Vitamin D, a steroid hormone, from theory to practice

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This paper is dedicated to the memory of Prof. Luboslav Stárka

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Short title: Vitamin D history

Summary

Recently deceased professor Luboslav Stárka was a world-renowned doctor who devoted his whole life to the study and therapeutic use of steroids, and was particularly interested in vitamin D. He knew from his own experience and from deep knowledge that this vitamin, one of the oldest steroids in development, must have a number of effects in addition to the undeniably positive effects on bones. He commissioned our task force to address the issues surrounding vitamin D, leading to years of studies with robust results made possible by the use of chromatography coupled with mass spectrometry (LC-MS), a so-called gold standard of measurement that is a cornerstone of recent scientific studies. This led to a whole series of scientific publications, the aim of which was to point out the possibility of using the abilities of vitamin D and thus also the gift that nature has given us.

Key words: Vitamin D, Vitamin K, immunity, neuroprotection

Introduction

For more than two decades, the so-called extraosseous effects of vitamin D have been intensively studied as a factor affecting a whole range of neurological, immunological and endocrinological problems. This fact divided the professional public into supporters and opponents of the extraosseous effects of vitamin D (and its active metabolite calcitriol 1,25-dihydroxyvitamin D). Some inconsistent and contradictory study results, the lack of record-based clinical trials and inconsistent measurements of the storage form of vitamin D, calcidiol (25-OH D) [1] led some doctors to see the vitamin D boom as a fad or a marketing strategy by pharmaceutical manufacturers.

Skeletal effects of vitamin D

The undeniable effects of vitamin D on bone were and are recognized without reservation, and in this sense the education that led the general public to spend more time in healthy air and avoid the sun less was also positively received. Of course, dermatologists rightly continue to warn against excessive sun exposure. However, it is also necessary to realize that the substrate (7-dehydrocholesterol) from which vitamin D (cholecalciferol) is formed decreases with age. From the age of 60, the production of vitamin D is completely insufficient, and geographical location of the Czech Republic in Central Europe also contributes to deficiencies, as vitamin D is formed only from approximately mid-April to mid-September [2] in sunny weather, often leading to a lack of calcium needed for incorporation into osteoblasts. To prevent osteoporosis, especially in women, a year-round substitution (600 IU/day, in winter 1000 IU/day) of vitamin D from the age of 50 is recommended by the State Health Institute of the Czech Republic (SZÚ) and European Food Safety Authority (EFSA). Misinterpretations may also have occurred due to inconsistent reporting of weight units (mg) or in international units (IU), while the following applies: [mg = x/40, where x = amount of vitamin D in IU units]. Similar problems can also arise when assessing serum vitamin D levels, as what is being measured is no longer vitamin D, but its metabolite calcidiol (25-OH vitamin D). Thanks to the fact that calcidiol is stable for several weeks it serves as a storage substance in the body, from which calcitriol, the most well-studied active metabolite is formed until further hydroxylation at the 1-α position. In contrast, the stability of calcitriol ranges in the order of minutes. Calcidiol values are generally reported in either ng/ml of serum or in nmol/l [ng/ml = x/2.5, where x = amount of vitamin D in nmol/l].

Neuroprotective effects of vitamin D

Vitamin D may act like a neurosteroid hormone [3], and indeed meets the criteria for a neurohormone. The amount and genetic development of specific receptors for vitamin D

(VDR) and its own transport protein (VDBP) may play important roles in the polymorphism and development of some very serious diseases [4].

As a neurosteroid, calcitriol is capable of rapid non-genomic action by acting on plasma membranes, and also genomic (long-term) action by acting on nuclear receptors. Calcitriol is produced mainly in the proximal tubule of the kidneys, but also locally in a number of other tissues of the body including the brain, with activity depending on the number of receptors in individual tissues. In the mid-1990s, reports on the positive effect of vitamin D3 on brain-derived nerve growth factor (BDNF) began to multiply, first in animal experiments [5]. Over time, it became clear that BDNF is mainly synthesized in the brain, and its expression increases with physical activity and exercise [6].

BDNF promotes neuroprotection and neuroregeneration. In depression, obesity and after brain injuries, the serum level of BDNF decreases, and recently BDNF has been reported to play a critical role in the pathophysiology of depression [7]. In one study using a depression model, the administration of vitamin D improved depressive feelings [8]. This supports the negative correlation between vitamin D levels and depression that our working group under the leadership of prof. Stárka had demonstrated as early as in 2015 [9]. Lately, the role of vitamin D in mental health and depression has led to increased research interest, but controlled clinical studies are still lacking [10].

The Vitamin D council of the USA (a non-profit organization devoted to disseminating information to the public about the effects of vitamin D) has coordinated with our laboratory to study issues of young people with autism and /or ADHD. In our work we have observed beneficial effect of vitamin D on mental condition [11], but because of the nature of the studies, the results must be taken with caution. A reduction of depressive states was reported by patients and doctors after taking vitamin D only, but the lack of proper controls and issues surrounding self-reporting by the patients and their parents prohibited proper statistical evaluations. While vitamin D deficiency is undoubtedly a risk factor for autism, the mere determination of vitamin D in blood is not a sufficient marker to evaluate the vitamin D status. In cases where levels of VDBP are altered, measurements of bioavailable vitamin D may better determine vitamin D status more accurately [12].

Elevated blood amino acid homocysteine (Hcy) levels are currently considered a risk factor for vascular and coronary heart diseases, and negatively impact nervous system function. Research has testified to a negative role of Hcy in findings of significant correlations between vitamin D levels, homocysteine and BDNF in patients with ischemic stroke [13]. A double-blinded, randomized, placebo-controlled trial in 2021 found a reduction in the risk of

cardiovascular and liver diseases after receiving 1250 µg vitamin D3 (60,000 IU) per week for 2 months [14].

The beneficial effects of vitamin D supplementation for people over 65 years of age and dosage recommendations are summarized in the Journal of the American Geriatrics Society [15]. Reductions of depressive symptoms after the use of vitamin D (most often 2000 IU /day) were described in 2022 by several dozen reviews [16]. Outdoor activity in fresh air and vitamin D supplementation has been demonstrated to yield positive results, including lowered Hcy, and increases in serotonin and neurosteroids positively modulating $GABA_A$-receptors [17].

Immunoprotective effects of vitamin D

One of the first reports of the antimicrobial effects of vitamin D in the treatment of *Mycobacterium tuberculosis* (MT) in 1949 suggests there is an association between low levels of vitamin D and tuberculosis infections [18]. In response to MT infections, the importance of the vitamin D induction of cathelicidin and its activation by Toll-like receptor signaling has been demonstrated [19]. In addition to other factors, a general susceptibility to microbial infections is dependent on the production or substitution of vitamin D. Toll-like receptor activation of human macrophages was shown to up-regulate the expression of the vitamin D receptor and vitamin D-1-hydroxylase genes, leading to induction of the antimicrobial peptide cathelicidin and the killing of intracellular MT [20]. The latest review of the role of vitamin D concerning prophylaxis and treatment in tuberculosis patients [21] confirmed the expression of pro-inflammatory and anti-inflammatory cytokines (a reduction of pro-inflammatory cytokines TNFα and IL-17 and increases of anti-inflammatory IL-10 and TGF-β) and thus slowing down the replication of MT. Considering the low incidence of side effects even at high dosages and its low cost, it would be advisable to assess vitamin D levels both in patients with active tuberculosis and high-risk groups. Further clinical trials are needed to study vitamin D efficacy *in vivo*, eliminating confounding factors, determining dosages and finding the most suitable methods of administration.

At the beginning of 2020, when the world was exposed to the cruel effects of SARS-CoV-2 and vaccination against the virus had not yet been developed, general reports began to appear about the positive effects of vitamin D supplementation, not only against COVID-19, but also against influenza [22]. Since then, the number of positive reports on the protective effect of vitamin D has grown to several hundred. Some summary studies and meta-analyses have not demonstrated a statistically significant preventive effect of vitamin D on acquiring COVID-19. In patients with COVID-19, however, they clearly support the positive effects of vitamin D supplementation [23] on stays in intensive care units and overall reductions in mortality. Nevertheless, summary studies and meta-analyses from 2022 still do not give a

clear answer on whether vitamin D supplementation can prevent or "only" reduce the severity of COVID-19 infection [24]. Further studies leading to a recommended dosage of vitamin D, duration of use, as well as method of application for both the prevention and treatment of COVID-19 are needed.

During 2021, our laboratory conducted a study that investigated the relationship between serum vitamin D levels and COVID-19 disease. The study [2] was conducted in the midst of the COVID-19 pandemic at our healthcare facility. Briefly: blood samples were taken from probands in the winter and again in summer, during which they filled out a short questionnaire. The levels of vitamin D were determined in the collected serum samples using LC/MS-MS instrumentation. A statistical evaluation of the results of our observation study showed that a minimum calcidiol concentration of 80 nmol/l is the critical limit for the disease of COVID-19. Our and other published results have shown that for elderly patients who no longer create sufficient amounts of vitamin D even when exposed to the sun in the summer (i.e., people over 60 years of age), taking a supplement of vitamin D allows them to overcome this vitamin D deficiency and increase the serum concentrations to > 80 nmol/L [25].

New aspects of vitamin D

Concerns among the professional public about vascular calcification with long-term use of vitamin D have declined thanks to studies on the effects of vitamin K2. Vitamin D3 increases the absorption of calcium from the gut to be available for bone construction, but it is mainly vitamin K2 that induces calcium transport into the bones and prevents it from being stored in blood vessels and soft tissues. The calcification of soft tissues and blood vessels could result in reducing elasticity, increasing the risk of high blood pressure, aortic stenosis, cardiac hypertrophy, heart attack, and ischemia of the lower limbs. However, as review paper [26] summarized the evidence on the effect of vitamin K and its protection against vascular calcification. The mechanism of action of vitamin K2 consists in influencing proteins from bone cells - osteoblasts. It is necessary for the activation of osteocalcin, which integrates calcium into bones and teeth and thus prevents the accumulation of calcium in blood vessels. Vitamin K2 is synthesized by intestinal bacteria, unlike vitamin K1 (necessary for blood clotting), which the body obtains exclusively from green vegetables [27]. Works from recent years [28] have provided evidence of the insufficient production of vitamin K2 and thus the necessity of occasional supplementation. Preference should be given to vitamin K2 in the form of menaquinone MK-7, which has a long half-life (2-3 days).

Another notable recent aspect of vitamin K2 is its relationship to Alzheimer's disease. In an intensive study on the action of vitamin K2, a molecular mechanism was discovered showing

protection against apoptosis induced by β-amyloid proteins, one of the main causes of this disease [29].

Conclusion

Professor Stárka and his collaborators long sought to increase awareness of the wide range of effects of vitamin D among both professionals and the lay public. They have drawn attention to the fact that age limits the sufficient production of vitamin D, and that there are no concerns of overdosing during the time-limited use of vitamin D in the fight against infections. Their studies have demonstrated the need for outdoor activity and exposure to healthy air and sunshine, during which levels of vitamin D naturally increase. In addition, they have also contributed to knowledge on relationships between vitamin D levels and the adverse effects of mental fatigue that often lead to depression.

Studies by Dr. Stárka's group have shown the importance of measuring calcidiol levels, which should be at a minimum of 80 nmol/l. During epidemics, levels of vitamin D should be increased to at least 120 nmol/l. People over the age of 60 should take vitamin D supplements year-round, at least 1000 IU/day depending on serum levels.

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