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Review

The renaissance of vitamin D

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There is no doubt that vitamin D plays a crucial role in the maintenance of musculoskeletal system. But the function of this ancient molecule presumably ranges far beyond hormone-like regulation, as it could be generated by simple unicellular organisms. First, we are going to discuss the role of vitamin D as a global regulator of homeostasis from a historical perspective, but later we will focus on current views and its relevance to human physiology and pathology. Three milestones are defining the impact of vitamin D on science and humanity. Firstly, discovery that vitamin D is the cure for rickets, brought us supplementation programs and rapid irradiation of this devastating disease. Secondly, detail description of photoproduction of vitamin D, its subsequent metabolism and interaction with vitamin D receptor VDR, provided mechanistic background for future discoveries. Finally, recent large epidemiological studies provided indirect, but strong evidence that optimal level of vitamin D in serum has beneficial effects on our health and protects us from multiple diseases, including cancer. Furthermore, existence of alternative pathways of vitamin D metabolism and multiple intracellular targets broadens our understanding of its physiological activities and offers new and very promising tools for prophylactics and treatment of many diseases of civilization. Although vitamin D (and its derivatives) should not be regarded as a cure-all for every human disease, its beneficial effects on the human health have to be taken under consideration.

Key words: vitamin D, skin, vitamin D deficiency, vitamin D supplementation, vitamin D analogs

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HISTORICAL PERSPECTIVE

Vitamin D is probably one of the oldest hormone that exists on earth (Bikle, 2011). Some of the earliest life forms have the capacity to produce vitamin D_2, when exposed to the sunlight. Impressively, vitamin D_2 producing phytoplankton survived, unchanged in the Atlantic Ocean, for more than 750 million years. There are many speculations, why these organisms need such excessive amounts of vitamin D_2. One of the most probable is that ergosterol may acted as natural "sunscreen" protecting cells against an ultraviolet (UVA and UVB) radiation (Holick, 1989; Holick, 2003).

The modern history of vitamin D in human health began during Industrial Revolution, in the 17th century. City lifestyle and growing air pollution decreased an access to the sun resulting in massive outburst of rickets. In that time, the disease was recognized as a major health problem among children. Severe growth retardation, widening of the ends of the long bones, and bowing and bending of the legs are only a few clinical signs of rickets (Holick, 1994).

The importance of exposure to the sunlight and its association with the prevention and cure of this bone deforming disease in children was first recognized by Polish physician Jędrzej Śniadecki, in 1822. In his observations Śniadecki concluded that children living in the polluted, sunless centre of Warsaw (Poland), more often suffered from rickets than compared to children living in the sunny rural areas. He hypothesized, that lack of sufficient sun exposure was responsible for the development of rickets among children. What is more, he claimed that direct exposure to sunlight might be one of the most efficient methods to prevent and cure rickets. However, in the nineteenth century his studies were considered as incomprehensible and remained largely unnoticed. Of note, it was great Polish biochemist, Professor Włodzimierz Mozolowski, who brought back Śniadecki`s observations to the scientific community (Mozolowski, 1939).

Another scientist, who predicted the relationship between exposure to the sunlight and occurrence of rickets, was a British epidemiologist, Theobald Palm. In 1890, he combined notes from his travels with the opinions of colleagues and postulated that there is a negative dependence between latitude and occurrence of rickets. In his observations, he firmly highlighted that despite the poverty, in sunny, tropical areas there were significantly reduced number of rickets cases, than compared to "rich" urban countries. Similarly to Śniadecki, Palm was a supporter of the beneficial effects of the sun to bone health. He also strongly encouraged moving infants and children afflicted with rickets from large towns to sunnier rural areas (Palm, 1890). Once again, the observations and benefits arising from Palm's findings remained unnoticed. It had taken another 30 years before significance of Śniadecki's and Palm's observations was explained.

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Abbreviations: 1α,25(OH)$_2$D$_3$, calcitriol (1α,25-dihydroxyvitamin D$_3$); 20-OH D$_3$, 20-hydroxyvitamin D$_3$; 25-OH D$_3$, calcifediol (25-hydroxyvitamin D$_3$); 7-DHC, 7-dehydrocholesterol (provitamin D$_3$, cholesta-5,7-dien-3β-ol); 7-DHP, 7-dehydropregnenolone (pregna-5,7-dien-3β-ol); CYP11A1, cytochrome P450scc; CYP24A1 or 24OHase, 24-hydroxylase; CYP27A1 or 25OHase, 25-hydroxylase; CYP2R1 or 25OHase — 25-hydroxylase; CYP27B1 or 1αOHase, 1α-hydroxylase; DBP, vitamin D-binding protein; IP$_3$, inositol trisphosphate; L$_3$, lumisterol 3 (9β,10α-cholesta-5,7-diene-3β-ol); MARRS receptor, Membrane-Associated Rapid Response to Steroid binding protein (other names: ERp57, GRp58, Pdia3); MED, minimal erythemal dose; RORα/γ, retinoic acid-related orphan receptors α and γ; RXR, retinoid X receptor; T$_3$, tachysterol or 3 (*6E*-9,10-secocholesta-5(10),6,8-trien-3β-ol); UVA/B, ultraviolet radiation A and B; VDR, vitamin D receptor, VDRE, vitamin D response elements

In 1919, Huldschinsky showed that exposure to ultraviolet radiation from a mercury arc lamp resulted in regression of severe rickets. Furthermore, he demonstrated that the therapeutic effect of ultraviolet radiation was not limited to irradiate place, but had an equal effect on whole organism. For instance, the exposure to mercury arc lamp of just one arm resulted in the cure of rickets in both arms (Huldschinsky, 1919; Huldschinsky, 1928).

Two years after Huldschinsky findings, Hess and Unger conducted another valuable study. They exposed children suffering from rickets on a roof of a New York City hospital to sunshine for various periods. It resulted in significant improvement of the health of rachitic children as reported by X-ray examination (Hess & Unger, 1921). The results of above-mentioned studies prompted US government to establish an agency responsible for promoting an exposure of children to the sun in order to prevent rickets (Fig. 1). What is more, in the 1930s–1950s parents could buy ultraviolet lamps in local pharmacies to protect children from developing rickets (Hess, 1936; Eliot & Park, 1938). At that time, the popularity of UV irradiation was constantly growing. In 1924, Steenbock suggested that not only children and animals could be irradiated in order to prevent rickets but also irradiate food seems to be effective (Steenbock, 1924). This led to the UV irradiation of cows, their diet, and ultimately their milk to acquire food with antirachitic properties. Thanks to the fortification of milk with vitamin D rickets was very quickly eradicated in the United States and Europe. In the 1930s and 1940s in addition to milk, many other products fortified with vitamin D like bread, hot dogs, Twang soda, and even Schlitz beer were popular. Unfortunately, potential excessive consumption of vitamin D was blamed for the outbreak of hypercalcemia in Great Britain and US, in the 1940s. This resulted in tightening of the regulations concerning fortification of dairy products with vitamin D in Europe (Holick, 2006b). Interestingly, it seems now that the outburst of hypercalcemia may not be attributed to an excessive supplementation, but also could be explained by coexistence of relatively rear diseases, including: Williams' syndrome (Wacker & Holick, 2013), primary hyperparathyroidism (Michels & Kelly, 2013) or even sarcoidosis (Nunes *et al.*, 2007). It could be also caused by specific mutation of CYP24A1 coding the main catabolic enzyme for vitamin D (Jacobs, 2014), resulting in abnormally high level of vitamin D in serum.

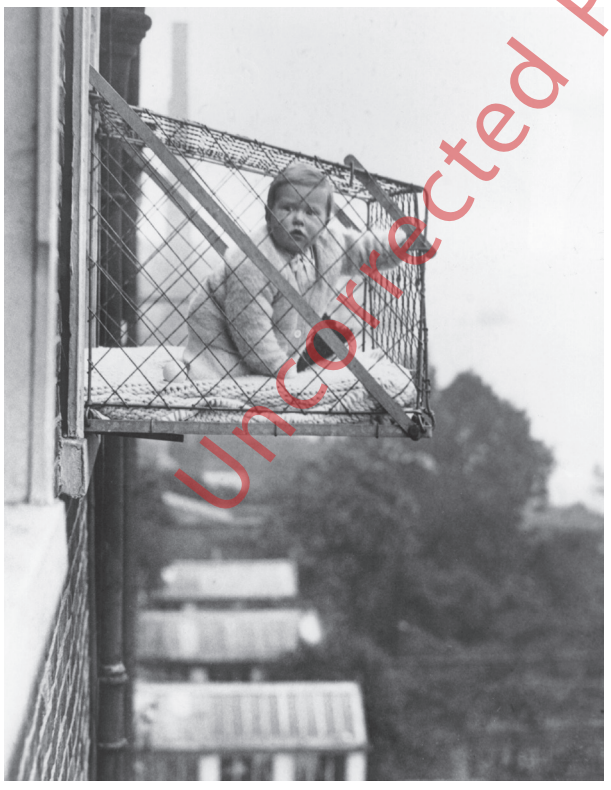

Figure 1. An example of the wire cage fixed to the tenement window, so that babies can benefit from sunshine. London, 1934.

Simultaneously to ongoing studies on the effect of the sun on bone health, another potential antirachitic agent was found — a cod liver. The effectiveness of cod liver oil in prevention of the disease has been particularly valued on the coastlines of the Scandinavian countries and the United Kingdom (Ihde, 1975). Nevertheless, Schutte was a first physician, who prescribed cod liver oil as specific agent preventing rickets in 1824. From this time to the end of the century, German and French physicians have widely recommended cod liver oil as an antirachitic agent. Nevertheless, at the beginning of the 20th century, the usefulness of cod liver oil was questioned, probably due to a poor quality or impurity of prescribed cod liver oil (Guy, 1923). The first scientific approach to prove the anti-rachitic properties of cod liver oil was made by Edward Mellanby and Elmer McCollum. In classic, animal experiments, they accredited the antirachitic function of cod liver oil to fat-soluble vitamin A or other similar substance (Mellanby, 1919). However, in 1922, McCollum demonstrated that vitamin A do not possessed previously ascribed antirachitic functions. He was aware that oxidation destroys fat-soluble vitamin A, while similarly treated cod-liver oil preserved its protective action against the development of rickets. Thus, McCollum concluded that the antirachitic substance must be a distinct one from fat-soluble vitamin A. The newly discovered antirachitic factor from cod liver oil was named vitamin D, as it was fourth in the sequence of discovery of vitamins (McCollum *et al.*, 1922).

In retrospect, the establishment of the fact that cod liver oil and sunlight were different but similar in their ability to prevent and treat rickets, was a significant advance in the study of vitamin D.

NATURALLY OCCURRING VITAMIN D ANALOGS

Although the idea of fat-soluble vitamin D as antirachitic factor became very clear at the beginning of the 20th century, the actual vitamin structure was not solved until 1932. First, Askew and co-workers succeeded in isolation of vitamin D_2 (D_2 or ergocalciferol) (Askew *et al.*, 1931). Independently, a German group led by Windaus in 1935 had isolated 7-dehydrocholesterol (Windaus, Lettre & Schenck, 1935) and two years later vitamin D_3 (Windaus & Bock, 1937). Interestingly, although Windaus was involved in discovery of vitamin D_3 and its synthesis pathway, he received Nobel Prize in Chemistry on the account of his discoveries concerning structures of sterols and their relationship with vitamins, in 1928 (Wolf, 2004). Interestingly, he passed his patent`s rights to the production of vitamin D by UV-irradiation of yeast derived ergosterol to Merck and Bayer companies. As a result, well known Vigantol is on the market since 1927 (Haas, 2007).

It was however still do not clear, whether the vitamin D is normally produced in the human body or could be found only in natural products. Henry Steen-

bock was one of the pioneers in vitamin D research, who in an addition to early experiments with food irradiation (Steenbock, 1924) was later focused on the physiological activity of this fat-soluble vitamin. After his retirement in 1955, Hector DeLuca took over his laboratory and similarly to his predecessor, vitamin D became the centre of his attention. In 1968, he isolated an active vitamin D metabolite and identified it as 25-hydroxyvitamin $D_{3\,(25\text{-}OH\ D_3)}$ (Blunt *et al.*, 1968). A few years later, he demonstrated that previously identified substance was produced in the liver (Gray *et al.*, 1971). Further collaboration of Hector DeLuca with Michael Holick resulted in numerous discoveries. They identified the major circulating form of vitamin D, 25-hydroxyvitamin D_3, (Holick *et al.*, 1972a). Another achievement of a millstone was the discovery of biologically active metabolite of vitamin D — 1α,25-dihydroxyvitamin D_3 (Holick *et al.*, 1971). This was followed by the identification of other vitamin D metabolites, including: 24,25-dihydroxyvitamin D_3, (Holick *et al.*, 1972b), 1α,24,25-trihydroxyvitamin D_3 (Holick *et al.*, 1973) and 25,26-dihydroxyvitamin D_3 (DeLuca *et al.*, 1970). Those key discoveries enable us to understand the mechanisms associated with production and metabolism of vitamin D.

THE SKIN — FINAL LINK BETWEEN SUN AND VITAMIN D

Since Sniadecki's times it was speculated that sun is essential for the product of the antirachitic factor, which was later described as vitamin D. However, it was Michael Holick, who for the first time showed effective synthesis of vitamin D in the skin subjected to ultraviolet radiation (Holick *et al.*, 1977; Holick *et al.*, 1979). His subsequent studies also confirmed experimentally, that the latitude or seasonal changes affect production of vitamin D in the skin (Webb *et al.*, 1988). Interestingly, the influence of skin pigmentation on the efficiency of vitamin D production is still under debate. In individuals with black skin phototype, the production of previtamin D_3 was found to be reduced to 20% of the white skin phototype (Fitzpatrick, 1988). More recent studies on the US population showed that mean serum concentrations of $25(OH)D_3$ were approximately 25 nmol/L less in African-Americans than in Caucasian (Looker *et al.*, 2008). Therefore, darker-skinned individuals require longer exposures to achieve the same plasma $25(OH)D_3$ concentration. However, even people with highly pigmented skin can obtain relatively high $25(OH)D_3$ concentrations in the serum, as it was observed among Gambia population (Prentice, 2008).

Interestingly, it was the production in the skin subjected to the sunlight not supplementation, that was found to be the most efficient source of vitamin D. The exposure of the skin to only 1 minimal erythemal dose (MED) of the sunlight results in production of at least 20 000 Units of vitamin D (Holick, 2008). Furthermore, skin production of vitamin D does not cause the symptoms of an overdose, because the excessive exposition to UVB light leads to its photodegradation. The main products are 5,6-transvitamin D_3, and suprasterols I and II (Webb *et al.*, 1989). Other photoproducts including 5,7,9(11)-trienes were described, recently (Chignell *et al.*, 2006; Zmijewski *et al.*, 2009).

Thus, almost 250 years after Sniadecki`s observations, it became obvious that beneficial role of the sunlight in rickets is attributed to skin production of vitamin D_3.

CLASSIC PATHWAY OF PRODUCTION AND METABOLISM OF VITAMIN D

It is well established that a biologically active form of vitamin D_3 is $1\alpha,25(OH)_2D_3$ (Fig. 2). It is produced in multistep process involving photochemical isomerisation of 7-dehydrocholesterol (cholesta-5 ,7-dien-3β-ol, 7-DHC) followed by enzymatic hydroxylation of vitamin D. Initially, under the UVB radiation, the B-ring of 7-DHC is photolysed, what leads to formation of previtamin D_3 (Holick *et al.*, 1977). The reaction takes place in keratinocytes of the base layer of the epidermis (Holick *et al.*, 1977; Slominski *et al.*, 2012c). Then, previtamin D_3 isomerizes to vitamin D_3, tachysterol$_3$ (T_3) and lumisterol$_3$ (L_3). Vitamin D_3 can be released to the circulation, where it is transported by vitamin D-binding protein (DBP) (Lehmann, 2009). Circulating vitamin D is activated by subsequent hydroxylations. First hydroxylation to 25-hydroxycholecalciferol (25-OH D_3) takes place in the liver and is carried out by mitochondrial or microsomal 25-hydroxylases (CYP2R1, CYP27A1). Next, in the kidneys 25-OH D_3 is hydroxylated by mitochondrial 1α-hydroxylase (CYP27B1) to $1\alpha,25(OH)_2D_3$ (calcitriol) — the fully active form of vitamin D_3 (Takeyama *et al.*, 1997). Additionally, a number of distinct tissues and organs, such as intestines, also have the ability to activate vitamin D by its hydroxylation (Hewison *et al.*, 2004). It should be emphasized, that the skin is the only known organ equipped with complete machinery of vitamin D_3 production and metabolism including enzymes responsible for 25- and 1α-hydroxylation as well as the vitamin D receptor (Bouillon *et al.*, 2008; Luderer & Demay, 2010). The level of 25-OH D_3 and $1\alpha,25(OH)_2D_3$

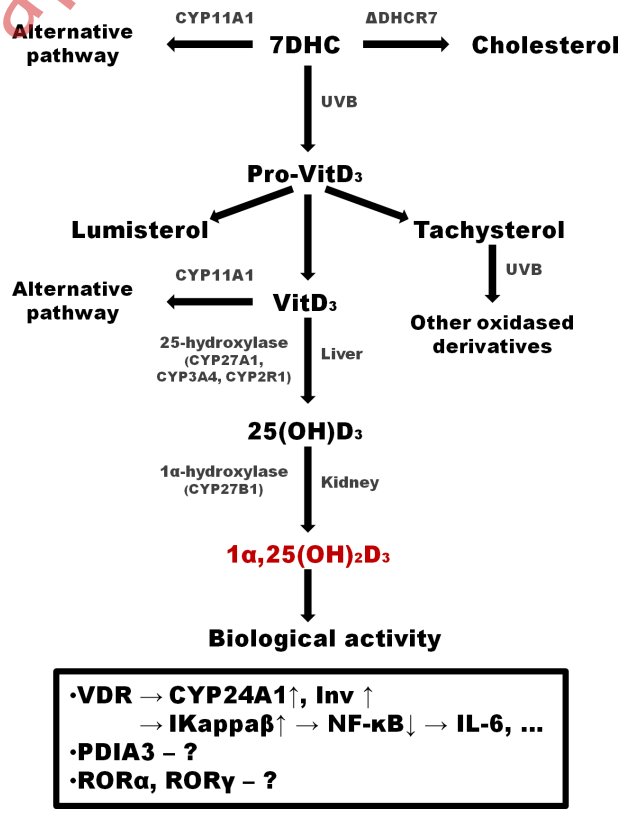

Figure 2. Scheme of Vitamin D classical and alternative metabolism.

in circulation and tissues is regulated by 24-hydroxylase (CYP24A1), which transforms them to $24,25(OH)_2D_3$ or $1\alpha,24,25(OH)_3D_3$, respectively. Further catabolism results in formation of water-soluble calcitroic acid, which is exerted in urine (Reddy & Tserng, 1989).

Vitamin D_2 is exclusively formed from ergosterol by fungi and phytoplankton subjected to UVB radiation (Holick *et al*., 1982). Some plants have also limited capacity to produce vitamin D2 (Japelt *et al*., 2013). Similarly to 7-DHC, the photolysis of ergosterol results in formation of three main products: vitamin D_2, lumisterol$_2$ and tachysterol$_2$ (Kalaras *et al*., 2012). Furthermore, acquired vitamin D_2 undergoes hydroxylation at position C25 in the liver and C1 in the kidney to produce biologically active $1,25(OH)_2D_2$ (Holick, 2003; Zhu & DeLuca, 2012). It has to be added, that vitamin D_2 is a major form of dietary vitamin D in humans, especially in Western Europe and USA (Holick, 2003; Bikle, 2011). However, the question whether vitamin D_2 is an ideal replacement for D_3 is still open to debate (Leventis & Kiely, 2009).

ALTERNATIVE PATHWAY LEADING TO NOVEL VITAMIN D ANALOGS

Although classical pathway of vitamin D synthesis and metabolism was established long time ago, new class of vitamin D derivatives was recently described. Collaboration of Andrzej Slominski and Robert Tuckey (recent reviewed Slominski *et al*., 2013b) resulted in discovery of novel metabolic pathway for 7-DHC and vitamin D in animals (Fig. 2). The alternative route is initiated by the enzymatic action of cytochrome P450scc (CYP11A1) on 7-dehydrocholesterol (Slominski *et al*., 2004; Slominski, Kim *et al*., 2013a; Slominski *et al*., 2014). It was shown that cytochrome P450scc (CYP11A1) in analogy to the conversion of cholesterol to pregnenolone may also catalyse the transformation of 7-DHC to 7-DHP (7-dehydroprognenolone). This conversion requires hydroxylation of 7-DHC at the C22 and C20 positions, followed by cleavage of side chains resulting in formation of 7-DHP (Slominski *et al*., 2004). It seems that 7-DHP may be further modified by classical steroid metabolic enzymes (17α-hydroxylase and 17, 20-liase), leading to the formation of new steroidal 5,7-dienes with modified side chains (Slominski *et al*., 2009). All of those compounds can serve as precursors for the vitamin D-like derivatives after exposure to the UVB radiation (Zmijewski *et al*., 2008; Zmijewski *et al*., 2009; Zmijewski *et al*., 2011). Interestingly, not only 7-DHC but also ergosterol, vitamin D_3 and vitamin D_2 can be metabolise by cytochrome P450scc,what results in formation of a new class of hydroxylderivatives (Slominski *et al*., 2005b; Slominski *et al*., 2005a; Slominski *et al*., 2006; Slominski *et al*., 2013a).

The CYP11A1 was shown also to act on C20 of vitamin D analogs (e.g. vitamin D_2 and D_3) generating family of novel vitamin D hydroxyderivatives (Slominski *et al*., 2005; Tuckey *et al*., 2008). Furthermore, it was shown that major product of hydroxylation of vitamin D, namely 20-hydroxyvitamin D_3(20-OH D_3) (Guryev *et al*., 2003; Slominski *et al*., 2005b) can be hydroxylated by CYP11A1 to 20,23-dihydroxyvitamin D_3 ($20,23(OH)_2D_3$) (Slominski *et al*., 2005b; Tuckey *et al*., 2011). It has to be stress out that CYP11A1-initiated metabolism of vitamin D_3 was detected both, *in vitro* (Tuckey *et al*., 2008) and *in vivo* (Slominski *et al*., 2012a). Futhermore, several new studies showed that Cyp450scc generated vitamin D hydroxyderivatives are biologically active. Importantly, it was shown that they are less prone to induce hypercalcemia, therefore they are currently investigated as potential anti-leukemia (Slominski *et al*., 2010) and anti-melanoma factors (Slominski *et al*., 2012a). Moreover, 20-OH D_3 exhibits anti-proliferative and pro-differentiation activities in human epidermal keratinocytes, as it was shown recently (Zbytek *et al*., 2008; Slominski *et al*., 2011). Nevertheless, further research is needed in order to establish physiological role of alternative vitamin D metabolites and their potential applications in therapy (recent review: Szyszka *et al*., 2012).

INTERCELLULAR MECHANISM ACTIVATED BY VITAMIN D AND ITS ANALOGS

Final puzzle in the studies on vitamin D was to establish, how it is possible that one, relatively simple molecule expresses such a variety of biological functions. It turns out that similarly to other steroid hormones, vitamin D activates its canonical nuclear receptor. Exploration of the classical pathway began in 1969, when Haussler and Norman discovered the nuclear receptor for $1,25(OH)_2D_3$ (Haussler & Norman, 1969). Over the following years, scientists revealed expression of the vitamin D receptor (VDR) in many target tissues (Cavalier, 2009). More than twenty years later VDR expression was also shown in epidermal keratinocytes (Milde *et al*., 1991). According to well-established "genomic pathway", vitamin D exerts its biological activity by binding with nuclear receptor — VDR, which after stimulation forms a dimer with 9-cis-retinoic acid receptor — RXR (retinoid X receptor). The complex is then translocated to the nucleus and acts as a transcriptional factor by binding to a VDR-responding element (VDRE). Now, it is well established that several co-activators or co-repressors interact with VDR-RXR complex and regulates its activity (Silvagno *et al*., 2013). Initially, VDRE was discovered in the promoter region of bone-specific osteocalcin gene (Morrison *et al*, 1989). It seems that this was symptomatic for the vitamin D history. Since then, the VDR action as transcription factor was extensively investigated by both mRNA and miRNA microarrays. For example, in squamous cell carcinoma cells over 900 genes was shown to respond to $1\alpha,25(OH)_2D_3$ (Wang *et al*., 2005). It was demonstrated that $1\alpha,25(OH)_2D_3$ can regulate genes controlling extracellular matrix structure and its remodelling, cell adhesion or inducting a basal keratinocyte phenotype (Lin *et al*., 2002).

Having in mind ancient origin of vitamin D, its interaction with VDR seems to be quite recent adapted pathway regulating its activity. In fact, so-called, non-genomic mechanism of rapid vitamin D response has been described recently. This mechanism does not directly affect gene expression or require additional protein synthesis. Rapid vitamin D response was shown to modulate intracellular calcium levels, affects activity of several intracellular signalling pathways, through activation of selected phosphate kinases and phosphatases. These activities take minutes and occur in the cytoplasm of the cell rather than in the nucleus. Potential mechanism of non-genomic response involves interaction of vitamin D to $1\alpha,25(OH)_2D$ membrane–associated rapid response steroid-binding protein (1,25 D-MARRSBP), also known as the protein-disulfideisomerase-associated 3 (PDIA3) or endoplasmic reticulum stress protein 57 (ERp57) (Nemere *et al*., 2004). PDIA3 activates phospholipase C in a G protein-coupled process and results in production of inositol trisphosphate (IP_3) and diacylglycerol. These two

cellular messengers mediate the rapid release of calcium from the cellular stores (Nemere *et al.*, 2012).

Recently, new targets for vitamin D have been discovered. It was that secosteroids (such as 20-OH D_3 and $20,23(OH)_2D_3$) can act as antagonists of retinoic acid-related orphan receptors α and γ (RORα and RORγ) (Slominski *et al.*, 2014). RORα and RORγ are the members of the nuclear receptors ROR subfamily, which take part in the regulation of a number of physiological processes — affects several immune functions, metabolism, and cerebellar development (Jetten, 2009). RORs are expressed in a variety of tissues, including testis and kidneys (Jetten, 2009). Moreover, their presence was also confirmed in human skin cells (Slominski *et al.*, 2014). Crystallography provided insightful structure of the ligand binding pockets of RORs (Stehlin *et al.*, 2001). Those observations not only confirmed that RORs can function as the ligand-dependent transcription factors, but also imply that RORs might be new interesting therapeutic targets for vitamin D analogs. Especially if we take under consideration several reports indicating a potential role of RORs in osteoporosis, autoimmune diseases, asthma, cancer, and obesity (Jetten, 2009). Thus, it seems that our relatively simple model for vitamin D, shown on Fig. 2 is going to be modified in forthcoming years in order to explain complexity of vitamin D metabolism and its pleiotropic activities.

THE RENAISSANCE OF VITAMIN D_3

The classic physiologic function of vitamin D_3 is to maintain calcium and phosphorus homeostasis, ensuring proper metabolic functions of bone mineralization and neuromuscular transmission (Holick, 2006a). Until recently, vitamin D was considered as "the bone vitamin" and was used predominantly for the treatment of diseases such as rickets or osteomalacia. As a result of vitamin D discovery, rickets was successfully eradicated by proper supplementation (Holick, 2013). However, it has to be underline that vitamin D is also crucial for maintenance of nervous and cardiovascular and immunological systems, as well as plays important role in skin physiology, to name only a few. Recent 20 years brought us constantly increasing number of epidemiological studies indicating that maintenance of the optimal level of 25-OH D_3 in the serum is simply essential for our health. Amongst others, vitamin D was found to be a protecting agent against multiple types of cancer (Garland *et al.*, 2006), bacterial infections (Bikle, 2008); autoimmune (Munger *et al.*, 2006) or cardiovascular diseases (Wang *et al.*, 2008; Tukaj *et al.*, 2012). It seems that vitamin D affects at least in part all major human function at the cellular, organ and whole body levels (Fig. 3). Multiple epidemiological studies provide strong evidence that monitoring of vitamin D status is the key factor in proper supplementation. For instance, recent Polish study (Gdansk region) showed that amongst 448 volunteers only 2.5% had optimal concentration of 25-OH D_3 in serum (more than 30 ng/μL) in winter of 2012. Moreover, lack of proper supplementation and food fortification with vitamin D in Poland resulted in very low mean of 25-OH D_3 concentration (14.3 ± 6.6 ng/μL). Interestingly, only individuals with recent episodes of UVB exposure had sufficient concentration of vitamin D (Kmiec, 2014). This finding as many other, strongly suggested the necessity of proper supplementation (Webb *et al.*, 2010; Trofimiuk-Muldner *et al.*, 2012). Furthermore, routinely suggested 400–800 Units of vitamin D, per day, may not be sufficient for efficient supplementation. Thus, higher doses up to 2000 U are recommended according to recently published guidance for Central Europe (Pludowski, 2013). This detail recommendation concerning supplementation for different groups was published, recently in English (Pludowski *et al.*, 2013a) and in Polish as well (Pludowski *et al.*, 2013b). It has to be also mentioned that monitoring of 25-OH D_3 concentration in the serum is essential for optimal supplementation. It is especially important for several groups of individuals vulnerable to vitamin D deficiency, including pregnant woman, children, and el-

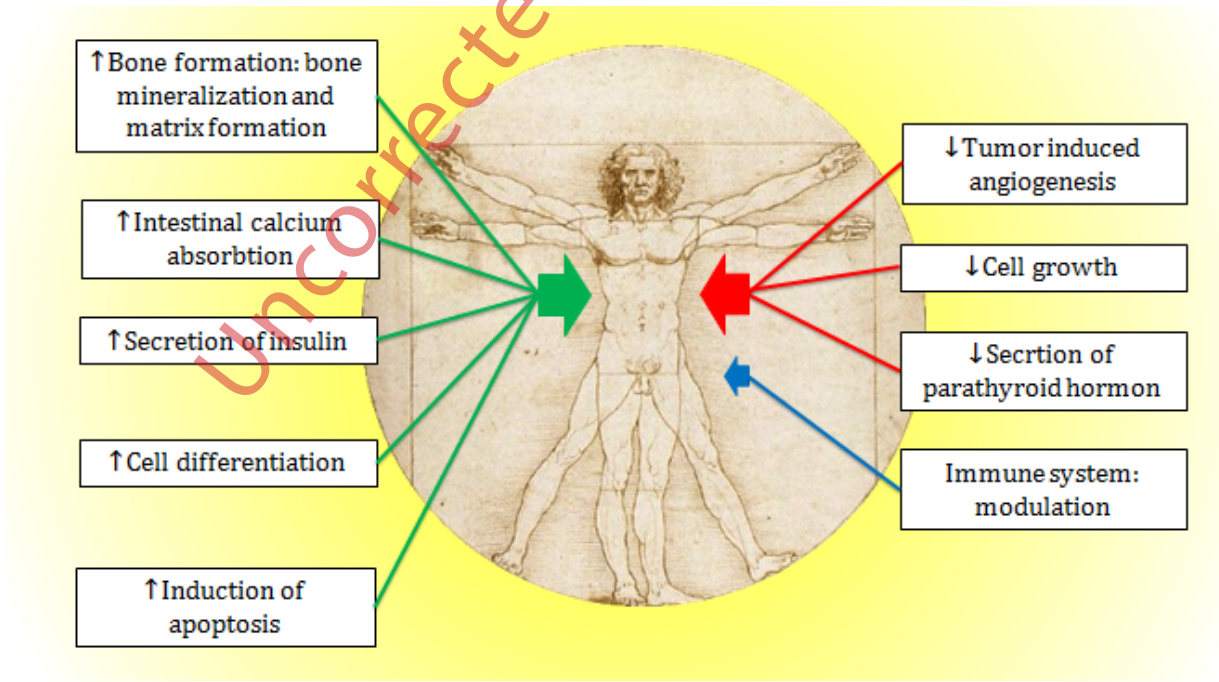

Figure 3. Overview of biological functions of vitamin D.

derly citizens (Czech-Kowalska *et al.*, 2012; Karczmarewicz *et al.*, 2013).

Since Śniadecki we know that sunlight has beneficial influence on our health. It was estimated that exposure to 1 minimal erythemal dose (MED) results in production of around 20 000 units of vitamin D (Holick, 2004). However, due to very high prevalence of skin cancer including melanoma, extensive exposure of the skin to the sunlight is not recommended. Nevertheless, as little as approximately 15 minutes long exposure of arms and legs in a sunny day (0.25–0.50 MED) is sufficient to generate equivalent of ~2 000–4 000 IU of vitamin D (Pludowski *et al.*, 2013). The skin as a natural source of vitamin D plays a prominent role in maintaining of optimal vitamin D serum level. Thus, balance between cancer prevention and the role of the sun-derived vitamin D has to be established. In any case, the supplementation and healthy, vitamin D reach food is always beneficial for our health.

BEYOND SUPPLEMENTATION

Pleiotropic properties of "sunny vitamin" suggest that vitamin D should not only be considered an essential supplement, but also can be used in a treatment of multiple diseases including cancer (Feldman *et al.*, 2014).

Although, the biologically active form of vitamin D_3 may be a promising anticancer agent, administration of $1\alpha,25(OH)_2D_3$ in its effective therapeutic doses (more than 50 000 units/day) in most cases may lead to development of side effects, such as hypercalcaemia and hypercalciuria. That is why a numerous laboratories worldwide are carrying out studies related to $1\alpha,25(OH)_2D_3$ analogs with little or no impact on the calcium homeostasis, but retaining its therapeutically crucial properties (Hansen *et al.*, 2000). It is worth to mentioned that novel vitamin D analogs such as 20-hydroxyvitamin D and other with shortened cholesterol side-chain were shown to be low calcemic and are intestivly tested as antymelanoma agents (see for recent reviews: Szyszka *et al.*, 2012; Slominski *et al.*, 2013b). Other approach is to compute the optimal structure for new vitamin D analogs. For instance, recent study by Kamel and Kolinski focused on assessment of the free binding energy of VDR receptor with $1\alpha,25(OH)_2D_3$ and its analogs, in order to estimate the relative binding affinity of the most potent analogue (Kamel & Kolinski, 2012). Both empirical and practical studies resulted in introduction of several vitamin D analogs into the treatment of several human diseases. The best example might be calcipotriol (Daivonex®), which is successfully used in the treatment of psoriasis (Leyssens *et al.,* 2014). Currently calcipotriol and it analogs are tested as potent antiproliferative agants on leukemia, breast cancer (Milczarek *et al.*, 2013) and colon cancer (Milczarek *et al.*, 2014).

CONCLUSIONS

In spite of more than 100 years and thousands of published articles, the overall impact of vitamin D and its derivatives on human health is not fully understood. Having in mind its ancient origin, it is obvious that vitamin D regulates multiple pathways and has an impact on human physiology and internal homeostasis. Proper activity of multiple organs depends on optimal vitamin D level, thus it should be not a surprise that vitamin D deficiency is an important factor involved in development of multiple human diseases.

Recent studies revealing existence of alternative pathways of vitamin D metabolism and activity broaden our knowledge concerning pleiotropic impact of vitamin D and its derivatives on human physiology and pathology. Furthermore, newly discovered low-calcemic vitamin D analogs such as 20-OH D_3; or intracellular targets like PDIA3 or RORs, provide new opportunities for the therapy of multiple human diseases.

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