$

and 3-epi-25(OH)$D_3$ concentrations correlated positively [14, 15].

Similarly to previous studies, a strong positive correlation between calcidiol and 24,25$(OH)_2D_3$ was demonstrated here [16, 17]. Increased conversion to inactive vitamin D form in persons with higher vitamin D levels was reported previously [18]. The ratio of 25(OH)$D_3$ to 24,25$(OH)_2D_3$ (or its reciprocal) has been proposed as an indicator of 24-hydroxylase deficiency and response to vitamin D supplementation [19]. Loss-of-function mutations in CYP24A1, the gene that encodes 24-hydroxylase, result in a range of clinical disorders, among them idiopathic infantile hypercalcaemia (IIH) and adult-onset nephrocalcinosis and nephrolithiasis [20]. Because serum 24(OH)$D_3$ is low not only in 24-hydroxylase deficiency but also in vitamin D deficiency, the ratio of calcidiol to its catabolite may indicate enzymatic deficiency: normal range of the ratio rarely exceeds 20, whereas in IIH it is greater than 80 [19]. In respect to assessing response to vitamin D supplementation using the 25(OH)$D_3$ to 24,25$(OH)_2D_3$ ratio, its basal lower values correlated with lesser increment in calcidiol after treatment [16, 21].

In our study almost half of the participants (47%) declared vitamin D supplementation. It may be speculated that participants using highest doses (5000 IU/day, n = 5) were treated for vitamin D deficiency, while the remainder merely supplemented to prevent it. In spite of supplementation or treatment and collection of samples during summer, still more than 50% of the study participants did not reach vitamin D sufficiency. Assuming patient compliance, our data point to the need for testing 25(OH)D levels to achieve target concentrations. In Poland, at 51°45'N latitude, Sewerynek et al. recently investigated (among others) the effect of two doses of cholecalciferol on vitamin D status in healthy women aged 20–30 years over a period of three months between January and May (total n = 106, only 67 adhered to therapy). Interestingly, 1500 IU of cholecalciferol increased mean 25(OH)D from 12.6 to 29.4 ng/ml (baseline deficiency group), while 800 IU increased mean 25(OH)D from 25.2 to 36.9 ng/ml (group with baseline 25(OH)D > 20 ng/ml) [22]. These results hint at the effect of vitamin D synthesis due to sunlight apart from supplementation.

The questionnaire examination in this study yielded data that indicate the effect of natural UVB exposure on vitamin D status. Interestingly, among participants who supplemented cholecalciferol, there were no statistically significant correlations in investigated parameters related to sunlight. Other authors found that self-reported sun exposure correlates with calcidiol levels [23, 24]. In a recent study, Vignali et al. developed an algorithm assessing vitamin D status, which used simple pieces of information: age, BMI, duration of daily sunlight exposure of the face, hands, and legs, attitude toward going outdoors ("often"/"sometimes"/"seldom"), and participation in a beach holiday in the past year. Their study was performed at 40°50'N latitude in August and March; 620 participants were recruited; vitamin D supplementation was an exclusion criterion. Vitamin D status in one of four concentration ranges (< 10, 10–20, 20–30, > 30 ng/ml) was predicted correctly in over 90% of participants [25]. A similar tool might be developed for predicting vitamin D status for other latitudes; it would probably require the consideration of air pollution, among others.

Research data concerning associations between autoimmune thyroid disease (and thyroid cancer) are inconclusive [2]. In our study 25(OH)D levels were not measured longitudinally; therefore, it is difficult to draw conclusions about the association between vitamin D status and thyroid disorders, although hypothyroid study participants had lower calcidiol levels than euthyroid. In a recent study, Mirhosseini et al. reported a significant decrease in thyroid stimulating hormone (TSH) concentrations (indicating reduction in overt and subclinical hypothyroidism), anti-thyroid peroxidase, and anti-thyroglobulin titres after a one year follow-up among approximately 11.000 Canadians who took part in a program aimed at reaching 25(OH)D levels of at least 40 ng/ml. This target was attained by over 72% of participants and mean vitamin D dose was approximately 4000 IU/day [26]. Other studies, including small patient samples in Poland, also indicate associations between AITD and vitamin D status [27, 28].

The present study has a number of limitations. Most importantly, thyroid function measures were not available for all participants, clinical data were acquired by questionnaire and in many instances missing information concerned doses of vitamin D supplements, and co-morbidities were poorly reported (i.e. ones that accompanied thyroid disease); in the study convenience sampling was applied; also, the methodology of the questionnaire examination related to sun exposure was simplistic (fortnight recall and not diary data).

In conclusion, to our knowledge, in the current study, for the first time in Poland, vitamin D status was examined among patients with thyroid diseases during summer. Furthermore, we report simultaneous analysis of four vitamin D derivatives including 3-epi-25(OH)$D_3$ and 24,25$(OH)_2D_3$ concentrations. Vitamin D sufficiency was recorded in fewer than 30% of the examined study participants, despite cholecalciferol supplementation in almost half of the subjects. Bearing in mind the associations between vitamin D status and thyroid disorders, in particular AITD, treatment and supplementation doses of vitamin D should be verified by testing 25(OH)D to reach recommended vitamin D status.

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