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# ANALYSIS OF VITAMIN D CONTENT IN DIETARY SUPPLEMENTS AVAILABLE IN THE EU

# ANALIZA ZAWARTOŚCI WITAMINY D W SUPLEMENTACH DIETY DOSTĘPNYCH W KRAJACH UE

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Authors' contribution
Wkład autorów:
A. Study design/planning
zaplanowanie badań
B. Data collection/entry
zebranie danych
C. Data analysis/statistics
dane – analiza i statystyki
D. Data interpretation
interpretacja danych
E. Preparation of manuscript
przygotowanie artykułu
F. Literature analysis/search
wyszukiwanie i analiza literatury
G. Funds collection
zebranie funduszy

**Summary**

**Background.** Vitamin D occurs in two forms: D2 (ergocalciferol) and D3 (cholecalciferol). Deficiency of vitamin D can lead to rickets in children, while in adults to osteoporosis, depression and multiple sclerosis. The aim of the pilot study was to analyze dietary supplements in terms of the actual content of vitamin D3.
**Material and methods.** The object of the study was ten random samples of dietary supplements from different manufacturers containing in their composition different values of vitamin D3. The manufacturers included a declaration on the content of vitamin D3 on the packaging. The actual content of vitamin D3 was determined using high-performance liquid chromatography (HPLC). The content of vitamin D3 was determined based on a standard curve. Sigma Aldrich's Cholecalciferol standard was used.
**Results.** In all the analyzed dietary supplements, the actual content of vitamin D is much lower than declared by the manufacturer. The carried-out tests revealed that the content of vitamin D3 in the analyzed supplements varied from 1.02 to 59.56 µg. In three cases borderline low values of vitamin D3 were noted. Furthermore, supplement 6 and 9 contained too low dosage of vitamin D3 to supplement a daily demand of an organism for this vitamin.
**Conclusions.** Controlling the shortage of vitamin D in an organism decreases the risk of occurrence of civilization diseases. The results of own pilot studies and studies of other authors prove that this type of research should be continued in a wider scope on various food categories.
**Keywords:** vitamin deficiency, vitamin D, dietary supplements, supplementation

**Streszczenie**

**Wprowadzenie.** Witamina D występuje w dwóch postaciach: D2 (ergokalcyferol) i D3 (cholekalcyferol). Niedobór witaminy D może prowadzić do krzywicy u dzieci, natomiast u dorosłych do osteoporozy, depresji oraz stwardnienia rozsianego. Celem badania pilotażowego była analiza suplementów diety pod kątem rzeczywistej zawartości witaminy D3.
**Materiał i metody.** Obiektem badań było dziesięć losowo wybranych próbek suplementów diety pochodzących od różnych producentów, zawierających w swoim składzie różne wartości witaminy D3. Producenci umieścili na opakowaniu deklarację o zawartości witaminy D3. Rzeczywistą zawartość witaminy D3 określono metodą wysokosprawnej chromatografii cieczowej (HPLC). Zawartość witaminy D3 oznaczono na podstawie krzywej wzorcowej. Zastosowano standard cholekalcyferolu firmy Sigma Aldrich.
**Wyniki.** We wszystkich analizowanych suplementach diety rzeczywista zawartość witaminy D jest znacznie niższa niż deklarowana przez producenta. Przeprowadzone badania wykazały, że zawartość witaminy D3 w analizowanych suplementach wahała się od 1,02 do 59,56 µg. W trzech przypadkach stwierdzono granicznie niskie wartości witaminy D3. Ponadto suplementy 6 i 9 zawierały zbyt małą dawkę witaminy D3, aby uzupełnić dzienne zapotrzebowanie organizmu na tę witaminę.
**Wnioski.** Kontrolowanie niedoboru witaminy D w organizmie zmniejsza ryzyko wystąpienia chorób cywilizacyjnych. Wyniki własnych badań pilotażowych i innych autorów dowodzą, że tego typu badania powinny być kontynuowane w szerszym zakresie w różnych kategoriach żywności.
**Słowa kluczowe:** niedobór witaminowy, witamina D, suplementy diety, suplementacja

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**Introduction**

Vitamin D is a fat-soluble vitamin which occurs in two forms: D2 (ergocalciferol) and D3 (cholecalciferol). Vitamin D2 occurs in food of animal origin, while vitamin D3 is mainly synthesized in skin through sunlight [1-3]. Vitamin D is also defined as the sun vitamin. In chemical terms it is a steroid whose activity is similar to that of a hormone. It regulates the functions of more than 200 genes and is necessary for the correct growth and development of an organism [4,5]. The body's demand for vitamin D is met both through the consumption of vitamin D contained in food and dietary supplements as well as through remaining exposed to sun for a sufficiently long period of time in order for an adequate volume of it to be created [1]. The amount of time necessary for the skin to produce an adequate volume of vitamin D depends on the strength of UVB rays (that is, one's geographic location), the length of time of spent in the sun as well as the volume of pigment in one's skin. A solarium ensures various levels of radiation, both in case of UVA and UVB and therefore it does not provide a sufficient dosage of vitamin D [1]. The main source of vitamin D in case of children and adults is exposure to natural sunlight. The cause of shortages of this vitamin is insufficient exposure to sunlight. Persons with naturally dark skin complexion have a natural protection against sun and require at least three to five times longer time of exposure to absorb the same amount of vitamin D as persons with white skin complexion [4]. Thus, climate, location, age, lifestyle, and skin pigmentation significantly impact the production of vitamin D by an organism. However, one must bear in mind the issues related to the prevention of skin cancer, which is still has a high ranking in epidemiology [6].

Production of vitamin D3 in skin is not an enzyme process. Cholecalciferol (vitamin D3) is formed of 7-dehydrocholesterol under the influence of sunrays [7]. Vitamin D3 is a naturally occurring form and originates from the skin synthesis, where 7-dehydrocholesterol, the precursor of cholesterol, transforms into pro-vitamin D3 under the influence of UVB radiation, and subsequently through thermal isomerization into vitamin D3 [8]. Vitamin D3 is metabolized to 25-hydroxyvitamine D3 by the liver under the influence of liver hydroxylase and subsequently it is transformed through kidney into 1,25-dihydroxyvitamine D3 [9,10].

Not many food items contain sufficient dosage of vitamin D. Therefore, without daily exposure to sunlight or a diet enriched with adequate supplementation, a significant risk of its shortage exists [6,11]. Only 20% of vitamin D is delivered to the organism by dietary products. The remaining 80% should be produced in the skin through UVB radiation. The modern diet is rather poor in terms of consumption of wild fish, which is significantly richer in vitamin D than farmed fish. Due to lifestyles characterized by low exposure to sunlight as well as a highly developed array of cosmetics with sun filters, the impact of the deficit of vitamin D manifests in the population's metabolism. As shown by research, even in sunny countries such as Greece, a high level of vitamin D shortage occurs. This is related to the angle of sun ray incidence in the period from autumn to spring. As indicated by Papadimitriou, such situation may lead to the insufficient production of vitamin D during normal sunlight exposure [12]. Shortage of vitamin D is a factor which substantially increases the risk of metabolic bone diseases such as rickets and osteomalacia. However, in recent times more and more attention has been drawn to the impact of a low level of vitamin D on the pathogenesis of various diseases, such as auto-immunological diseases, cardiovascular diseases and contagious diseases as well as some cancers. Supplementation in vitamin D indicates high efficiency with respect to rickets and osteomalacia as well as a decrease in the risk of bone fractures among elderly persons [8]. Shortage of vitamin D is rather widespread among patients in the elderly age, residing in countries with temperate climate, especially in winter and in early spring, due to the decreased skin synthesis. Shortage of vitamin D causes irregularities in metabolism of calcium and phosphorus in bones [13]. Dietary recommendations for Central Europe concerning vitamin D revealed in 2017 (Table 1) show a necessity of supplementing vitamin D in newborns, regardless of the child-rearing practice. The required dosage in case of newborns is 400 IU/day (10 µg/day), newborns up to 6 months 400-600 IU/day (10-15 µg/day).

Between 6 and 12 months the supplementation of vitamin D depends on the newborn's diet. As the child grows, its organism's demand for vitamin D increases. In the case of children and adolescents between 1 to18 years of age the recommended dosage of vitamin D is 600-1000 IU/day (15-25 μg/day). Among adults, supplementation in vitamin D should be within the range of 800-2000 IU/day (20-50 μg/day). However, the basic role is played by body mass. Higher doses of vitamin D are recommended for overweight persons [2].

**Table 1.** Human eating standards in terms of vitamin D3 [2]

| Group<br>Gender, age (years) | μg cholecalciferol / person / day |
|---|---|
| Newborns | |
| 0-0.5 | 10 |
| 0.5-1 | 10 |
| Children | |
| 1-3 | 15 |
| 4-6 | 15 |
| 7-9 | 15 |
| Boys | |
| 10-12 | 15 |
| 13-15 | 15 |
| 16-18 | 15 |
| Girls | |
| 10-12 | 15 |
| 13-15 | 15 |
| 16-18 | 15 |
| Men | |
| ≥19 | 15 |
| Women | |
| ≥19 | 15 |
| Pregnancy | |
| <19 | 15 |
| ≥19 | 15 |
| Lactation | |
| <19 | 15 |
| ≥19 | 15 |

To identify shortage of vitamin D in an organism, a serum test in terms of concentration of one of the forms of vitamin D-25 [OH] D is conducted. The level of vitamin D necessary for optimizing the absorption of calcium in intestines (34 ng/ml) is lower than the level required for the correct functioning of neuromuscular system (38 ng/ml). However, it is assumed that the lower boundary of the level 25[OH] D in serum ought to amount to no less than 30 ng/ml [14].

The aim of this pilot study was to compare samples of dietary supplements from different manufacturers containing in their composition different values of vitamin D3 with the level of such values as declared on their packaging.

## Material and methods

The materials for the tests consisted of 10 randomly selected diet supplements containing vitamin D3, coming from various manufacturers and generally available in pharmacies in Poland and on the EU market, purchased in the second quarter of 2017. Selected supplements were within the expiry date. At this pilot study each sample was tested twice. Tests were carried out on fresh, just-opened samples, not those stored after having been opened. The manufacturers declared the content of vitamin D3 on the packages. The content of vitamin D3 was marked with HPLC method according to the elaborated testing procedure, in compliance with the norm PN-EN 12821:2002. A Dionex liquid chromatograph equipped with UV detector with a wavelength of 265 nm was applied. A column with phase-inverted AQUASIL C18 by Thermo Scientific was used for the division, with the dimensions of 250 mm x 4.6 mm 5μm along with security stanchion LC-18 and a mobile phase methanol/water in ratio of 93:7 (v/v). Samples were subjected to saponification by means of a methanol solution of potassium hydroxide in the presence of BHT (butylated hydroxyl toluene) as antioxidant, and subsequently via extraction with hexane prior to conducting the analysis. Extracts were evaporated while the residues were dissolved in methanol and subjected to chromatographic analysis. The content of vitamin D3 was marked against the master curve. The standard Cholecalciferol by Sigma Aldrich Company served as a model.

## Results

The carried-out tests revealed that the content of vitamin D3 in the analyzed supplements varied from 1.02 to 59.56 μg. In three cases (supplement 1.2 and 10) borderline low values of vitamin D3 were noted. Furthermore, the dosage of vitamin D3 in supplements 6 and 9 was too low to supplement a human body’s daily demand for this vitamin (Table 2). Supplements 3, 4, 5, 7 and 8 (Table 2) contained a dosage of vitamin D3 sufficient for ensuring the correct functioning of the body. Similar results were obtained within the pilot tests carried out by Verkaik-Kloosterman and others who tested diet supplements as well as special food designation products for newborns in terms of vitamin D [15].

**Table 2.** Content of vitamin D3 in the tested supplements based on own elaboration

| Supplement / Number of days until best-before date | Declared content by producer | | Marked content | |
|---|---|---|---|---|
| | [I.U.] | [μg/capsule] | [I.U.] | [μg/capsule] |
| **Supplement 1/ 315-522** | 200 | 5 | 158.32 | 3.958 |
| **Supplement 2/ 348-377** | 400 | 10 | 183.36 | 4.584 |
| **Supplement 3/ 518-563** | 2000 | 50 | 1299.84 | 32.496 |
| **Supplement 4/ 166-533** | 2000 | 50 | 1501.52 | 37.538 |
| **Supplement 5/ 288-413** | 4000 | 100 | 2382.4 | 59.56 |
| **Supplement 6/ 745-13** | 1000 | 25 | 411.32 | 10.283 |
| **Supplement 7/ 342-378** | 2000 | 50 | 759.28 | 18.982 |
| **Supplement 8/ 379-317** | 2000 | 50 | 1047.76 | 26.194 |
| **Supplement 9/ 501-229** | 1000 | 25 | 411.92 | 10.298 |
| **Supplement 10/ 348-590** | 66.668 | 1.6667 | 40.6 | 1.015 |

## Discussion

Lhamo et al., pursuant to the carried-out tests and observations, discovered that Indian medical students society's awareness of the topic of threats related to the shortage of vitamin D is on a very low level. Persons participating in the test were not aware that at present the shortage of vitamin D reaches the dimensions of an epidemic on a world scale, regardless of demographic indicators or geographic regions [9]. Kulie et al. in their work considered the relationship between low levels of vitamin D and many diseases. It was proved that a low level of vitamin D is related to the increased frequency of occurrence of cardiovascular diseases, cancers, and auto-immunological diseases such as multiple sclerosis. Furthermore, a combination of vitamin D and calcium is necessary for maintaining healthy bones. Multivitamin supplements available without prescription often contain 400 I.U. of vitamin D3. Alternative supplements of vitamin D3 available without prescription may also be found in amounts of 400, 800, 1000 and 2000 I.U. [1]. Jungert et al. focused in their studies on identifying the key sources of vitamin D in food and on the factors impacting its consumption. The main sources of vitamin D turned out to be fish products, eggs, oils, bakery products and dairy products. Moreover, no significant differences determining the share of food product groups in the consumption of vitamin D were noted apart from household income [16,17]. Specialists nowadays focus ever-more attention on the level of vitamin D among the sick, thereby helping society to raise its awareness about threats related to vitamin D shortages. Vitamin D supplementation might also be associated with improved clinical outcomes, especially when administered after a diagnosis of COVID-19 [18]. Due to the broad scope of vitamin D functions, it is critical to apply the right dosage in the supplementation, in accordance with the needs of a given organism. One must bear in mind that all types of treatments must be consulted with a specialist. The pharmaceutical market offers supplements with varying contents of vitamin D, and the producer's incorrect analysis of the product's content as presented on information labels may lead to pathological states within one's body. Yet another issue is the insufficient level of information about the actual content of vitamin D in food products. The data concerning nutrients is often outdated as well as not being standardized. Moreover, enriching food in vitamin D would not ensure the right level of consumption proper for all groups. A high level of vitamin D in a diet is not required in the summer when the volume of exposure to the sun is sufficient. For this reason, introducing food enriched in vitamin D but limited to the winter months could be adequate, but it would be difficult to implement.

## Conclusions

Half of the analyzed supplements of a diet contained a dosage of vitamin D which can meet the demand of an organism for this vitamin. Furthermore, one must bear in mind that applying diet supplements without medical consultation may lead to occurrence of pathological processes in an organism. Nonetheless, controlling the shortage of vitamin D in an organism decreases the risk of occurrence of civilization diseases. The results of own pilot studies and studies of other authors prove that this type of research should be continued in a wider scope on various food categories.

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**References:**


1. Kulie T, Groff A, Redmer J, Hounshell J, Schrager S. Vitamin D: an evidence-based review. J Am Board Fam Med. 2009; 22(6): 698-706. https://doi.org/10.3122/jabfm.2009.06.090037
2. Jarosz M. [Nutrition standards for the Polish population]. Warsaw: Institute of Food and Nutrition; 2017 (in Polish).
3. Ismailova A, White JH. Vitamin D, infections and immunity. Rev Endocr Metab Disord. 2022; 23(2): 265-277. https://doi.org/10.1007/s11154-021-09679-5
4. Nair R, Maseeh A. Vitamin D: the „sunshine vitamin". J Pharmacol Pharmacother. 2012; 3(2): 118-126.
5. Pludowski P, Takacs I, Boyanov M, Belaya Z, Diaconu CC, Mokhort T, et al. Clinical practice in the prevention, diagnosis and treatment of vitamin D deficiency: a Central and Eastern European Expert Consensus Statement. Nutrients. 2022; 14(7): 1483. https://doi.org/10.3390/nu14071483
6. Guillaume J, Souberbielle JC, Chazot C. Vitamin D in chronic kidney disease and dialysis patients. Nutrients. 2017; 9(4): 328. https://doi.org/10.3390/nu9040328
7. Bikle DD. Vitamin D metabolism, mechanism of action, and clinical applications. Chemistry & Biology. 2014; 21(3): 319-329. https://doi.org/10.1016/j.chembiol.2013.12.016
8. Van Den Ouweland J. Analysis of vitamin D metabolites by liquid chromatography – tandem mass spectrometry. Trends in Analytical Chemistry. 2016; 84: 117-130. https://doi.org/10.1016/j.trac.2016.02.005
9. Lhamo Y, Chugh PK, Gautam-Sandhya R, Tripathi CD. Epidemic of vitamin D deficiency and its management. J Environ Public Health. 2017; 2517207. https://doi.org/10.1155/2017/2517207
10. Hector DL. The metabolism and functions of vitamin D. Adv Exp Med Biol. 1986; 196: 361-375. https://doi.org/10.1007/978-1-4684-5101-6_24
11. Żukiewicz-Sobczak W, Sobczak P, Siluch M, Weiner M, Pawłowicz-Sosnowska ET, Wojtyla-Buciora P, et al. Analysis of the chemical composition of energy drinks for contents of stimulant and filling compounds. Przemysł Chemiczny. 2018; 97(4): 560-564. https://doi.org/10.15199/62.2018.4.9
12. Papadimitriou DT. The big vitamin D mistake. J Prev Med Public Health. 2017; 50: 278-281. https://doi.org/10.3961/jpmph.16.111
13. Utami GL, How HC. Vitamin D deficiency. Singapore Med J. 2015; 56(8): 433-437. https://doi.org/10.11622/smedj.2015119
14. Alshahrani F, Aljohani N. Vitamin D: deficiency, sufficiency and toxicity. Nutrients. 2013; 5: 3605-3616. https://doi.org/10.3390/nu5093605
15. Verkaik-Kloosterman J, Marije-Seves S, Marga C. Vitamin D concentrations in fortified foods and dietary supplements intended for infants: implications for vitamin D intake. Food Chemistry. 2017; 221: 629-635. https://doi.org/10.1016/j.foodchem.2016.11.128
16. Jungert A, Spinneker A, Nagel A, Neuhäuser-Berthold M. Dietary intake and main food sources of vitamin D as a function of age, sex, vitamin D status, body composition, and income in an elderly German cohort. Food Nutr Res. 2014; 58. https://doi.org/10.3402/fnr.v58.23632
17. Bouillon R, Manousaki D, Rosen C, Trajanoska K, Rivadeneira F, Richards JB. The health effects of vitamin D supplementation: evidence from human studies. Nat Rev Endocrinol. 2022; 18(2): 96-110. https://doi.org/10.1038/s41574-021-00593-z
18. Pal R, Banerjee M, Bhadada SK, Shetty AJ, Singh B, Vyas A. Vitamin D supplementation and clinical outcomes in COVID-19: a systematic review and meta-analysis. J Endocrinol Invest. 2022; 45(1): 53-68. https://doi.org/10.1007/s40618-021-01614-4