Vitamin D deficiency: Causes & Treatment

Chapter 4

Vitamin D in Supplements and Medicines

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Abstract

Vitamin D deficiency is highly prevalent condition worldwide with potential serious consequences. Vitamin D supplementation is recommended for all age groups by various guidelines. However, the use of vitamin D supplements is associated with a certain risk of lack or insufficient efficiency as well as adverse effects, mostly due to manufacturing and labelling errors. The obtained results from our own independent analysis of 23 preparations in various pharmaceutical dosage forms, available on the European market are in accordance with the published studies from all around the world and reveal high variability in the determined vitamin D content in individual preparations. The variations were more significant in supplements compared with medicines. The determined vitamin D content was higher than stated on the label in most of the tested preparations, reaching up to 156% in our study and up to 200% in the published data. Even though the use of vitamin D overages is a routine, it poses a serious danger of excessive vitamin D intakes. These can be responsible for toxic effects, mediated mostly through hypercalcaemia. This chapter hereinafter summarizes 15 reports of vitamin D intoxication due to manufacturing or labelling errors. Such results are not only concerning but also reveal the urgent need for a more strict regulation of vitamin D supplements. Their quality control is of particular importance in the supplementation of infants, which are the most sensitive population to the potential harm of vitamin D deficiency or vitamin D toxicity.

Abbreviations: Anses: French Food Safety Agency; cps: capsule; D-A-CH: Germany, Austria, Switzerland; DRV: Dietary Reference Values; DS: dietary supplements; ES: Endocrine Society; FAO: Food and Agriculture Organization of the United Nations; Gulf: United Arab Emirates and the Gulf region; HPLC: High-Performance Liquid Chromatography; ICH: International Conference on Harmonization; IOM: Institute of Medicine; m: month; NCM: Nordic Council of Ministers; NHRMC: Australian National Health and Medical Research Council; NL: Netherlands; OTC: Over the Counter medicines; SACN: UK Scientific Advisory Committee on Nutrition; SCF: Scientific Committee for Food; SE: standard error; tbl: tablet; VDD: Vitamin D deficiency; VDI: Vitamin D insufficiency; UAE: United Arab Emirates; UL: Upper tolerable limits; WHO: World Health Organization; y: years; 25(OH)D: 25-hydroxyvitamin D.

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1. Vitamin D Deficiency and Prevalence

Vitamin D Deficiency (VDD) is defined as serum concentration of its major circulating form 25-hydroxyvitamin D [25(OH)D] of less than 20 ng/mL and vitamin D insufficiency (VDI) as 25(OH)D level between 21 and 29 ng/mL [1,2]. According to these definitions, 20–100% of U.S., Canadian, and European elderly men and women are estimated to be vitamin D deficient [2–4].Vitamin D deficiency and insufficiency are also highly prevalent in young and middle-aged adults worldwide, as VDD occurs in 4-55% and VDI in 25-80% of adults [5]. Pregnant and lactating women are especially at risk of vitamin D deficiency. A recent systematic review of vitamin D status revealed that the prevalence of VDD in pregnant women by WHO region is 10-54% and of VDI 50-90% [6,7], where the prevalence of VDD in breastfeeding women is 25-85% [8–10]. The main reports on vitamin D deficiency in children reveal high prevalence of VDD (1-85%) and VDI (30-90%) also in the paediatric age group [11–17]. Therefore, vitamin D deficiency is currently considered a pandemic, as it is estimated to affect around 1 billion people [3].

2. Causes of Vitamin D deficiency

The main source of vitamin D for the human kind is endogenous synthesis in the skin following exposure to sunlight. Therefore, vitamin D deficiencies are most often caused by decreased exposure of the skin to sunlight. Other factor, such as use of sunscreens, aging, skin pigmentation, season, latitude and time of the day also decrease its endogenous synthesis [18]. Furthermore, VDD is associated with some conditions (such as breastfeeding and obesity) and diseases (skin diseases, malabsorption, celiac disease, Crohn's disease, cystic fibrosis, liver or renal failure…). VDD can also be a consequence of the use of some medications, including antifungal medications, anticonvulsants, glucocorticoids and medications to treat AIDS/HIV [1].

3. Consequences of Vitamin D Deficiency

Severe vitamin D deficiency can lead to rickets in infants and small children and osteomalacia in adults. Both manifest as a consequence of impaired bone mineralisation, caused by inefficient absorption of dietary calcium and phosphorus. Typical symptoms include hypocalcemic seizures, muscle weakness, growth failure, decreased bone mass and characteristic bone changes or fractures. Less severe vitamin D deficiencies are associated with secondary

hyperparathyroidism, hypocalcaemia and hypophosphatemia. Other, nonspecific symptoms include irritability, lethargy and developmental delay. Prolonged VDI leads to low mineral bone density and an increased risk of osteoporosis, especially in post-menopausal women. Besides these well-known effects on musculoskeletal system, vitamin D deficiency is also related to several non-skeletal health problems, including many chronic conditions and diseases, which are associated with the modern way of life. Low vitamin D levels are largely described in allergies and autoimmune diseases (such as multiple sclerosis and Crohn's disease) as well as diabetes (type I and II) [19]. VDD is also associated with neurological and psychiatric diseases, including depression, schizophrenia, dementia, fibromyalgia, Alzheimer's and Parkinson's disease, as well as with the pathogenesis of different types of cancers (colorectal, prostate, breast and ovarian cancer) [20]. Moreover, several clinical trials and epidemiological studies have shown a strong association between VDD and increased risk of cardiovascular diseases and metabolic syndrome [21].

4. Vitamin D Supplementation

Food sources, naturally rich with vitamin D are quite rare and include fatty fish, red meat, eggs and wild mushrooms. Further sources of dietary vitamin D are fortified foods. In the United States and Canada, milk, infant formula, some bread products, orange juices, cereals, yogurts and cheeses are fortified with vitamin D [4]. In most European countries the fortification of foods with vitamin D is forbidden, due to an outbreak of vitamin D intoxication in young children in the 1950s. Nonetheless, some countries with high prevalence of vitamin D deficiency, such as Sweden and Finland, fortify milk products and margarine. Currently, there is growing support and development of strategies for fortifying foods with vitamin D in other European counties as well [4]. However, within the food fortification as a strategy to reduce VDD, problems associated with the stability of vitamin Darise. It is known that vitamin D is susceptible to degradation, especially when exposed to light, oxygen, elevated temperature and humidity [50]. The stability of vitamin D in foodstuffs during cooking has been shown to vary widely, resulting in vitamin D loss by 10-60% [22]. The content of vitamin D in fortified foods is also questionable, as it was found to vary from 50% to 150% of the declared to the declared value [23].

Therefore, the most effective and controlled way to prevent and treat VDD and VDI is vitamin D supplementation. Recommendations on vitamin D supplementation have been changing during the past two decades, following the latest scientific and clinical developments. Previously, vitamin D supplementation was recommended only for population groups at high risk of deficiency and for the treatment of VDD. These groups include infants and young children (<5 years), pregnant and breastfeeding women, teenagers, elderly population (≥65 years), particularly institutionalised ones, people with limited exposure to the sun (e.g. those who cover their skin for cultural reasons) and people with darker skin pigmentation. The

amount of vitamin D produced by exposure to sunlight in the summer months was presumed to be adequate for achieving sufficient serum 25(OH)D concentrations during winter for most people. However, it is now known that this is not the case. A combination of various factors: limited dietary sources of vitamin D, reduced sun exposure and increased use of sunscreens for the prevention of skin cancer, use of some medications, body fat content, age as well as several diseases has recently led to a widespread VDD in all age groups [2,4].

4.1. Vitamin D supplementation in the general population

According to the current clinical guidelines, vitamin D supplementation is recommended in all life stage groups at suggested daily amounts, depending on age and clinical circumstances. Dietary Reference Values (DRVs) are defined as the complete set of nutrient recommendations and reference values, such as population reference intakes, the average requirement, adequate intake level and the lower threshold intake [24]. Recommendations on DRVs for vitamin D by the UK Scientific Advisory Committee on Nutrition (SACN) [25], German Nutrition Society for the German-speaking countries (Germany, Austria, Switzerland, D-A-CH) [26], the French Food Safety Agency (Anses) [27], Nordic Council of Ministers (NCM) [28], Health Council of the Netherlands (NL) [29], the Scientific Committee for Food (SCF) for the European Community [30], U.S. Institute of Medicine of the National Academy of Sciences (IOM) [31], the Endocrine Society in the USA (ES) [32], Australian National Health and Medical Research Council and New Zealand Ministry of Health (NHRMC) [33], WHO/FAO [34] and in the UAE and the Gulf region [35] are summarized in **Table 1**.

Table 1: Overview of Dietary Reference Values (DRV) for vitamin D

		Life stage group						
Authority		Infants	Children		Adults	Elder	Pregnancy	Lactation
SACN	Age	0- <12 m	1-17 y		≥18 y			
	DRV (IU/day)	340-400	400		400	400	400	400
D-A-CH	Age	0- <12 m	1-18 y		≥19 y	≥65 y		
	DRV (IU/day)	400	800		800	800	800	800
Anses	Age	0- <12 m	1-3 y	4-19 y	20-74 y	≥75 y		
	DRV (IU/day)	800-1000	400	200	200	400-600	400	400
NCM	Age	0- <12 m	1-18 y		18-74 y	≥75 y		
	DRV (IU/day)	400	400		400	800	400	400
NL	Age	0- <12 m	1-18 y		18-69 y	≥70 y		
	DRV (IU/day)	400	400		400	800	400	400
SCF	Age	0-11 m	1-17 y		18-64 y	≥65 y		
	DRV (IU/day)	400-1000	0-600		0-400	400	400	400

IOM	Age	0- <12 m	1-18 y	19-70 y		≥71 y		
	DRV (IU/day)	400	600	600		800	600	600
ES	Age	0- <12 m	1-18 y	19-70 y		≥70 y		
	DRV (IU/day)	400-1000	600-1000	1500-2000		1500-2000	600-2000	600-2000
NHRMC	Age	0- <12 m	1-18 y	19-50 y	51-70 y	≥70 y		
	DRV (IU/day)	200	200	200	400	600	200	200
WHO / FAO	Age	0- <12 m	1-18 y	19-50 y	51-65 y	≥66 y		
	DRV (IU/day)	200	200	200	400	600	200	200
GULF	Age	0- <12 m	1-18 y	19-65 y		≥65 y		
	DRV (IU/day)	400-600	600-1000	800-2000		1000-2000	1500-2000	1500-2000

m-months, y-years.

As can be seen in **Table 1**, the recommended intakes for vitamin D are similar or within the same range, for most of the listed countries. The DRV for vitamin D by the Endocrine Society in the USA are considerable higher, because it recommends achieving serum 25(OH)D concentrations above 30 ng/mL, with preferred range 40-60 ng/mL on behalf of the additional health benefits in reducing the risk of common cancers, autoimmune, cardiovascular and infectious diseases and type 2 diabetes [32]. Similarly, the recommended DRV for vitamin D are noticeable higher for the UAE and the Gulf region, where the residents are exposed to various risk factors for VDD, such as diet, lack of exercise, cultural habits and avoiding sun exposure due to excessive heat [35]. The goal for this area is to achieve and maintain serum 25(OH) D concentration of 30–50 ng/mL, whereas other authorities recommend supplementation to maintain 25(OH)D concentrations above 20-30 ng/mL [36].

In general, it can be concluded that obtaining the current recommended levels of vitamin D throughout the year, through diet or endogenous synthesis is nearly impossible and that all presented authorities recommend preventive vitamin D supplementation for all age groups. Although there are a variety of guidelines on vitamin D supplementation for almost every part of the world, several factors, besides environmental, should be considered when selecting the adequate DRV. These include skin pigmentation, exposure to sun, health care system, dietary habits, clothing and cultural habits, age, body weight and potential coexisting diseases.

4.2. Vitamin D supplementation in groups at risk of vitamin D deficiency

Therapy with higher vitamin D doses than the DRV (**Table 1**) is recommended for the aforementioned groups at high risk of deficiency as well as for individuals with diagnosed conditions such as: rickets, osteomalacia, osteoporosis, bone pairs, bone deformations, disorders of calcium-phosphorus metabolism, treatment with corticosteroids, antiretroviral and antiepileptic drugs, liver and renal failure, hyper- and hypoparathyroidism, diabetes type 1 and

2, growth hormone deficiency, development delay, diseases of the nervous system, autoimmune, cardiovascular and metabolic diseases. In these cases the dosing of vitamin D should be individualized depending on the serum 25(OH)D concentration in order to maintain optimal concentration of >30 ng/mL. It is recognized that for every 100 IU of vitamin D ingested, the blood level of 25(OH)D increases by approximately 0.6 to 1 ng/mL [37,38].

4.3. Vitamin D supplementation in groups at risk of vitamin D hypersensitivity

Vitamin D hypersensitivity syndrome is a condition, responsible for vitamin D accumulation along with increased efficiency of intestinal calcium absorption and mobilization from the skeleton [39]. It is biologically characterized by a tendency towards hypercalcemia and hypercalciuria with low parathormone levels. Vitamin D hypersensitivity is associated with mutations of the CYP24A1 and SLC34A1 genes as well as with conditions such as primary hyperparathyroidism, granulomatous diseases (especially sarcoidosisor tuberculosis), chronic fungal infections and some cancers (especially lymphoma). In such cases, supplementation should be supervised by a knowledgeable medical professional and carried out in an individual manner, by monitoring the calcium and 25(OH)D levels. The latter should not exceed serum concentrations of 30 ng/mL [38–40].

4.4. Upper tolerable limits of vitamin D

Endogenous vitamin D synthesis is regulated so that prolonged sunshine exposure does not lead to vitamin D toxicity. However, considering the extensive and increasing number of vitamin D supplements, available in pharmacies and on the Internet along with the current guidelines, medical personnel recommendations and media campaigns, there is a possibility of excessive vitamin D intake. These can be responsible for toxic effects, mediated through hypercalcaemia due to bone resorption of calcium and increased intestinal calcium absorption. Symptoms of vitamin D toxicity range from mild (thirst, nausea and polyuria) to severe (calcification of soft tissues, bone demineralization, associated with increased incidence of falls and fractures, seizures, increased rates of pancreatic and prostate cancer and increased risk of mortality) [41,42]. For these reasons, regulatory agencies worldwide have established guidelines for Tolerable Upper Intake Level (UL), as a simple and effective tool to prevent vitamin D overuse by the healthy population. UL is defined as the highest average chronic daily intake of vitamin D unlikely to pose a risk to adverse health effects for almost all individuals in the population. UL values for vitamin D by European Food Safety Authority (EFSA) [43], U.S. Institute of Medicine of the National Academy of Sciences (IOM) [30], the Endocrine Society USA (ES) [31], Australian National Health and Medical Research Council and New Zealand Ministry of Health (NHRMC) [32] and in the UAE and the Gulf region [35] are presented in **Table 2**.

Table 2: Overview of Upper tolerable limits (UL) for vitamin D.

		Life stage group							
		Infants		Children		Adults	Elder	Pregnancy	Lactation
EFSA	Age	0- <12 m		1-10 y	11-17 y	≥18 y			
	UL (IU/day)	1000		2000	4000	4000	4000	4000	4000
IOM	Age	0-6 m	6-12 m	1-3 y	4-8 y	9-70 y	≥71 y		
	UL (IU/day)	1000	1500	2500	3000	4000	4000	4000	4000
ES	Age	0- <12 m		1-18 y		19-70 y	≥70 y		
	UL (IU/day)	2000		4000		10000	10000	4000-10000	4000-10000
NHRMC	Age	0- <12 m		1-18 y		19-70 y	≥70 y		
	UL (IU/day)	1000		3200		3200	3200	3200	3200
GULF*	Age	0- <12 m		1-10 y	11-18 y	≥18 y			
	UL (IU/day)	1000		2000	4000	4000	4000	4000	4000

*UL limit for obese adults and obese elderly people is 10000 IU/day; for institutionalized people it is 5000 IU/day.

5. Preparations with Vitamin D

In preparations in pharmaceutical dosage forms (dietary supplements and medicines) vitamin D is used as vitamin D2 (ergocalciferol) or vitamin D3 (cholecalciferol). The latter is the preferred form of vitamin D for supplementation, as it was proved more effective in raising and maintaining serum 25(OH)D levels [44–47]. However, these finding are disputable, as other studies have found them to be equally effective [48,49]. Preparations containing only vitamin D are available as most proper preparations for breastfed infants who have no need for supplementation with other vitamins. In addition to these, there are many available preparations with a combination of vitamin D with other vitamins (multivitamin preparations). Vitamin D is commercially available in various dosage forms including capsules (oil- and water-soluble), tablets, concentrates, chewable and gummy forms, sprays, injections and oral drops, as the only dosage form acceptable for infants [50]. Vitamin D amounts in different preparations vary greatly, reaching even 500 IU per drop in oral solutions. These preparations should be used with caution because of the ease of dispensing excessive vitamin D amounts to infants with just a few drops.

The available vitamin D preparations are registered as dietary or food supplements, non-prescription medicinal products (Over the Counter medicines - OTC) or prescription medicinal products. These differ in their definition, legislation, intended use and place of issuance.

The main difference is that supplements are used to supplement the diet by increasing the total dietary intake and unlike medicines, supplements are not intended to treat, diagnose, prevent, or cure diseases. In the EU, food supplements are defined with the Food Supplements Directive 2002/46/EC, which also establishes a list of allowable vitamins and minerals and sets labelling requirements. Other regulations are governed by individual EU Member States [51]. In the USA these preparations are referred to as dietary supplements (DS) and are regulated by Federal Food, Drug and Cosmetic Act [52] and Dietary Supplement Health and Education Act [53].The used term in Australia is complementary medicines and they are regulated with the Australian Regulatory Guidelines for Complementary Medicines [54], whereas DS in New Zealand are managed with the Dietary Supplements Regulations as part of the Food Act [55].

In general, DS are regulated as food and therefore have fewer restrictions than registered medicines. In most countries, in contrast with medicines, evidence of quality, efficacy and/or safety is not required for DS. Nonetheless, this is an upgrading field and as such, it is currently under examination and discussion by various regulatory agencies. The FDA has thus implemented a current Good Manufacturing Practice policy to ensure the quality of DS [56]. The regulation in Australia is even more stringent, as it requires pre-market approval based on quality and safety assurance [57].

One of the main aspects in the quality assurance process is content evaluation of the active ingredient in the sense of verifying the accuracy of the labelled amount. Since the European Pharmacopoeia as well as the national pharmacopoeias of the EU Member States mostly describes general test procedures, the European Medicines Agency (EMA) establishes maximum acceptable tolerance limits in the active substance content in medicines up to the end of the proposed shelf life of ± 5%. Release limits wider than ± 5% would need to be justified based on experimental results [54]. The acceptance limit for DS according to the European Commission is 80-150% of the label claim [55]. The USP acceptance limit for medicines is mostly 90-110% [58]. The acceptance limits for vitamin D in both forms, in medicines, according to their USP monographs are 100-120% (vitamin D2) and 90-120% (vitamin D3) of their labelled amount. USP provides different acceptance limits for DS with vitamin D for different dosage forms. Therefore, vitamin D tablets and capsules should contain 90-165%, whereas oral solutions should contain 90-120% of the labelled amount [56].

6. Vitamin D Content in Commercial Preparations

Production of quality commercial preparations with vitamin D can be quite a challenge, mainly due to its instability, causing losses during manufacture and storage [50]. In favour of producing commercially acceptable products, the technique that manufacturers often implement is the addition of an ‘overage’ of the stability-sensitive vitamin D. ‘Overage’ is defined as the difference between the formulated and declared levels and is usually added to ensure

the labelled content at the end of the declared shelf life [59]. However, according to the ICH guidelines on Pharmaceutical Development, any overage in the manufacture of medicines should be justified considering the safety and efficacy of the product. Information should be provided on the amount of overage, reason for the overage (e.g., to compensate for expected and documented manufacturing losses) and justification for the amount of overage [60]. The well-established use of vitamin D overage in preparations, on one hand, and its intrinsic instability and degradation during manufacture and storage, on the other hand, cause diversity in its content in commercial preparations. The re-emergence of diseases associated with VDD in the last decade, has led to a considerable rise in the usage and as well as in the number of commercially available vitamin D preparations. In relation to this, there is also an increased interest in their quality evaluation, in terms of content analysis, which is the scope of several recently published studies. In an initially published study on this topic, vitamin D content was evaluated in 4 vitamin D dietary supplements on the Polish market. The determined vitamin D content was in the range 107-138% compared with the labelled content [61]. The results obtained from a study on 16 pharmaceutical preparations with vitamin D (not specified weather they are DS, OTC or prescription medicines) from the Jordanian market revealed that only 3 preparations had vitamin D content >90%. The determined content ranged from 5 to 94% of the stated dose [62]. Comparable results were obtained in an Indian study of 14 pharmaceutical preparations (again, not specified). Vitamin D content varied from 9 to 165% of the labelled claim. Only 4 preparations were found to be within the acceptable range (90 to 125%) as defined by Indian Pharmacopeia [63]. Similar variability was determined in 15 dietary supplements from the Dutch market, specifically intended for infants. Compared to the declared values, vitamin D content ranged from 8% to 177% [23]. Similarly, a study of 15 vitamin D preparations in the USA revealed significant variations in vitamin D content in the individual preparations (9-140%), as well as between tablets from different containers with the same lot number of the same preparation (52-136% of the labelled claim) [64]. The results from a more comprehensive USA study of 54 multivitamin supplements showed similar variability in vitamin D content, ranging from 7 to 172% according to the declared amount [65]. A study on 12 dietary supplements with vitamin D from the New Zealand market revealed an even greater variability in its content, ranging from 8 to 201%. However, the content of vitamin D in the two prescription medicines included in the study was 90±4% and 97±2% of the labelled amount [66]. Difference in quality, in terms of the labelled amount accuracy between medicines and supplements was also observed in a recently published Slovenian study on 3 medicines (vitamin D content: 100-131%) and 3 supplements (vitamin D content: 100-153%) with vitamin D [67].

The actual vitamin D content in numerous preparations, registered as medicines or supplements, was also evaluated as a part of our research work. The tested pharmaceuticals in various dosage forms are available on the European market; the majority was purchased

in Slovenia. Vitamin D content was determined according to a published stability-indicating HPLC method [67]. Samples from each preparation were prepared and analysed in at least triplicate. For individual preparation, more batches with different lot numbers, were purchased and analysed. The obtained results, expressed as mean content of the analysed batches ±SE, when more batches were analysed or as mean content of the single tested batch are summarized in Table 3. More detailed results on vitamin D content, expressed as a percent of the declared amount, including the individual results of the tested batches are presented in **Figure 1**.

Table 3: Vitamin D content in supplements and medicines available on the European market, determined in our laboratory.

Mark	Dosage form	Registered as	Label claim	Number of analysed batches	Average amount found± SE (standard error)
1	Oral drops	Dietary supplement	30760 IU/mL	1	42232 IU/mL
2	Oral drops	Veterinary medicine	25000 IU/mL	1	29906 IU/mL
3	Oral drops	Prescription medicine	20000 IU/mL	4	20147 ± 209 IU/mL
4	Oral drops	Prescription medicine	14400 IU/mL	1	14043 IU/mL
5	Oral drops	Dietary supplement	12320 IU/mL	1	14774 IU/mL
6	Oral drops	Dietary supplement	12000 IU/mL	1	7755 IU/mL
7	Oral drops	Veterinary medicine	5000 IU/mL	1	6830 IU/mL
8	Oral drops	Prescription medicine	4000 IU/mL	4	4223 ± 17 IU/mL
9	Oral drops	Dietary supplement	4000 IU/mL	3	3999 ± 34 IU/mL
10	Oral drops	Dietary supplement	2400 IU/mL	1	2682 IU/mL
11	Oral drops	Prescription medicine	2000 IU/mL	5	2598 ± 47 IU/mL
12	Oral drops	Dietary supplement	320 IU/mL	1	345 IU/mL
13	Oral drops	Dietary supplement	200 IU/mL	1	240 IU/mL
14	Oral spray	Dietary supplement	1000 IU/mL	2	998 ± 22 IU/mL
15	Syrup	Dietary supplement	200 IU/mL	3	204 ± 3 IU/mL
16	Syrup	Dietary supplement	40 IU/mL	1	42 IU/mL
17	Tablets	Non-prescription medicine	500 IU/tbl	1	648 IU/tbl
18	Tablets	Non-prescription medicine	400 IU/tbl	1	488 IU/tbl
19	Tablets	Dietary supplement	400 IU/tbl	3	623 ± 7 IU/tbl
20	Tablets	Dietary supplement	400 IU/tbl	3	483 ± 13 IU/tbl
21	Tablets	Dietary supplement	200 IU/tbl	1	293 IU/tbl
22	Tablets	Non-prescription medicine	80 IU/tbl	1	97 IU/tbl
23	Capsules	Dietary supplement	600 IU/cps	1	728 IU/cps

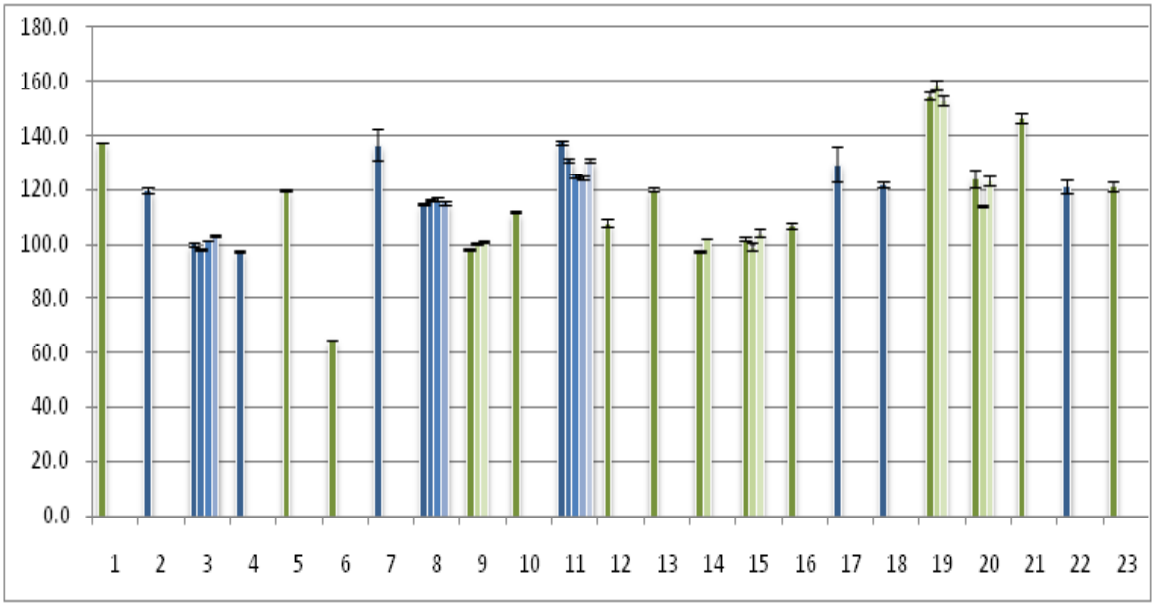

Figure 1: Vitamin D content (expressed as a percent of the declared amount with the belonging SE, n ≥ 3) in medicines (blue) and supplements (green), available on the European market and determined in our laboratory.

The obtained results are additional confirmation that in general, medicines, which are more strictly regulated, are also superior to supplements in the sense of lower variability between different batches and disparity in the actual and declared content (**Figure 1** and **Table 3**). The determined vitamin D content in the tested medicines ranged 98-130% and was similar in veterinary medicines (between 120 and 137%), whereas supplements showed substantial variability: 65-156% of the labelled amount (**Figure 1**). The determined vitamin D content was found consistent with the label claim (100 ± 5%) in approximately 20% of the tested preparations (**Figure 1**). It is also evident, that the use of vitamin D overage is a common practice among manufacturers, as only 1 out of 23 tested preparations has significantly lower vitamin D content, whereas 15 preparations had vitamin D contents higher than 110% of the declared amount (**Figure 1**). Vitamin D overages of 20% were most frequently used, which were observed in roughly one third of the tested preparations. Special attention should also be drawn at the declared vitamin D content, particularly in the tested oral drops and syrups, ranging from 40 IU/mL up to 30760 IU/mL (**Table 3**). The dose of these high-dose preparations, recommended by the manufacturer, itself exceeds most of the generally accepted Dietary Reference Values (**Table 1**). Such high concentrations in combination with inaccurate or inappropriate dosing, pose a high risk of excess vitamin D intake, which can lead to unwanted or ever toxic effects, especially if used for infants or small children. The used vitamin D overage is an additional risk factor and cause for concern. These high-dose preparations should therefore be used with extreme caution and avoided for the supplementation of infants and small children.

The main cause of the frequently used vitamin D overages lays in its instability. It is generally accepted that many environmental factors, including light, oxygen, elevated temperature and humidity affect its stability [50]. During the manufacture process and storage in closed original containers it is protected from these external influences. However, once the original containers are opened, vitamin D is exposed to these factors, resulting in its degrada-

tion and decreased content. Nonetheless, shelf life after opening is generally not defined by the manufacturers and is a common reason for disagreement between medical professionals. Despite the fact that the evaluation of vitamin D stability in commercial preparations is an important aspect of their quality, especially in the case of considerably lower initial content (around 60% in preparation 7, **Figure 1**), this research area still remains quite unexplored. To our knowledge, there is currently only one published study evaluating the effect of storage conditions on the shelf life of commercial preparations with vitamin D. The results reveal that its content generally decreases after opening the preparations to an extent dependent on the formulation and storage temperature. Considerable decrease of around 10% in vitamin D content was observed in one liquid vitamin D preparation, registered as medicine in the first 10 days of storage at ambient temperature (25 °C). The decrease was even more extensive with increases in the storage temperature, which may occur in real life [50].

Stability issues are generally more evident in solutions compared with solid dosage forms. Considering that solutions are the only dosage form acceptable for infants, they should be verified in terms of vitamin D content and shelf life after opening in order to avoid insufficient supplementation and possible consequent complications. Therefore, disparity between the actual and declared vitamin D content in preparations in both directions can be associated with serious consequences due to either excessive or insufficient vitamin D intake, especially in the most sensitive, paediatric population. The importance of appropriate regulation, via stringent quality control, emerges in such cases. The regulatory framework should ensure that vitamin D preparations (both medicines and supplements) are appropriate for use, based on benefit risk profile and that they are of adequate quality.

7. Quality Control Assessment and Toxicity

Vitamin D supplementation has gained increased acceptance due to the current guidelines and recommendations by the healthcare professionals as well as the high prevalence of vitamin D deficiency. However, it is associated with a certain risk of potential adverse toxic effect, particularly when using errantly manufactured or wrongly labelled preparations. A review of the literature revealed an increased number of publications on vitamin D toxicity after 2001 and especially after 2009. Vitamin D supplementation is generally considered safe, supported by studies revealing that even doses of 10000 IU/day, administered through longer time periods are not associated with vitamin D toxicity [4]. A recent review article reports that the most common causes of vitamin D toxicity are errors in formulation or fortification, followed by inappropriate prescribing or dispensing and errors in administration [68]. Examples of vitamin D toxicity as a result of errors in preparations are summarized in **Table 4**.

Table 4: Reports of vitamin D intoxication as a result of inaccurately manufactured or labelled supplements.

Country, year, reference	Affected patients	Symptoms	Vitamin D content (fold)*
USA, 2001, [69]	1 adult	Hypercalcemia symptoms	26 to 434
USA, 2004, [70]	1 adult, 3 children	Fatigue, constipation, back pain, forgetfulness, nausea, vomiting	470
Netherlands, 2010, [71]	2 adults	Life-threatening hypercalcemia	100 to 1000
Dominican Republic, 2011, [72]	9 adults	Hypercalcemia	400
USA, 2011, [73]	2 adults	Fatigue, excessive thirst, polyuria, muscle aches, poor mentation	117**
Ecuador, 2012, [74]	1 adult	Submandibular pain and hypercalcemia	Unknown
Australia, 2013, [75]	1 adult	Nausea, vomiting, abdominal discomfort	1000
Turkey, 2013, [76]	3 children	Abdominal pain, vomiting, poor appetite, failure to gain weight, irritation, constipation	Unknown
Italy, 2013, [77]	3 adults	Hypercalcaemia and renal insufficiency	880
Turkey, 2013, [78]	7 children	Symptoms of hypercalcemia	4000
Italy, 2014, [79]	2 children	Abdominal pain, constipation and vomiting	Higher than declared
Brazil, 2014, [80]	1 adult	Worsening of renal function, pruritus, muscle weakness, lack of appetite, weight lost	2000
USA, 2015, [81]	1 child	Emesis, diarrhoea, lethargy, dehydration	3
Netherlands, 2016, [82]	1 adult	Headaches, nausea, reduced appetite, weight loss	78
Brazil, 2016, [83]	1 adult	Hypercalcemia, acute renal function impairment	Unknown

*Fold of the determined vitamin D content according to the label claim;
**Along with the manufacturing error, there was a labelling error, recommending 10 cps instead of one cps per day.

As can be seen in **Table 4**, typical symptoms of vitamin D intoxication are a consequence of hypercalcemia and include fatigue, constipation, nausea, vomiting, polyuria and muscle weakness; more severe symptoms are associated with renal failure, which can be life-treating. Vitamin D intoxication, as a result of errors in pharmaceutical preparations, has been reported in both adult and paediatric population. As demonstrated in **Table 4** inaccurately manufactured and labelled vitamin D supplements are reported worldwide and pose a serious health problem, as the determined vitamin D amounts diverge significantly from the declared contents. The determined vitamin D amounts in these particular preparations were 3-4000 folds higher that stated on the label. Such case reports on vitamin D intoxication in conjunc-

tion with errant manufacture and labelling has not been published for vitamin D preparations, registered as medicines. Even though vitamin D intoxication is a rare condition, it is nearly always linked to supplement use and can be life-threatening. Therefore, greater awareness and caution is needed when using vitamin D supplements. Evidently, there is a substantial need for improvement in the quality of vitamin D supplements, in conjunction with more specific, strict and effective legislation to ensure their safety and also appropriate supervision of preparations, registered as supplements.

8. Conclusion

Vitamin D sufficiency is crucial for health maintenance due to its numerous functions, including calcium homeostasis and bone mineralization along with many non-skeletal effects, especially in autoimmune, cardiovascular diseases and cancer. Vitamin D deficiency is highly prevalent condition worldwide. Consequently, vitamin D supplementation is strongly recommended by numerous agencies and scientific organizations and has become widely accepted by the general population. Despite the wide therapeutic index, vitamin D supplementation can lead to toxicity and life-threatening hypercalcemia, as a result of excess vitamin D intake, especially in individuals with vitamin D hypersensitivity syndrome as well as in infants and children. In spite of that, the quality and safety of vitamin D preparations cannot be taken for granted. Due to vitamin D instability, the manufacture of vitamin D preparations is challenging and requires certain pharmaceutical expertise. The shortcut approach, which is used by most manufacturers, is the addition of vitamin D overage. The literature review along with the presented results from the currently most extensive study on vitamin D preparations available in the European market reveal that variations in the determined content compared to the stated amount were higher in dietary supplements compared to medicines. The presented cases of vitamin D intoxication, as a result of inaccurately manufactured or labelled supplements, emphasize the urgent need for a more strict regulation in the field of supplements, in general, and especially in the case of vitamin D supplements. Another, equally important aspect in the need for appropriate supervision by routine quality control and content determination in vitamin D preparations is the possible insufficient supplementation. Lower vitamin D intakes, than expected may be a result of either low initial content or vitamin D instability and its degradation during storage. As can be concluded from the obtained results, lower initial vitamin D contents are seldom in commercial preparations, but the differences between the actual and declared content can be very broad. Such deviations should be particularly taken into consideration when supplementing infants, as preparations are often their main source of the indispensable vitamin D.

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