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VITAMIN D AND ITS STATUS IN VEGETARIANS AND VEGANS

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Vitamin D is an essential hydrophobic micronutrient. There are at least seven different forms of vitamin D, however, only D_2 and D_3 are considered to be its biologically active forms in the human body.
Besides its primary functions of calcium homeostasis and bone metabolism, vitamin D exerts a number of additional biological effects. For instance, it is a powerful immunomodulator.
Lack of vitamin D is primarily associated with osteoporosis and rickets. Moreover, vitamin D deficiency can aggravate the risks of cardiovascular diseases, autoimmune diseases, and even tumour genesis.
At the same time, vitamin D can be extremely toxic if taken in excess. As a consequence of vitamin D hypervitaminosis, severe impairment in calcium metabolism may occur and can result in neuropsychiatric abnormalities, gastrointestinal, renal, or cardiovascular disorders.
The human body mainly obtains vitamin D_3 by endogenously biosynthesizing it from cholesterol in the skin in presence of ultraviolet radiation. The richest food source of vitamin D_3 is oily sea fish. Much smaller amounts of calciferol are found in dairy products, eggs, or liver. Mushrooms are also capable of accumulating vitamin D under the influence of sunlight, but they store vitamin D in its less active form – vitamin D_2.
Despite the presence of different forms of vitamin D in various foods, it is extremely difficult to compensate for its need if relied only on food, even for people who regularly include fish in their diet. And while vegetarians and vegans consume significantly less vitamin D and generally have lower serum concentrations, the entire population of extratropical regions is highly susceptible to calciferol deficiency, regardless of their diet.
Therefore, it seems reasonable to regularly screen the vitamin D supply, especially for people living in high latitudes, no matter which diet they follow.

Key words: *cholecalciferol, ergocalciferol, calcidiol, 25(OH)D, calcitriol, 1,25(OH)$_2$D, vegetarianism, veganism.*

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INTRODUCTION

Vitamin D is a group of fat-soluble secosteroids responsible for increasing intestinal absorption of calcium, magnesium, and phosphates; and contributes to multiple other biological effects. In humans, the most important compounds in this group are vitamin D_3 (also known as cholecalciferol) and vitamin D_2 (ergocalciferol) (Figure). Other forms are not regarded to be crucial so some of them even do not have any trivial names. Vitamin D_2 is mostly produced by mushrooms (like *Cantharellus tubaeformis*) or human-made and added to foods, whereas vitamin D_3 is synthesized in the skin of humans from 7-dehydrocholesterol. Similarly, vitamin D_3 can be acquired by consuming animal tissues as well [1].

PHYSIOLOGICAL ROLE OF VITAMIN D

Vitamin D is essential for maintaining bone health, and for muscle function and balance. It increases bone density by promoting calcium absorption and prevents the disturbance of neuromuscular coordination. Moreover, it has been hypothesised that the active form of vitamin D binds to the nuclear receptor of the muscle tissue [2].

After binding to the nuclear receptor, vitamin D induces protein synthesis, resulting in muscle cell growth and increased muscle strength. Hence, it plays a dual role in preventing fractures: it directly increases bone density, and it improves muscle strength [3] and balance. Both factors reduce the risk of bone fracture on falling [4].

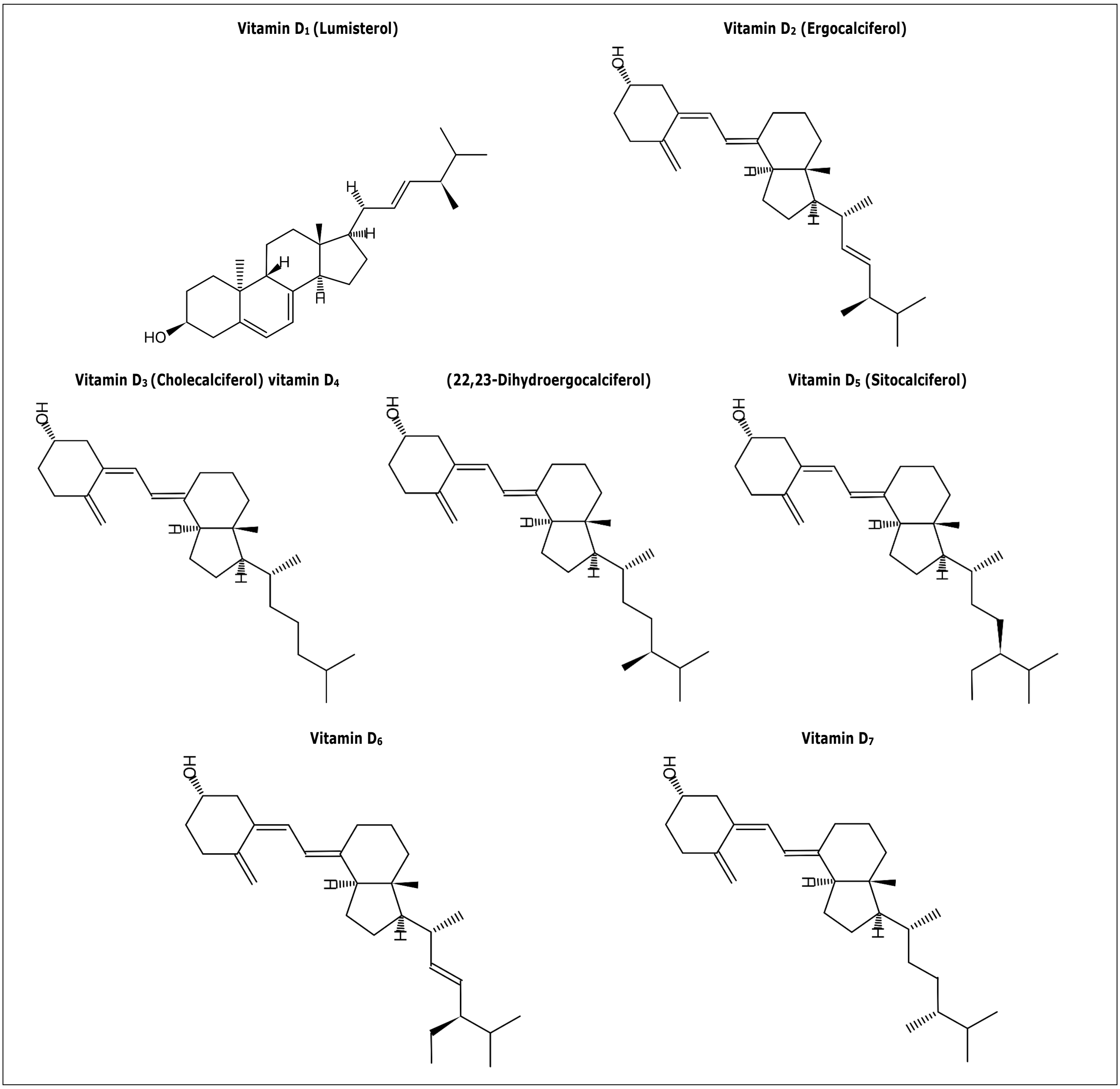

Figure. Structures of different forms of vitamin D

Vitamin D exerts its biological functions far beyond calcium homeostasis and bone metabolism through paracrine/autocrine actions and with the help of the presence of the vitamin-D receptor (VDR) in almost all cells of the body. 1,25-dihydroxyvitamin D (1,25(OH)$_2$D or calcitriol) can take part to initiate biological responses eventually affecting many organs and systems. Vitamin D has also been understood to strengthen the immune system affecting both the innate and adaptive immune responses [5, 6]. It is worth reminding that, in the pre-antibiotic era, very high doses of vitamin D (200000–300000 IU/day) were used to treat tuberculosis [7]. Experimental data suggest that vitamin D suppresses bacterial growth [8], decreases pro-inflammatory cytokines, and inhibits respiratory tract infections [9,10]. The first clue to link adaptive immunity with vitamin D is the expression of VDR on T and B-cells. It also has been found that the number of these receptors increases as B- or T-cells proliferate. Through those receptors, vitamin D can influence the phenotype of T-cells by inhibiting Th1 cells. Similarly, the active form of vitamin D has also been shown to enhance cytokines associated with Th2 cells [11]. In the case of innate immunity, 1,25$(OH)_2$D provokes the expression of cathelicidin (LL-37) that promotes the intracellular killing of bacteria. [12, 13].

Vitamin D has also been seen to prevent or delay auto-immune diseases, such as type 1 diabetes [14] or multiple sclerosis [15, 16]. In population studies, it has been well established that the rate of auto-immune diseases and cancers are directly proportional to higher latitudes and are significantly more prevalent at northern latitudes of Europe, the U.S., or the southern part of Australia, correlating well with the increased prevalence of vitamin D deficiency at higher latitudes [17]. Vitamin D reduces the incidence of cancer in several ways. Firstly, calcitriol inhibits tumour angiogenesis. Secondly, it helps to firmly adhere the cells to enhance intercellular communication via gap junctions, which inhibits proliferation through "contact inhibition".

The next mechanism is related to the ability of 1,25$(OH)_2$D to release ionized calcium in a pulsatile manner. Such release of calcium is found to help in terminal differentiation and apoptosis of the cell. Similarly, epidemiologic data have correlated an association between vitamin D deficiency and an increased rate of all-cause and cardiovascular mortality [18].

A significant role of vitamin D in multiple health outcomes has been studied and supported by plausible biological mechanisms and large prospective studies, but the results are yet not consistent. A large number of these positive associations are quite well established. However, results still need to be proven on a large scale by conducting long-term randomized clinical trials.

HYPOVITAMINOSIS, VITAMIN D DEFICIENCY DISORDERS

The insufficiency of vitamin D has long been known to mankind for its association with osteomalacia, osteoporosis, or rickets [19]. This occurs due to the fact that the lack of vitamin D causes less calcium and phosphorus to be absorbed from the gastrointestinal tract which consequently increases the parathyroid levels [20].

Furthermore, lack of vitamin D causes neurological diseases, coronary heart disease, type II diabetes, depression, autoimmune diseases, various inflammatory disorders, high blood pressure, or age-related cognitive decline [21].

HYPERVITAMINOSIS D, VITAMIN D TOXICITY

Vitamin D toxicity (VDT) can be life-threatening if not promptly identified. The unintentional overdosing due to the misuse of pharmaceutical products is the most common cause of exogenous VDT. Some studies have reported that there are no side effects of vitamin D up to the intake of 50,000 IU/day [22, 23]. But the US Institute of Medicine has stated that VDT can be more common in individuals consuming regular dose of more than 50,000 IU/day which could elevate serum 25(OH)D concentrations to 500 nmol/l (200 ng/ml). In the USA it is not recommended to take more than 4,000 IU/day regularly (Table 1) [1], and in Russia – not more than 2,000 IU/day [24].

Table 1. ***Tolerable Upper Intake Levels (UILs) for vitamin D for different age groups***

Age	<6 months	7–12 months	13 years	4–8 years	9–10 years	>11 years
Institute of Medicine [1]	25 μg	38 μg	63 μg	75 μg	100 μg	
European Food Safety Authority [25]	25 μg		50 μg			100 μg

Note: vitamin D intakes are usually expressed in micrograms (μg) or International Units (IU). However, μg is the first choice for the measurement of vitamin D in Europe. The value should be multiplied by 40 to convert μg to IU.

The clinical manifestations of VDT can vary a lot but are primarily related to hypercalcemia [26, 27]. Symptoms of VDT are manifested in the form of neuropsychiatric abnormalities like difficulty in concentration, confusion, drowsiness, apathy, depression, psychosis, or in extreme cases, a stupor or even coma. All these symptoms are associated with the fact that vitamin D plays an important role in neurotransmission and synaptic plasticity [28, 29]. The gastrointestinal symptoms of VDT include recurrent vomiting, polydipsia, abdominal pain, anorexia, constipation, or pancreatitis. In the case of the cardiovascular system, VDT can cause hypertension, shortened QT interval, ST-segment elevation, or bradyarrhythmias with first-degree heart block on the electrocardiogram. VDT also impedes renal functions and manifests symptoms like hypercalciuria, polydipsia, dehydration, polyuria, nephrocalcinosis, or renal failure.

SOURCES OF VITAMIN D AND ITS DAILY REQUIREMENT

According to Russian recommendations [24], children and adults should consume at least 10 μg of vitamin D per day, whereas for elderly (>60 years old) this level is ≥15 μg/day. In the UK 15 μg/day has been recommended for all age groups [30].

The US Institute of Medicine has developed the Recommended Dietary Allowance (RDA) for vitamin D for different age groups [1]: <1 year – 10 μg; 1–70 years – 15 μg; >70 years – 20 μg.

There are 3 major groups of sources of vitamin D. They are: sunlight-dependent endogenous production, nutritional sources, and supplements. The major replenishment of the vitamin occurs from the synthesis of cholecalciferol in the skin from 7-dehydrocholesterol through a chemical reaction that is dependent on sun exposure (specifically UV-B radiation). The vitamin D_3 so produced is converted to 25(OH)D (Calcidiol) in the liver with the help of several enzymes like CYP2R1, CYP27A1 (25-OHase), or sterol 27-hydroxylase (CYP27A1) [31, 32].

The calcidiol has a half-life of around 3 weeks, so it is taken as the indicator of vitamin D status. It is to be noted that the concentration of calciferol is not useful to judge the vitamin D status because its value rapidly fluctuates depending on recent exposure to vitamin D and it disappears from the circulation within the hours and reappears as calcidiol.

Finally, the 25(OH)D passes through the kidney and the enzyme 25(OH)-1α-OHase (CYP27B1) converts it into the biologically active form of vitamin D – $1,25(OH)_2D$ [31]. Vitamin D_2 (ergocalciferol) also has similar metabolism as vitamin D_3 (cholecalciferol) except the fact that vitamin D_2 can not be hydroxylated to calcidiol by sterol 27-hydroxylase (CYP27A1) [32].

Cholecalciferol and ergocalciferol can be obtained from a nutritious diet or through the intake of supplements. Only a few foods, such as the flesh of fatty fish (like salmon) and cod liver oil contain significant amounts of vitamin D [33]. Other sources like dairy products, eggs, or meat also contain vitamin D, but their vitamin D content is significantly lower than in fish. Unfortunately, even the consumption of fish does not provide enough vitamin D to achieve optimal vitamin D status without sunlight or supplements [33].

Followed by fish, mushrooms exposed to UV-radiation are the second richest source of vitamin D. But they provide the vitamin D in the form of vitamin D_2, which is supposed to be less efficacious [34]. Oliveri et al. have declared that the same amount of vitamin D_2 raises the total calcidiol level 28.6% less effectively than vitamin D_3 does [35]. At the same time, it was found that consumption of vitamin D2 only increases $25(OH)D_2$ levels but declines the total vitamin D concentrations [36]. The overall reduction of vitamin D was due to reduced serum $25(OH)D_3$ levels [37,38]. Indeed, Pinto et al. have calculated a 42% reduction of $25(OH)D_3$ after ergocalciferol supplementation [39]. Some mechanisms underlying this phenomenon have been proposed. The first one is due to competition of both forms of vitamin for 25-hydroxylase that converts ergo and cholecalciferol into 25-hydroxycalciferol. The second one is due to excessive degradation of 25(OH)D3 [40]. Next, metabolites produced by vitamin D_2 have less affinity towards vitamin D-binding protein. And it has also been surmised that this could lead to its shorter half-life [32]. Finally, the next mechanism is related to the enzyme - sterol 27-hydroxylase (CYP27A1). The enzyme can alternatively convert vitamin D_3 into calcidiol but not vitamin D_2. In this way, more of vitamin D_3 can be metabolised than vitamin D_2. The advertent action of the same enzyme can hydroxylase calciferol at position 24. This leads to the inactivation of vitamin D. For cholecalcitriol, 24-hydroxylation decreases its activity by 60% [41], whereas, for ergocalcitriol, the activity is decreased to 10 times [42]. So, the answer to the vexing question, if vegetarians and vegans can maintain the vitamin D status solely from mushrooms, probably has a negative answer.

Dietary recommendations typically calculate the required amount of vitamin D which is orally ingested, as the sun exposure in the population is variable. But recommendations for the timing of safe sun exposure are still a million-dollar question. Moreover, in winter above latitudes of 40° North or South, insufficient amounts of ultraviolet B radiation are found to pass the atmosphere. This implies that at least during winter, we have to consider vitamin D as an essential micronutrient that should be supplied via fortified food or supplements [43].

VITAMIN D INTAKE IN VEGANS, VEGETARIANS, AND OMNIVORES

All the studies are consistent with the fact that omnivores consume more vitamin D than vegetarians followed by vegans (Table 2).

Table 2. ***Intake of vitamin D in vegans, vegetarians, and omnivores***

References	Types of diet	Number of participants		Intake of vitamin D, μg/day
Elorinne et al. [45]	Vegans	22		5
	Omnivores	15		14
Davey et al. [46]	Vegans	M=770		0.88
		F=1342		0.88
	Vegetarians	M=3748		1.56
		F=12347		1.51
	Pescatarian	M=1500		2.90
		F=6931		2.78
	Omnivores	M=6951		3.39
		F=22962		3.32
Larsson et al. [48]	Vegans	M=15		3.7
		F=15		2.0
	Omnivores	M=15		7.7
		F=15		5.1
Chan et al. [49]	Vegetarians	Fair-skinned	93	8.77
	Flexitarians		34	8.80
	Non-vegetarians		88	10.07
	Vegetarians	Black-skinned	43	9.38
	Flexitarians		26	6.63
	Non-vegetarians		103	8.19
	Vegetarians	Fair-skinned from additives	94	5.69
	Flexitarians		34	5.23
	Non-vegetarians		91	5.90
	Vegetarians	Black-skinned from additives	48	6.44
	Flexitarians		29	3.72
	Non-vegetarians		104	4.51
Crowe et al. [47]	Vegans	87		0.7
	Vegetarians	417		1.2
	Pescatarian	208		2.2
	Omnivores	1359		3.1
Outila et al. [44]	Vegans	6		0.09
	Vegetarians	6		0.7
	Omnivores	16		4

The intake of vitamin D by vegans seems to widely vary from one study to another. The lowest consumption rate was found by Outila et al. (0.09 μg/day) [44], while the highest rate was found by Elorinne et al. (5 μg/day) [45]. Studies of Davey et al. [46] and Crowe et al. [47] almost agree upon each other (0.88 μg/day and 0.7 μg/day respectively), whereas Larsson et al. [48] suggest that intake in vegans is 3.7 μg/day.

Similarly, there is also a huge disparity in the consumption of vitamin D by vegetarians. Some suggest [49] it to max out at 9.4 μg/day, while another study [44] suggests it to be as low as 0.7 μg/day. Studies of Davey et al. [46] and Crowe et al. [47] are on par with each other with a slight variation of 0.36 μg/day.

Moreover, in the case of omnivores, a daily intake of up to 12 μg/day was found by Elorinne et al. [45], while it could be as low as 3.1 μg/day in another study done by Crowe et al. [47]. Similar results were obtained by Davey et al. [46] and Outila et al. [44] which yielded 3.33 μg/day and 4 μg/day respectively. For other researchers [48, 49], the values fluctuated between 4 to 10 μg/day.

VITAMIN D SUPPLY IN VEGANS, VEGETARIANS, AND OMNIVORES

Chan et al. assessed serum 25-hydroxyvitamin D concentrations and factors affecting them in vegetarians, flexitarians, and non-vegetarians. In the study, they collected data on sun exposure from 199 black and 229 non-Hispanic white adults. Eventually, they compared serum calcidiol concentration, sun exposure, dietary and supplemental vitamin D intake in the different dietary groups, and finally revealed that there was a significant difference in serum 25(OH)D between white and black subjects as fair-skinned subjects had higher calcidiol concentration by almost 25 nmol/L. For white-skinned subjects, the calcidiol concentration was around 77 nmol/L whereas, for black-skinned subjects, the figures were around 50 nmol/L [49]. In the case of several dietary groups, they were not significantly different.

In the study of Millet et al., there was no substantial difference in serum calcidiol level for the different dietary groups with an average of 34 nmol/L [50]. On the other hand, Ho-Pham et al. showed that omnivores are slightly richer in calcidiol (79 nmol/L) than vegans (65.25 nmol/L) [51]. But there was a huge discrepancy in the research of Elorinne et al. Omnivores had significantly higher vitamin D level (90 nmol/L) than vegans (54 nmol/L) [45].

Comparing the results from different authors, it can be said that vegetarians seem to have a lower vitamin D level than omnivores but higher than vegans (Table 3) [45, 49, 51, 52].

Table 3. ***Blood concentration of vitamin D in vegans, vegetarians, and omnivores***

References	Types of skin	Types of diet	Number of participants	Calcitonin concentration (nmol/L)	Percentage of subjects below the threshold
Gorbachov et al. [52]	–	Vegetarians	46	71.25	68.8
Elorinne et al. [45] (Normal level was >75 nmol/L)	–	Vegans (D_2 and D_3)	21	54	90
		Omnivores (D_2 and D_3)	18	90	22
		Vegans D_2	21	27	–
		Omnivores D_2	18	2	–
		Vegans D_3	21	31	–
		Omnivores D_3	18	90	–
Chan et al. [49] Sufficient >75 nmol/L; Insufficient 50–74.9 nmol/L; Deficient <50 nmol/L)	Fair-skinned total	Vegetarians	98	76.76	Insufficient=36.73 Deficient=12.24
		Flexitarians	35	77.25	Insufficient=37.14 Deficient=17.14
		Non-vegetarians	96	78.64	Insufficient=26.04 Deficient=17.71
	Black-skinned total	Vegetarians	52	48.65	Insufficient=21.15 Deficient=63.46
		Flexitarians	31	52.63	Insufficient=22.58 Deficient=61.29
		Non-vegetarians	116	51.51	Insufficient=27.59 Deficient=55.17
Ho-Pham et al. [51] (Deficient level was <20 ng/ml)	–	Vegans	88	65.25	27
		Omnivores	93	79	6.5
Millet et al. [50] (Lower limit was < 20 nmol/L)	–	Vegetarians	M=11	35.69	46
			F=26	34.94	34
		Non-vegetarians	M=33	33.19	29
			F=36	34.94	26

All of the studies are consistent with the fact that subjects following a vegetarian or vegan diet had a higher possibility of being below the threshold level as compared with omnivores. But it should be noted that even omnivores are likely to suffer from vitamin D deficiency because of low sunlight exposure in high latitudes and rare supplement use.

CONCLUSION

The main food source of vitamin D is oily sea fish. Since vegetarians and vegans do not consume fish, they have a significantly lower intake of vitamin D, lower serum concentrations, and a greater risk of developing its deficiency. However, it is to be noted that a small amount of cholecalciferol is supplied by dairy products or eggs in a vegetarian diet. Vegans can also get ergocalciferol from mushrooms, provided that they have been sufficiently exposed to sunlight.

Since the main source of vitamin D is still endogenous biosynthesis under the influence of UV radiation, all the people living in high latitudes with less sunlight exposure, including omnivores, have an extremely high risk of developing vitamin D deficiency. Pigmented skin prevents the cutaneal synthesis of vitamin D even in short periods of relatively high insolation in northern countries, which include the middle zone of Russia, not to mention the subarctic regions. It can be concluded that the greatest risk of vitamin D deficiency is in the dark-skinned vegans living in the far north/south.

Vitamin D supplementation, or at least screening its serum concentrations, seems to be necessary for residents of extratropical regions, regardless of the type of diet.

Conflict of interest

Authors declare no conflicts of interest.

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ВИТАМИН D И ЕГО СТАТУС СРЕДИ ВЕГЕТАРИАНЦЕВ И ВЕГАНОВ

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Витамин D – это эссенциальный гидрофобный микронутриент. Существует по меньшей мере семь различных его форм. В человеческом организме биологическую активность проявляют витамины D_3 и D_2. Помимо роли в регуляции кальциевого гомеостаза и костного метаболизма, витамин D оказывает ряд иных биологических эффектов. Так, витамин D является мощным иммуномодулятором. Недостаток витамина D приводит к развитию рахита и остеопороза. Кроме того, дефицит витамина D повышает риск сердечно-сосудистых и аутоиммунных заболеваний и даже опухолевого роста. В то же время витамин D может быть крайне токсичным в случае его избытка. Тяжелое нарушение метаболизма кальция в результате гипервитаминоза кальцитриола способно приводить к поражению нервной системы, желудочно-кишечного тракта, почек, сердечно-сосудистой системы. Основным источником витамина D_3 в организме является его эндогенный биосинтез из холестерола, инициируемый в коже ультрафиолетовым излучением. Главный пищевой источник витамина D_3 – жирная морская рыба. Гораздо меньшие количества кальциферола обнаружены в молочных продуктах, яйцах, печени. Грибы также способны под воздействием солнечного света кумулировать витамин D, однако менее активную его форму – витамин D_2. Несмотря на наличие форм витамина D в различных пищевых продуктах, компенсировать его потребность потреблением из пищи крайне затруднительно, даже для часто употребляющих рыбу людей. И хотя вегетарианцы и веганы потребляют существенно меньше витамина D и обычно имеют более низкие его сывороточные концентрации, дефициту кальциферола в высокой степени подвержено все население внетропических областей, независимо от характера питания. Разумным представляется регулярный скрининг обеспеченности витамином D жителей высоких широт, всех типов питания.

Ключевые слова: *холекальциферол, эргокальциферол, кальцидиол, 25(OH)D, кальцитриол, 1,25(OH)$_2$D, вегетарианство, веганство.*

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