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ORTHOPAEDIC HERITAGE

Vitamin D history part III: the "modern times"—new questions for orthopaedic practice: deficiency, cell therapy, osteomalacia, fractures, supplementation, infections

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Abstract

Purpose The nutritional basis for rickets was described between 1880 and 1915, at the same period of discovery of other "vital substances" or vitamins. In contrast, rickets could also be prevented or cured by sunshine. But as the capacity to produce vitamin D depends on exposure to ultraviolet B rays (UVB) from sunlight or artificial sources, vitamin D became one of the most frequently used "drugs" in the twentieth century to compensate for insufficient exposure to UVB of humans. Furthermore, as the understanding of vitamin D metabolism grew during the twentieth century, other concerns than rickets occurred for the orthopaedic surgeon: In recent history, deficiency is explored as being an associated factor of different bone pathologies as fracture or prosthetic infection. The aim of this review is to analyze these new data on vitamin D.

Materials and methods During the twentieth century, there were many concerns for the orthopaedic surgeon: sources and synthesis of vitamin D, regulation of the calcium deposition process for both children and adults, when vitamin D deficiency is observed, and what the best method of vitamin D supplementation is. As target genes regulated by vitamin D are not limited to those involved in mineral homeostasis, orthopedists recently discovered that vitamin D might prevent periprosthetic infection.

Results The primary source (80%) of vitamin D is dermal synthesis related to the sun. Dietary sources (20%) of vitamin D are fat fishe, beef, liver, and eggs. Vitamin D is produced industrially to be used in fortified foods and supplements. Maintenance of skeletal calcium balance is mediated through vitamin D receptors. Progenitor cells, chondrocytes, osteoblasts, and osteoclasts contain these receptors which explains the role of vitamin D in cell therapy, in the prevention of rickets and osteomalacia. Despite fortified foods, the prevalence of deficiency remains endemic in north latitudes. However, the definition of vitamin D insufficiency or deficiency remains controversial. Vitamin D has been evaluated in patients undergoing fractures and elective orthopaedic procedures Although supplementation may not be able to prevent or cure all the orthopaedic pathologies, oral supplementation is able to improve the vitamin D levels of deficient patients. These vitamin D level improvements might be associated with better functional and clinical outcomes after some surgical procedures and improvement of immunity to decrease the risk of infection in arthroplasties.

Conclusion Vitamin D deficiency is frequent and concerns millions of people in the world. It is therefore normal to find hypovitaminosis in various orthopaedic populations including trauma and arthroplasties. However, we do not know exactly if this phenomenon only reflects the general prevalence of vitamin D deficiency or has an influence on the outcome of some pathologies on specific populations at risk. After the success of treatment of rickets, it is disappointing that we are still wondering in the twenty-first century whether supplementation of a substance synthetized millions of years ago by plankton and necessary for growth of all the animals may improve (or not) clinical and functional outcomes of a simple fracture in humans.

Keywords Vitamin D and orthopaedic surgery · Cell therapy and vitamin D · Fracture healing and vitamin D · Osteomalacia · Osteoporosis · Arthroplasty infection and vitamin D · Supplementation

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Introduction

The vitamin D story thus started early in the evolution of life as an inert molecule as end product of a photochemical reaction. During the early evolution of vertebrates, it gained a second life as the substrate essential for normal calcium and bone homeostasis of terrestrial animals [1]. In the recent history of humans and owing to differences in lifestyle after the description of rickets by Glisson, two centuries were necessary to realize that rickets was a lack of an antirachitic nutrient obtained in the diet or by skin exposure to ultraviolet radiation [2]. Discovered at the beginning of the twentieth century [2] by Mellanby, McCollum, Steenbock, and Windaus, vitamin D was known to be centrally involved in the regulation of calcium and phosphorus homeostasis in higher vertebrates and was found to be an excellent strategy to prevent or cure the bone and growth problems of rickets. In the twentieth century, other beneficial health effects were also described. The "vitamin's" mode of action involves the regulation of gene expression in specific tissues; this activity is mediated by the nuclear receptor for vitamin D (VDR). When the modes of action were revealed in details, the question of extraskeletal effects of vitamin D regained attention [3–5]. Indeed, the vitamin D receptor (VDR) was found to be present in most nucleated cells and the key activating enzyme, 25-hydroxyvitamin D (25[OH]D)-1α-hydroxylase or CYP27B1, and its inactivating enzyme, 25-hydroxylase, CYP24A1, were found in at least ten different nonrenal tissues. Moreover, a very large number of genes were found to be under the direct or indirect control of vitamin D. Many of these genes and proteins were not related to calcium or phosphate handling or to bone cells but were clusters of genes involved in a wide variety of cellular functions, such as cell proliferation or differentiation, or with immune functions [5].

Therefore, as our understanding of mineral and vitamin D metabolism grew during the twentieth century, other concerns than rickets occurred for the orthopaedic surgeon: Sources and synthesis of vitamin D have many variations according to the different populations; the calcium deposition process to build strong bones is complex and under the regulation of vitamin D both for children and adults. Although rickets was eradicated as a health problem in the 1930s by the process of fortifying milk with vitamin D, it has been estimated that today more than one billion people worldwide are either vitamin D deficient or insufficient and that 50–100% of the elderly men and women in the USA and Europe are vitamin D deficient. Therefore, this deficiency impacts the orthopaedic practice for children and adults nowadays. Due to the pandemic deficiency, vitamin D became one of the most frequently used "drugs" to compensate for less exposure to UVB; however, vitamin D supplementation is not as simple as frequently believed. Target genes regulated by vitamin D are not limited to those involved in mineral homeostasis, but also include genes that are linked to highly diverse biological processes associated with the immune system, and orthopedists recently discovered that vitamin D could prevent periprosthetic infection.

Therefore, the aim of this paper was to analyze (1) why the production of vitamin D has so much variation, (2) how vitamin D affects the musculoskeletal system and calcium regulation, (3) how the modern world has created a vitamin D-deficiency pandemic, (3) the problem of vitamin D deficiency in orthopedic practice, (4) which is the best vitamin D supplementation, and (6) how a substance made by plant plankton 700 million years ago can prevent periprosthetic infection.

Production of vitamin D (sources and synthesis)

The production of vitamin D (Fig. 1) is directly linked to the diet, the sun, the skin, the liver, and the kidneys. Therefore, each of these factors have clinical implications for the orthopedist to detect the risk of vitamin D deficiency among patients. Vitamin D enters the body through dietary intake (about 20% of vitamin D_3 is assumed with diet) or is synthetized by the skin (80%) from 7-dihydrocholesterol following UVB exposure. As such, humans do not have a dietary requirement for vitamin D when sufficient sunlight is available.

The skin and the dietary intake

Look at the skin and body fat of your orthopaedic patient to evaluate the risk of hypovitaminosis

During exposure to the sun, ultraviolet radiation (290–315 nm) will be absorbed by 7-dehydrocholesterol (Fig. 2). This molecule is present in plasma membranes of epidermal keratinocytes and dermal fibroblasts. The sun's energy transforms 7-dehydrocholesterol in previtamin D_3 and in vitamin D_3. During this process, vitamin D_3 is ejected from the plasma membrane to the extracellular space. The vitamin D-binding protein present in dermal capillaries has affinity for vitamin D_3 and draws it into the circulation.

Anything that influences the amount of solar UVB photons that penetrate the skin or alters the quantity of 7-dehydrocholesterol present in the skin influences the production of vitamin D_3. The quantity of 7-dehydrocholesterol in the skin is relatively constant until 60 years of age, when it begins to decline. As elderly people have thinner skin, they have less capacity of vitamin D production [6, 7]. A 70-year-old person exposed to the same quantity of sunlight as a 25-year-old person makes around 25% of the vitamin D_3 that the 25-year-old person makes.

It is also noteworthy that skin type determines a person's effectiveness in producing vitamin D. Personal variations represent another group of influential factors affecting the

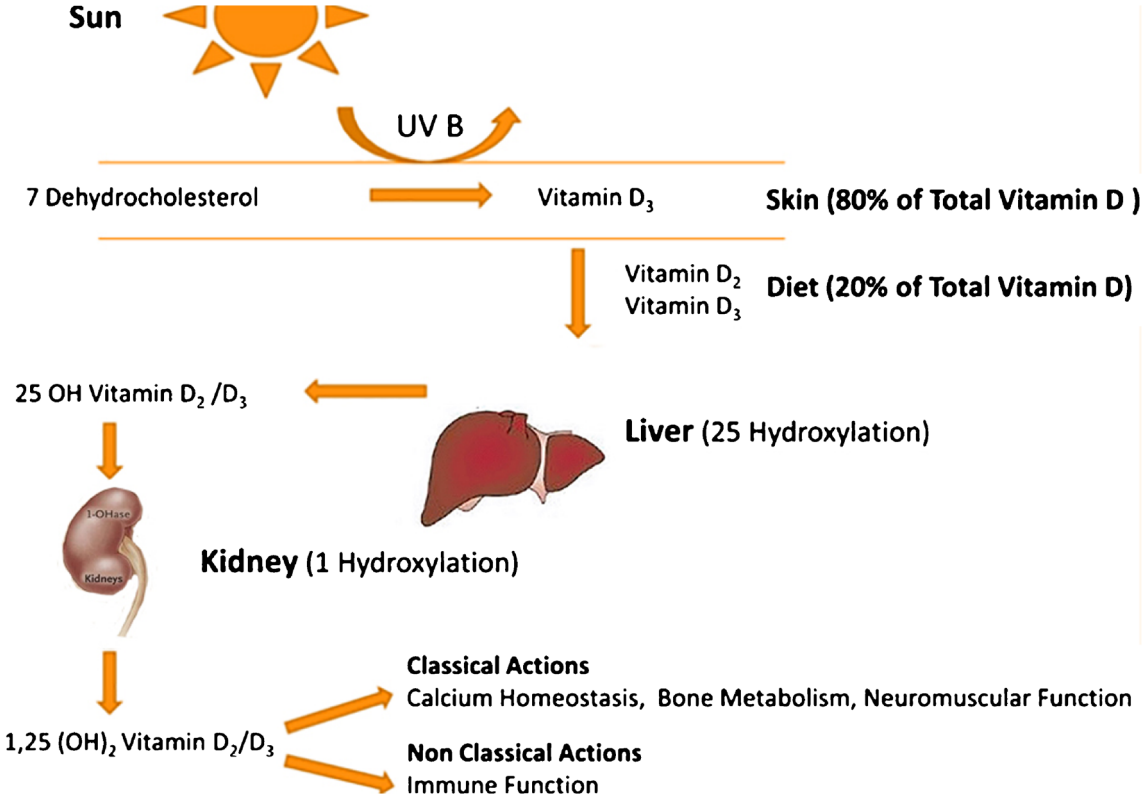

Fig. 1 Schematic representation of vitamin D production

vitamin D production in the skin, including light skin, smoking, and obesity, as overweight individuals have reduced vitamin D levels. Light skins produce up to sixfold the amount of vitamin D that is produced by dark skins. In addition, clothing habits, lifestyle, workplace (e.g., indoor versus outdoor), and sun avoidance practices have a strong impact on vitamin D synthesis. Hypovitaminosis D is prevalent in smokers and is probably due to premature skin aging: Smoking affects skin appearance in men and women, with increased wrinkling and increased elastosis decreasing the capacity of vitamin D production [8, 9]. Fat-soluble vitamin D_3 is stored in body fat. Excess vitamin D_3 that is produced can be stored in body fat and used during winter, when little vitamin D is produced in the skin. The reverse consequence for obese individuals is that the fat may be an irreversible sink for vitamin D, with risk of vitamin D deficiency.

The diet

Under these conditions where skin is not able to provide enough vitamin D, its hormone derivative calcitriol (vitamin D_2) can be considered a bona fide vitamin (Fig. 2) in that it must be supplied in the diet [10, 11]. The World Health Organization has defined the "international unit" (IU) of vitamin D_3 as the activity of 0.025 μg of the international standard preparation of crystalline vitamin D_3. But few foods contain vitamin D (Fig. 3). Vitamin D is oil soluble, which means you need to eat fat to absorb it. Some foods contain enough vitamin D: 200 IU of vitamin D per day satisfies the requirement for children. Fatty fishes, such as salmon (360 IU per 100 g serving), tuna, and mackerel, as well as beef, liver, and eggs, comprise the primary natural dietary sources of vitamin D_3. Foods high in vitamin D include also mushrooms exposed to sunlight. Although egg yolks contain vitamin D, amounts are variable (usually no more than 50 IU per yolk), and the cholesterol content of egg yolks is a poor source of vitamin D. Cod liver oil, which has been considered for three centuries to be critically important for bone health, is an excellent source of vitamin D_3. Vitamin D-fortified foods include dairy products, cereals, and juices, but very few foods are fortified with vitamin D. Fortified foods include milk (100 IU per 200 mL serving), orange juice (100 IU per 200 mL serving) and other juice products, some tofu, yogurt, and some breads and cereals. These sources are estimated to provide up to 20% of total vitamin D intake in Western populations; they can provide the total intake according to the food selected by the patient.

Role of sunlight

During exposure to the sun, 7-dehydrocholesterol of the skin is converted to previtamin D_3. PreD3 immediately is converted by a heat process to vitamin D_3. Excessive exposure to the sun degrades previtamin D_3 and vitamin D_3 in inactive photoproducts. Vitamin D_2 and vitamin D_3 obtained from dietary

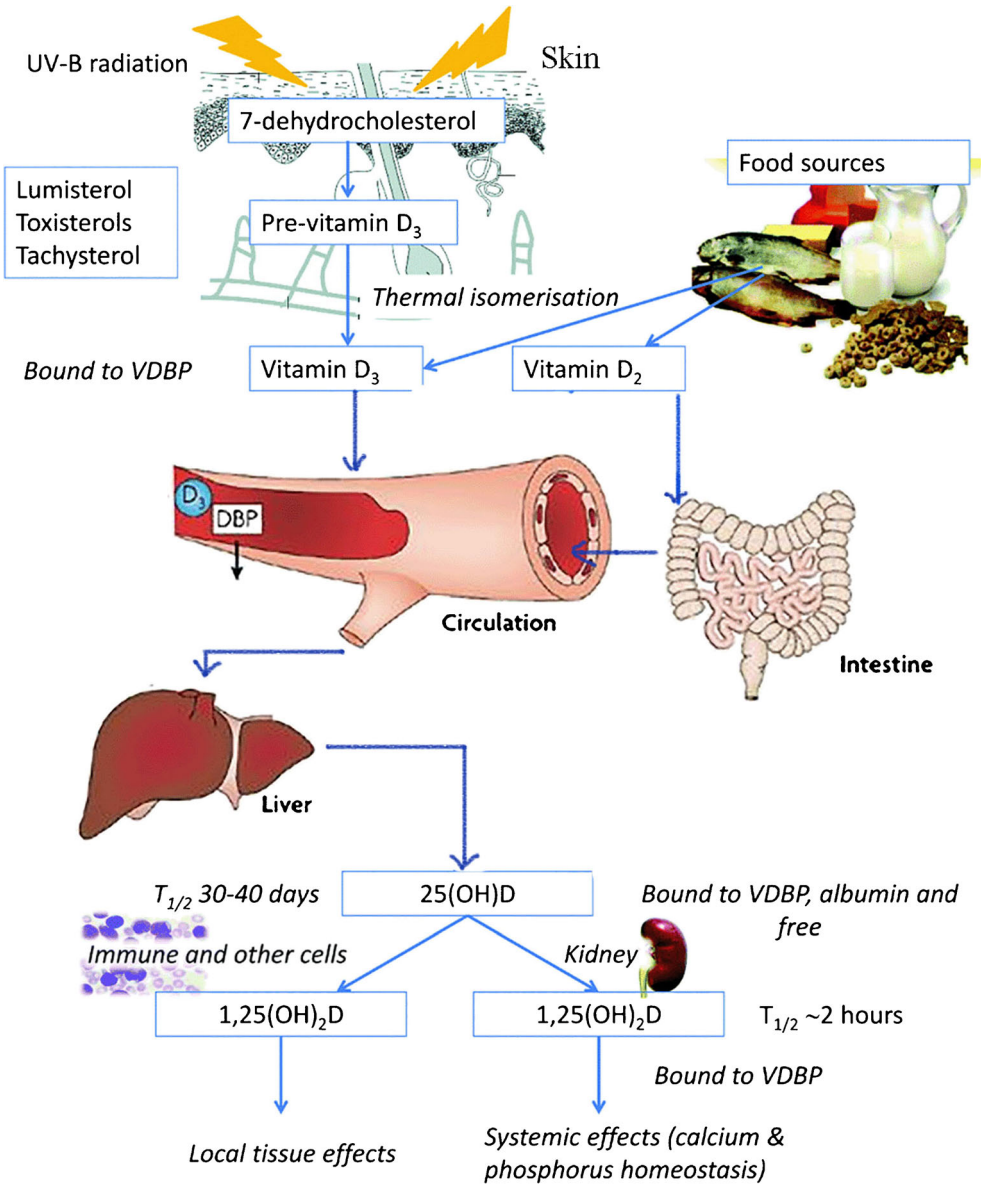

Fig. 2 Metabolism of vitamin D in circulation, liver, and kidneys

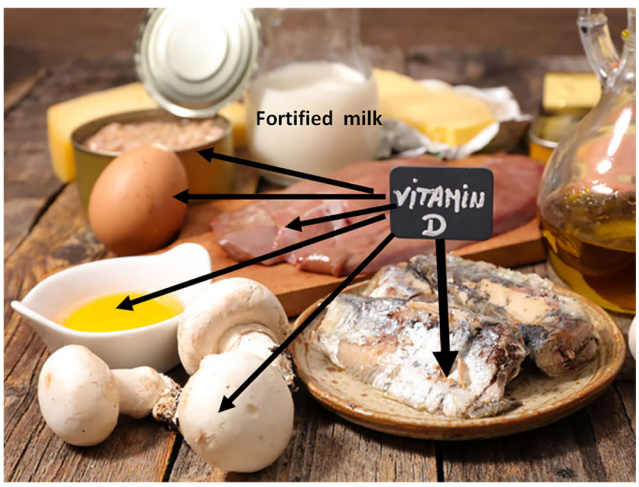

Fig. 3 Examples of food containing vitamin D

sources are incorporated in chylomicrons and are transported by the lymphatic system in the venous circulation (Fig. 2). Vitamin D (D represents D_2 or D_3) made in skin or ingested in the diet is stored in fat cells and then released from them. Prolonged sun exposure does not result in the production of excess quantities of vitamin D_3 to cause intoxication. The reason for this is that, during sun exposure, the previtamin D_3 that is formed and the thermal isomerization product vitamin D_3 that does not escape into the circulation absorb solar UV radiation and isomerize to several photoproducts that are thought to have little activity on calcium metabolism.

However, nutritional vitamin D becomes essential when sunlight is insufficient to meet daily needs. This has become particularly acute as more people reside in urban centers where they are exposed to sub-optimal levels of sunlight. In addition, clothing habits, lifestyle, workplace (e.g., indoor versus outdoor), and

sun avoidance practices have a strong impact on vitamin D synthesis. Air pollution, clothes, tall buildings, indoor dwelling, and sunscreens block ultraviolet light from the sun, and these factors all reduce the ability of the skin to synthesize vitamin D_3. Moreover, people living far north (or south) of the equator obtain little purposeful ultraviolet radiation during the winter months. The same is true for Middle-Eastern women who remain indoors or cover their entire body from sunlight. Regarding the amount of vitamin D production in human skin, it depends on several variables that include factors such as geographic latitude, time of day, season, weather conditions (cloudiness), pollution, and surface reflection which all interfere with the level of UVB radiation reaching the skin [6, 7].

Role of the liver and kidneys

Activation of vitamin D by the liver and kidneys

Vitamin D in the circulation [11–15] is bound to the vitamin D-binding protein (Fig. 2) which transports it to the liver where vitamin D is converted by the enzymes cytochrome P450 2R1 (CYP2R1) and cytochrome P450 27 (CYP27A1) to 25-hydroxyvitamin D_3 [25(OH)D3]. *This is the vitamin D circulating form that is used by clinicians to measure the vitamin D level; it is biologically inactive.* It is converted in the kidneys by the enzyme CYP27B1 to a biologically active form, 1,25-dihydroxyvitamin D [1,25(OH)2D]. The clinical implications for the orthopedic surgeon are that (1) a normal level of vitamin D in the serum does not mean that the vitamin D is active and (2) disorders of the liver and kidneys and malabsorption disorders disrupt this pathway, which may result in deficient or insufficient levels of active vitamin D. Similarly, pharmacologic agents with metabolism in the liver or kidneys such as glucocorticoids, antiretrovirals, and antiepileptics, in particular, diphenylhydantoin, increase catabolism of vitamin D and reduce serum concentrations.

Negative feedback by the kidneys

Synthesis of 1,25(OH)2D3 is strictly regulated in a renal negative feedback loop: high levels of 1,25(OH)2D3 and FGF-23 inhibit CYP27B1 and induce the cytochrome P45024A1(CYP24A1), which transforms 1,25(OH)2D3 into the inactive form 24(OH)D3 [11–15]. The clinical implication for the orthopaedic surgeon is that vitamin D intoxication by medical supplementation does not exist if the kidney function is normal.

How does vitamin D affect the musculoskeletal system?

All cells comprising the skeleton—progenitors, chondrocytes, osteoblasts, and osteoclasts—contain both the vitamin D receptor and the enzyme CYP27B1 required for producing extrarenal conversion of 25-hydroxyvitamin D (25OHD) to the biologically active metabolite of vitamin D, 1,25 dihydroxyvitamin D.

Progenitor cells

Human bone marrow stromal cells have molecular machinery [16–19] to metabolize and respond to vitamin D. Circulating 25OHD, by virtue of its local conversion to 1,25(OH)2D catalyzed by basal CYP27B1 in human mesenchymal stem cells (hMSCs), amplifies vitamin D signaling which in turn induces CYP27B1 in a feedforward mechanism to potentiate osteoblast differentiation (Fig. 4). Osteoblast differentiation in hMSCs is stimulated by both 1,25(OH)2D3 and 25OHD3; 25OHD directly acts on these osteoblast precursor cells or can be activated to 1,25(OH)2D3 in vitro. Vitamin D can also promote hMSC proliferation and migration in the directionally chondrogenic differentiation of BMSCs. This is of great importance for the application of BMSCs on the restoration of OA-induced cartilage injury. However, the vitamin D receptor in MSC showed a significant decrease with age, with as a consequence a reduction in osteogenic potential and chondrogenic differentiation with age. *The clinical implications for the orthopedic surgeon are the consequences for cell therapies.* Patients who are proposed for cell therapies [20–22] for osteonecrosis or osteoarthritis or nonunion should be checked for vitamin D deficiency and proposed for vitamin D supplementation if necessary.

Calcium regulation

Bone develops intramembranous (e.g., skull) or from cartilage (endochondral bone formation; e.g., long bones with growth plates). Intramembranous bone formation occurs when osteoprogenitor cells proliferate and produce osteoid, a type I collagen-rich matrix. The osteoprogenitor cells differentiate into osteoblasts, which then deposit calcium phosphate crystals into the matrix to produce woven bone. This bone is remodeled into mature lamellar bone. Endochondral bone formation is initiated by the differentiation of mesenchymal stem cells into chondroblasts that produce the proteoglycan-rich type II collagen matrix. These cells continue to differentiate into hypertrophic chondrocytes that shift from making type II collagen to producing type X collagen [23].

Children

In children [24–26], vitamin D deficiency causes a decrease in the efficacy of intestinal calcium absorption and results in a decrease in ionized calcium. Poorly mineralized matrix and abnormal chondrocyte maturation lead to the classic skeletal deformities of rickets including the inward or outward bowing

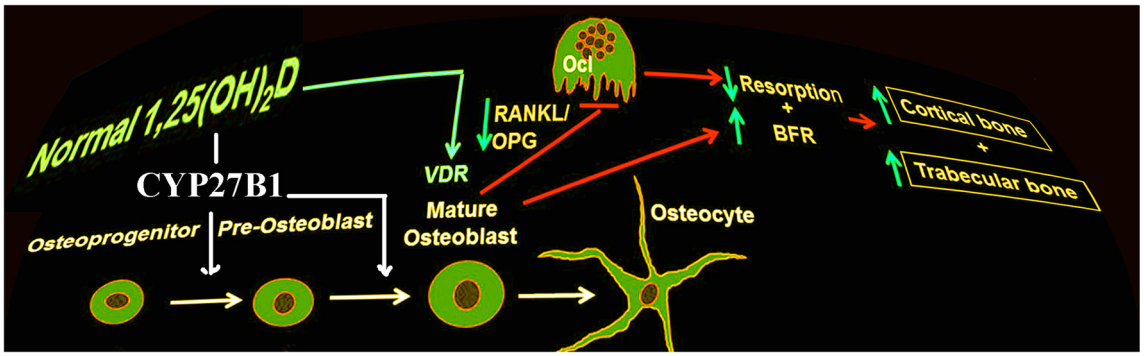

Fig. 4 Normal levels of 1,25(OH)2D act via CYP27B1 on precursors and via the vitamin D receptor (VDR) on mature osteoblasts to decrease the ratio of RANKL/OPG and reduce osteoclastic bone resorption

of the legs, widened epiphyseal plates at the end of the long bones and costochondral junctions, frontal bossing of the skull, and a delay in tooth eruption. The characteristic features of rickets are centered during the endochondral ossification, which is responsible for the longitudinal growth of long bones. In endochondral ossification, proliferating cartilage is progressively replaced by bone. The low-normal serum phosphorus concentrations with low-normal serum calcium concentrations often result in an inadequate calcium-phosphate product, which is important for the mineralization process. The growth plates or epiphyseal plates are the locations where the endochondral ossification occurs. No further longitudinal growth takes place once the growth plates are closed. Histology of the growth plate shows thickened, poorly defined architecture. There is, particularly on the metaphyseal side, a disarrangement of the growth plate with prolonged structures of uncalcified cartilage potentially extending into the metaphysis and wide osteoid seams associated with the irregular and granular junction between osteoid and mineralized bone. Terminal differentiation of the hypertrophic chondrocytes and the subsequent calcification of the matrix are markedly impaired in vitamin D deficiency (Fig. 5), leading to the flaring of the ends of the long bones and the rachitic rosary along the costochondral junctions of the ribs, classic features of rickets.

Adults

In adults, a decrease in intestinal calcium absorption and a decrease in ionized calcium are immediately recognized by the calcium sensor in the parathyroid glands [27–29], causing the parathyroid glands (Fig. 6) to increase the production and secretion of parathyroid hormone (PTH). PTH maintains serum calcium levels by increasing tubular reabsorption of calcium in the kidneys. Through its receptor on osteoblasts, PTH stimulates the formation of osteoclasts, which in turn dissolves the bone matrix and mineral to release the calcium into the extracellular space. This process, known as secondary

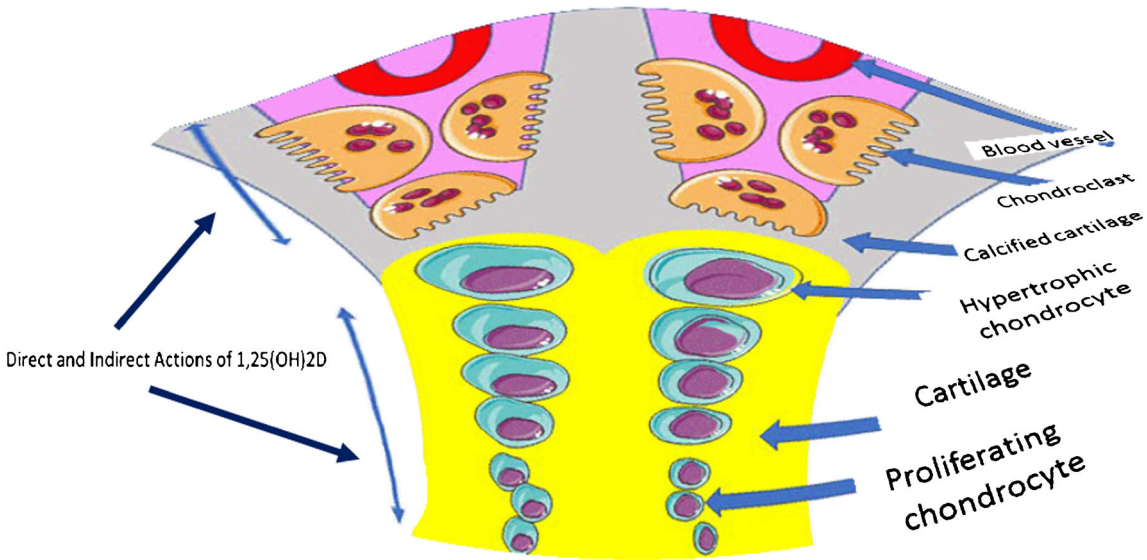

Fig. 5 Direct and indirect actions of 1,25(OH)2D on the growth plate. Reduced 1,25(OH)2D can result in decreased apoptosis of hypertrophic chondrocytes. Reduced 1,25(OH)2D can also reduce the availability of calcium and diminish calcification of cartilage

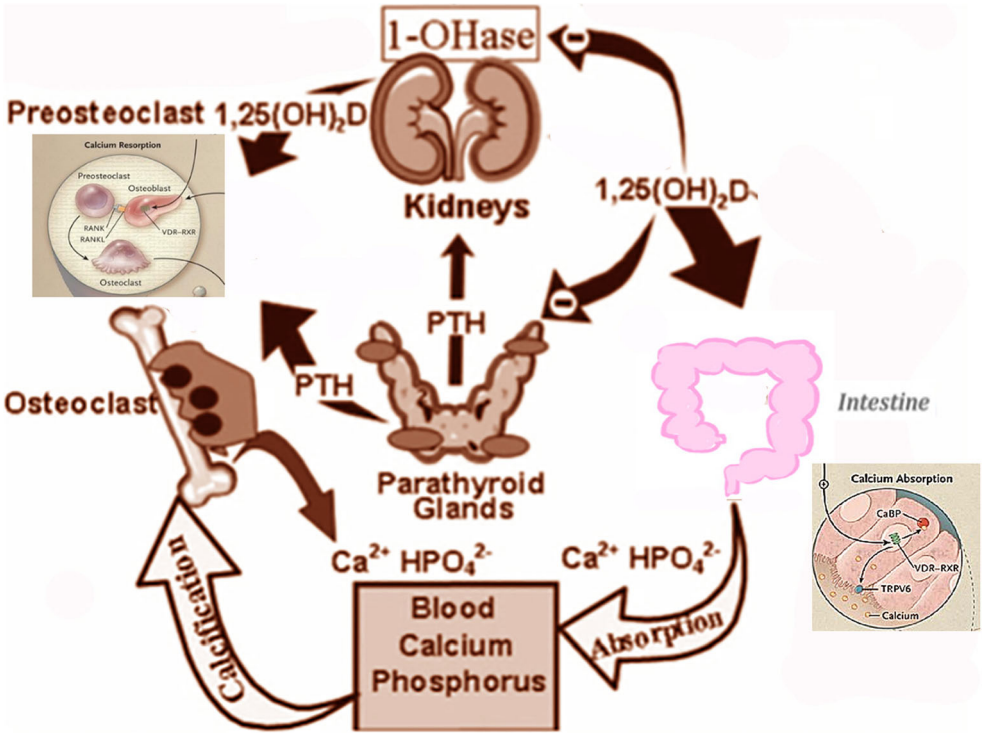

Fig. 6 Hormonal regulation of serum calcium (Ca) and phosphorus (P). A low serum calcium due to negative calcium balance can stimulate renal conversion of 25(OH)D to 1,25(OH)2D and can stimulate the release of PTH from the parathyroid glands. The increase in PTH can further stimulate 25OHD conversion to 1,25(OH)2D and can promote increased urine phosphorus which can result in decreased serum phosphate. PTH and 1,25(OH)2D can also produce bone resorption to elevate serum calcium. The increased 1,25(OH)2D can also enhance calcium absorption from the intestine and elevate serum calcium

hyperparathyroidism, has as consequence an inadequate calcium-phosphate product necessary for the mineralization of the collagen matrix leading to osteomalacia. In adults, there is enough mineral in the long bones and the epiphyseal plates are closed; thus, there are no obvious skeletal deformities. In addition, vitamin D deficiency results in a mineralization defect of the collagen produced by osteoblasts. The rubbery structure does not provide structural support and increases the risk of fracture. When the collagen matrix (not properly mineralized) becomes hydrated, this causes an expansion on the highly innervated periosteal covering. The consequence is that patients with osteomalacia complain of an aching in the bones. In addition, muscles have receptors for 1,25(OH)2D, and vitamin D deficiency also causes muscle weakness among adults. Such patients are misdiagnosed with fibromyalgia, chronic fatigue syndrome, or myositis.

Clinical implications for the orthopaedic surgeon

While rickets and osteomalacia remain clinically relevant problems, it is the role of vitamin D in the prevention/treatment of osteopenia/osteoporosis [30, 31] that commands the most clinical attention at present in developed countries. Neither a skeletal x-ray nor a bone density scan can distinguish between osteomalacia, osteopenia, and osteoporosis. They look the same, i.e., decreased bone mineral (calcium) content that can increase the risk of fractures.

However, the definition of the terms osteomalacia, osteoporosis, and osteopenia is not so evident for most of the orthopaedic surgeons: According to the new definitions decreed by the World Health Organization, osteopenia is a certain degree of lack of bone, and osteoporosis is a greater degree. Formerly, osteoporosis (Fig. 7) was a lack of bony matrix (osteoid) on which calcium hydroxyapatite could be deposited, and osteomalacia (Fig. 7) was a lack of calcium hydroxyapatite deposition on the matrix, the matrix itself being present and intact.

But osteopenia is not a diagnosis; it is a hedge word, an acknowledgment that radiographs of adults cannot distinguish osteoporosis from osteomalacia. The term "osteopenia" is being used synonymously with the diseases osteoporosis and osteomalacia. Osteopenia refers to "reduced bone mass due to a decrease in osteoid synthesis to a level insufficient to compensate normal osteolysis." It is used to describe a lower-than-average peak bone density on the radiograph alone or in the presence of the more severe disease as osteoporosis or osteomalacia.

Osteomalacia is caused by delayed or inadequate mineralization of osteoid in mature cancellous or cortical bone after epiphyseal closure. It is the adult equivalent of rickets. Osteomalacia is a diagnosis; in adults, it is generally caused by anything that interferes with vitamin D metabolism or calcium and phosphate absorption. Certain anticonvulsant medications interfere with the formation of 25-hydroxyvitamin D in the liver. Malabsorption syndromes can lead to hypophosphatemia as can familial hyperphosphatemic osteomalacia. In benign mesenchymal neoplasia, a substance is sometimes produced that interferes with renal tubular

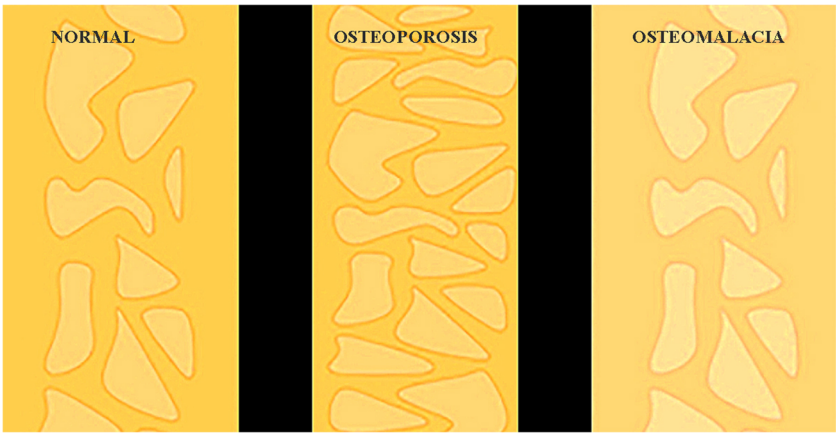

Fig. 7 Osteoporosis is thin and brittle but the composition is normal; in osteomalacia, the total volume of bone may be normal, but it is weak because it is poorly mineralized

functions, including the hydroxyglation of 25-hydroxyvitamin D. Puerperal osteomalacia can be the consequence of repeated pregnancies and lactation that exhaust calcium and phosphate stores.

For osteoporosis, causes are different from vitamin D deficiency and include diseases that result in a decrease in axial loading of bone and, therefore, a reduction in lamellar bone formation. Postmenopausal osteoporosis occurs from decreased estrogen levels, which leads to decreased cancellous bone synthesis more than cortical bone synthesis. It is also seen in men due to the gradual decreases in testosterone and estrogen levels that occur with age. Posttraumatic osteoporosis may also occur as a component of reflex sympathetic dystrophy in a limb. Other causes include hyperthyroidism, hyperparathyroidism, corticosteroid use, autoimmune disorders, and hematologic disorders.

How has the modern world created a vitamin D deficiency pandemic?

Before 1970, rickets was the only target of vitamin D

Early in the twentieth century (before 1920), more than 80% of children in industrialized Europe and North America were ravaged by the devastating skeletal consequences of rickets. In the 1920s, irradiation of yeast was able to promote antirachitic activity. Ergosterol was identified as the sterol in yeast that had antirachitic activity when irradiated; within a few years after this process of fortifying milk with vitamin D was implemented in the 1930s, rickets was eradicated as a health problem. In the early 1950s, in Great Britain, an outbreak of hypercalcemia in infants was thought to be due to the overfortification of milk with vitamin D. Although this was not proven, some hysteria about children intoxicated with vitamin D from milk prompted Great Britain to stop vitamin D fortification, and all other European countries followed. Since rickets was not commonly seen, physicians, regulatory and healthcare agencies, and the general public concluded that vitamin D deficiency was conquered. However, in Canada, when routine vitamin D fortification of milk was stopped in many dairies, cases of rickets were reported as increased in admissions to Montreal hospitals, leading to reintroduction of milk fortification with vitamin D by the federal regulation. Also, Finland and Sweden began to fortify their milk with vitamin D in the 1990s, but overall, fortification is not widely practiced in Europe [28, 29, 32].

With new targets, vitamin D thresholds are areas of controversy: What are the cutoff values for deficiency?

- *< 10 ng/mL?* For rickets, in the 1940s, 100 IU of vitamin D/day was considered sufficient to prevent overt skeletal deformities associated with rickets. Regulatory agencies in the USA and Europe later increased the dose twofold and recommended that 200 IU of vitamin D/day be required to satisfy the requirement for children. It was assumed that the same was true for adults. Since rickets is not commonly seen, physicians, regulatory and healthcare agencies, and the general public concluded that vitamin D deficiency was conquered. Clinically, when vitamin D deficiency is defined by the manifestation of bone diseases such as osteomalacia or rickets, it has been defined by a serum 25(OH)- vitamin level of less than 10 ng/mL. This was due to the fact that serum 1,25(OH)2- vitamin levels and calcium absorption drastically decrease at this level and also to the fact that rickets was not observed for a level higher than 10 ng/mL.

- *< 20 ng/mL?* However, a consensus [33–36] is lacking on how to define vitamin D insufficiency and deficiency. In the 1970s, clinical assays were developed for 25(OH)D in the serum. To determine the normal range for the assay, blood was collected from healthy adults who were presumed to be vitamin D sufficient and the mean ± 2 standard deviations was used as the normal range (10–55 ng/mL) at that time. In 1998, healthy adults with a blood level of 25(OH)D between 11 and

25 ng/mL were considered to be vitamin D sufficient. When they received 50,000 IU of vitamin D_2 weekly for 8 weeks, there was a substantial decrease in their PTH levels. On average, there was a 35% decrease in PTH levels in the adults who had blood levels less than 20 ng/mL. Thus, it was concluded that vitamin D deficiency should be defined as a 25(OH)D < 20 ng/mL. It was also observed that PTH levels began to plateau when 25(OH)D levels were between 30 and 40 ng/mL. Vitamin D deficiency is defined as a 25(OH) vitamin D level of ≤ 20 ng/mL and insufficiency as a level between 21 and 29 ng/mL. It is, however, only a marker of supply, not function, and represents sources of vitamin D including dietary and dermal synthesis. We do not know if the 25(OH)D serum levels less than 20 ng/mL, the theoretical cutoff value for vitamin D deficiency, have the same effect for child growth, risk of fracture fragility, occurrence of infection, and so on, and more we do not know the effect of supplementation when it is given.

- *< 50 nmol/L?* Recently, the practical guideline on vitamin D and bone health advocates use of target thresholds [35, 36] that are in line with the Institute of Medicine Report of 2010; the Institute of Medicine (IOM), now the National Academy of Medicine, established different cutoff values to differentiate vitamin D insufficiency and deficiency. Using a model that takes into account measures of bone health, fracture risk, and PTH levels, they suggested the following thresholds: serum 25OHD < 30 nmol/L for deficiency, between 30 and 50 nmol/L inadequate in some people and > 50 nmol/L sufficient for almost the whole population. The Endocrine Society clinical practice guideline on the evaluation, treatment, and prevention of vitamin D deficiency suggested higher thresholds for deficiency (50 nmol/L) and insufficiency (52.5–72.5 nmol/L). These thresholds are based on data looking at PTH cutoffs, intestinal calcium absorption, which are consistent with data on fracture rates although it is possible that for extraskeletal indications disease-specific targets would be preferable.

Based on the new definitions for vitamin D deficiency and insufficiency, it has been estimated that more than one billion people worldwide are either vitamin D deficient or insufficient. A multitude of studies have reported that 50–100% of the elderly men and women in the USA and Europe are vitamin D deficient. It has been estimated that more than 50 million teenagers in the USA are either vitamin D deficient or insufficient.

The clinical implication for the orthopaedic surgeon

Deficiency is present in all the areas of the world: Every country is a concern; every patient is a suspect of deficiency

There is concern that this represents a growing public health problem, characterized as pandemic in some quarters. Worldwide populations are changing, with an aging population in high-income countries, global migration patterns, and an increase in obesity and non-communicable diseases in low- to middle-income countries. People tend to reduce their sun exposure when given advice to avoid the sun to prevent risk of skin cancers. Adults work indoors; children and adolescents are spending more time indoors playing on computers instead of being outside. The use of sunscreens in Australia has resulted in a marked increase in vitamin D deficiency in children and adults. Eighty-seven percent of Australian dermatologists had a deficient blood level of 25(OH)D < 20 ng/mL at the end of the summer. Reports of vitamin D deficiency from countries as diverse as Great Britain, Austria, Germany, Finland, New Zealand, and India indicate the scope of the pandemic [37–46]. Even sunny Australia has up to 30 to 50% of vitamin D-deficient children and adults. Even where milk vitamin D fortification has been in place since many decades, as in the USA and Canada, there are still deficiencies related to latitude of residence, season, gender, age, and social conditions.

Do we need laboratory testing for all our patients: a lack of consensus and evidence?

The recent interest in vitamin D and its roles in classical processes (calcium, bone metabolism, and neuromuscular function) and nonclassical diseases (arthritis, cancer, cardiovascular, diabetes, psychiatric illness) has increased the demand for measurement of vitamin D and its metabolites. Laboratory testing to determine serum 25OHD concentrations is increasing, and concerns have been raised on the resulting health economic burden [47–50]. The cost per test is real, and with the increased frequency of testing of a large at-risk population, the overall liability could be considerable. Treatment decisions are not easy after testing. Although only a small portion of surgeons are routinely prescribing vitamin D, this proportion is higher than those testing for insufficiency. This suggests that some surgeons might believe it is more cost-effective to treat all patients rather than test. For example, if the assumption is that 75% of patients are deficient, then perhaps testing is not cost-effective and it is more cost-effective to simply treat all patients. Regardless, this practice is the minority and highlights the ongoing controversy of the significance of hypovitaminosis D and how to detect and treat this deficiency in the fracture population.

If measurement is done: which measurement?

There are well over 40 metabolites of vitamin D identified, and this could potentially result in difficulties in establishing assays for specific molecules of interest. In practice, however, the vast majority of metabolites have a very short half-life in the circulation and, thus, are currently of minimal interest and present little challenge in assay development.

- *Biologically inactive vitamin D*: The best way to determine whether a person is vitamin D (vitamin D represents vitamin D_2 and vitamin D_3) sufficient or deficient is to measure the circulating concentrations of 25(OH)D. 25(OH)D is produced in the liver and is the major circulating form of vitamin D. Its half-life is two weeks in the circulation, but it is only a measure of vitamin D status because 25-hydroxyvitamin D (25OHD) is biologically inactive.

- *Biologically active vitamin D:* 25(OH)D requires additional hydroxylation in the kidney to become active as 1,25-dihydroxyvitamin D [1,25(OH)2D], but serum concentrations of 1,25(OH)2D should never be used to determine vitamin status. The reasons are that the half-life of 1,25(OH)2D is < four hours in the circulation, and its concentrations are 1000-fold less than those of 25(OH)D. Most importantly, when somebody becomes vitamin D deficient, there is an increase in parathyroid hormone (PTH) secretion (Fig. 6), which stimulates the kidney to produce more 1,25(OH)2D. As a consequence, when a person becomes vitamin deficient, 25(OH)D concentrations decrease, but 1,25(OH)2D concentrations are in the normal range and sometimes are elevated. Therefore, 1,25(OH)2D concentrations are not very useful and can mislead physicians into thinking that patients are vitamin sufficient although they can be vitamin D deficient.

The problem of vitamin D deficiency in orthopaedic practice

The conquest of rickets was one of the most notable achievements of pediatrics after World War I [2]. The diminution of rickets began in the mid-twenties, and by 1940, rickets due to vitamin D deficiency was a matter of history. However, if the term rickets may sound like a headline in the history of medicine, in fact, deficiency of vitamin D is a current and underestimated problem for today's orthopaedic surgeons.

Children still should follow grandma's advice: "Drink up your milk, and go play outside"

Rickets remains a major health problem in many developing countries and among immigrants in developed countries [25, 26]. Affected children typically present at the age of 18 months with delayed motor development, hypotonia, and short stature, and they have knock-knees or bowed legs. The causes usually are inadequate exposure to sunlight because the children are clothed and kept indoors and has had prolonged breastfeeding without vitamin D supplementation (mother's milk has very little vitamin D as compared to cow milk). Additional dietary factors may reduce calcium and vitamin D absorption, such as vegetarian diet and high intake of phylate and fiber that are associated with reduced calcium and vitamin D absorption.

Physicians often misdiagnose (non-typical form) by only dosing calcium: Vitamin D deficiency results in decreased concentrations of calcium, which are recognized by the calcium sensor in parathyroid glands. This results in increased secretion of PTH. PTH maintains calcium by increasing reabsorption of calcium in the kidney. Vitamin D-deficient children have typically normal serum calcium concentrations. This is the reason why physicians often miss the diagnosis for vitamin deficiency, since they are monitoring calcium concentrations and not 25(OH)D concentrations. However, it is not only low serum calcium concentrations that cause rickets. Vitamin D deficiency causes secondary hyperparathyroidism and results in PTH-induced low serum phosphorus concentrations (Fig. 6) by loss of phosphorus in urine with decreased intestinal phosphorus absorption. The low phosphorus concentrations with low or normal calcium concentrations result in an inadequate "calcium-phosphate" product for the mineralization process. This causes the mineralization defect that results in rickets among children. It is necessary to dose the vitamin D for the diagnosis of rickets.

Vitamin D deficiency in fracture patients and the paradox of treatment by orthopaedic surgeons

Hypovitaminosis D is globally prevalent with an estimated one billion people being vitamin D deficient or insufficient worldwide. It is estimated that 70% of North Americans have hypovitaminosis D [36–38]. Two-thirds of geriatric and nongeriatric fracture patients have serum vitamin D levels that are too low to maintain adequate bone health. Vitamin D level has been screened in patients undergoing elective orthopedic procedures [51, 52]. The prevalence of hypovitaminosis D is high among this population: 40% of patients had a vitamin D insufficiency (< 30 ng/mL) and 10% were deficient (< 20 ng/mL). Thus, half of the population was found to have abnormally low vitamin D levels. These orthopaedic populations may only reflect the general prevalence of vitamin D deficiency; however, the orthopaedic literature [53, 54] has begun to report a beneficial relationship between vitamin D and patient outcomes.

There is little agreement among surgeons about whether fracture patients should routinely receive vitamin D as part of their initial fracture management. If it is well established that vitamin D plays an important role in bone metabolism, its role in acute fracture healing is less clear and there is limited evidence available to guide practice among orthopaedic surgeons. For fragility fracture, approximately all surgeons indicated they routinely prescribe vitamin D [55]. For non-fragility fracture patients, most of all respondents do not routinely prescribe vitamin D to younger patients. Similar controversies were found among respondents regarding the use of routine screening programs to detect hypovitaminosis D and for prescribing vitamin D to all fracture patients without

evidence of insufficiency [56–60]. The same controversy exists for prevention of fracture with vitamin D [61]. However, it is currently unknown whether postfracture vitamin D insufficiency is clinically significant for fracture healing. Since the Canadian and American general population prevalence of hypovitaminosis D has been reported as high as 65% and 77%, respectively, it is clinically plausible that recent reports of vitamin D insufficiency in fracture patients may actually represent a normal physiologic response accentuating an approximately 70% baseline prevalence of hypovitaminosis D in North Americans.

Another problem is to define the best period to test the level of vitamin D in a fracture patient? It is currently unknown whether postfracture vitamin D insufficiency is clinically significant. For example, in a prospective study of 73 tibial and femoral shaft fracture patients that presented with normal vitamin D levels at admission, Ettehad et al. [62] observed a 20% decrease in serum vitamin D concentrations after one week of injury. This decrease continued to persist, and by the end of the third week, the mean vitamin D serum level of the cohort had fallen into the insufficiency category (30 ng/mL). Since the Canadian and American general population prevalence of hypovitaminosis D has been reported as high as 65% and 77%, respectively, it is clinically plausible that recent reports of vitamin D insufficiency in fracture patients may actually represent a normal physiologic response accentuating an approximately 70% baseline prevalence of hypovitaminosis D in North Americans.

Which is the best vitamin D supplementation?

Sun, skin, and the unit definition of vitamin D

Unit definition

The World Health Organization has defined the "International Unit" of vitamin D_3 as the activity of 0.025 μg of the international standard preparation of crystalline vitamin D_3. Thus, 1 IU of vitamin D_3 is 0.025 μg, or 65 pmol. The "unit definition" of the active metabolite calcitriol was defined as equivalent in "molar terms" to that of vitamin D_3. Thus, one unit is 65 pmol of calcitriol, and, as such, the unit of calcitriol is more active than one unit of vitamin D itself.

How much production with sunlight?

Sunlight is the best source of vitamin D for humans [63–65]. The vitamin D effective radiation is described in terms of its action spectrum (i.e., the efficiency of each wavelength to synthesize vitamin D in skin) which covers a narrow spectral range (255–330 nm) with as maximum 295 nm (UVB). For example, a young adult with light skin (always burns, always tans) who is exposed to 1 minimal erythemal dose (MED) of 54 mJ/cm^2 exhibits a 50-fold increase in blood concentrations of vitamin D_3 within eight hours. A total body exposure to UVB radiation inducing a light pink color for 15–20 minutes is able to produce up to 250 μg vitamin D (10,000 IU). Dermal synthesis of vitamin D_3 decreases with increasing latitude, increasing age, use of sunscreen, and darker skin pigmentation. Dark-skinned populations in temperate climates have been found to demonstrate low levels of vitamin D. Melanin is an effective natural sunscreen. Because it absorbs UVB photons, people with increased melanin pigmentation require longer exposures to sun to make the same quantity of vitamin D_3, compared with light-skinned people. For example, an African American adult (with skin that never burns) who is exposed to 54 mJ/cm^2 does not exhibit any significant increase in circulating concentrations of vitamin D_3. This adult requires five to ten times the exposure and exhibited only a 30-fold increase in the blood concentration of vitamin D_3.

Exposure of a body in a bathing suit to one minimal erythemal dose (MED; i.e., slight redness of the skin) is equivalent to taking between 10,000 and 25,000 IU of vitamin D orally. However, adequate sunlight exposure may not be feasible or may be difficult to obtain at certain latitudes in the winter months. However, exposure of hands, face, arms, and legs to sunlight two to three times a week is more than adequate to satisfy the body's vitamin D requirement and enough to store some vitamin D_3 in the body fat.

Why hypovitaminosis in sun countries?

It is easy to understand hypovitaminosis in norther latitudes: Time of day, season, and latitude influence the production of vitamin D_3 [64–66]. In the winter, the sun's rays are entering at an oblique angle (zenith angle) and more UVB photons are absorbed by the ozone layer and do not reach earth (Fig. 8); the UVB photons pass through the ozone on a greater distance due to the more oblique angle. In addition, with a more oblique angle, there are few photons per unit area striking the earth. Above 37° latitude (Fig. 9) during the months of November through February, there are marked decreases (80 to 100%, depending on latitude) in the number of UVB photons reaching the earth's surface. Therefore, very little vitamin D_3 is produced in the skin during the winter.

In sun countries, more vitamin D_3 synthesis occurs in the skin throughout the year. But below 37° and closer to the equator, it is difficult to remain in the sunlight around noon; most people are inside at this time or use sunscreens which reduces the capacity of the skin to produce vitamin D_3 by 90%. But when people are outside, in the early morning or late afternoon, the zenith angle is so oblique (Fig. 8) that very

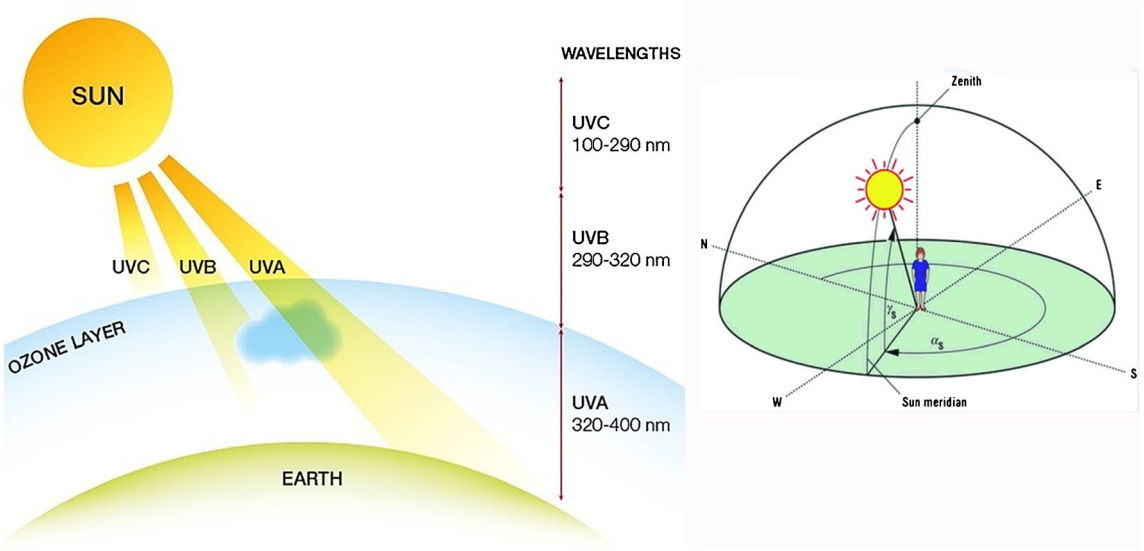

Fig. 8 Influence of the sun ray's obliquity angle (zenith angle) on absorption of ultraviolet by the ozone layer. Due to the presence of pollutants, water vapor, and (dust) particles in the lower layers of the local (city) atmosphere, most of the UVB light gets reflected back into sky and thus cannot easily reach the ground when the sun is at low positions in the sky, i.e., morning and evening. Hence, it strikes out our traditional assumption that standing in morning or evening sunlight (less intense or tolerable) for 15–20 minutes or so is enough to satisfy the body's vitamin D demand

little if any vitamin D_3 is produced in the skin even in the summer. This is why it is important to have safe sun exposure (short time) between the hours of 10:00 and 15:00 in the spring, summer, and autumn, because this is the only time when enough UVB photons reach the earth's surface to produce vitamin D_3 in the skin; this explains also why hypovitaminosis is so frequent in areas such as Australia.

How much oral supplementation for sunlight compensation?

The vitamin D requirements and oral supplementation for children or adults have not been precisely defined. Historically, it was defined on the basis of the vitamin D content in a teaspoon of fish oil every week, a quantity shown to

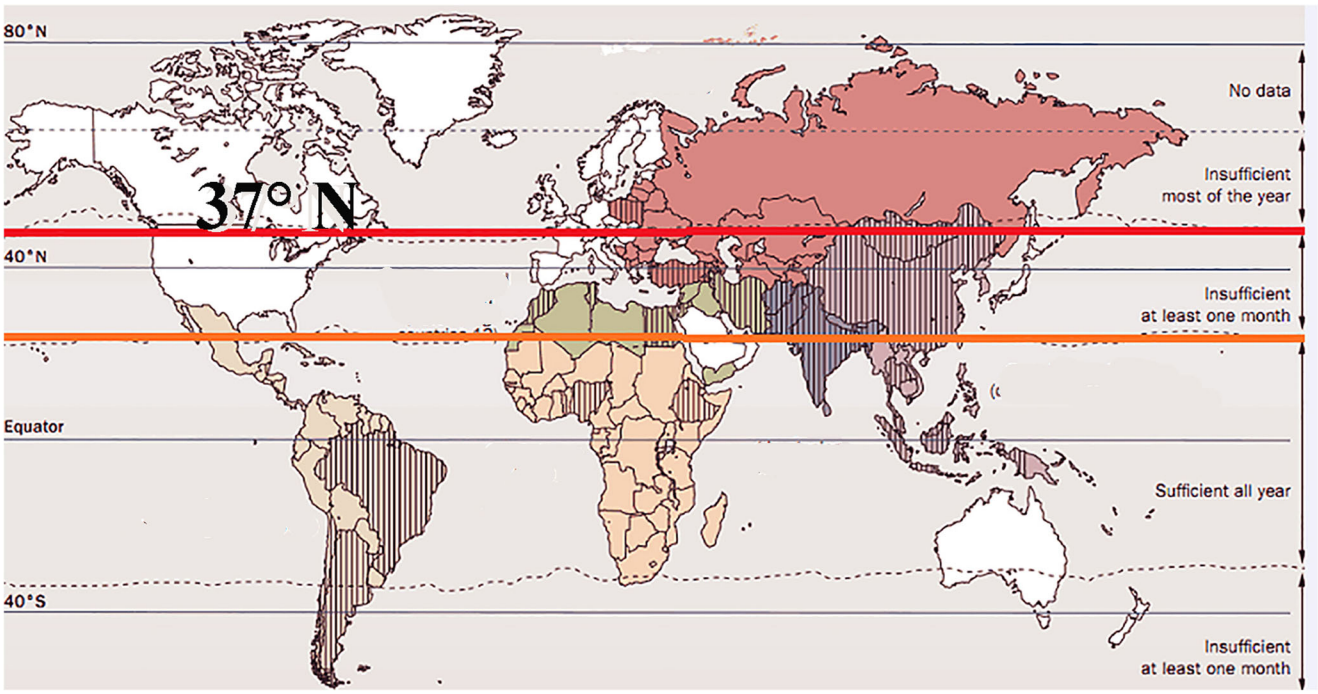

Fig. 9 Influence of latitude on the reception of ultraviolet light

be sufficient to prevent rickets. A more rigorous scientific definition is not available at this moment (Fig. 10).

In the 1940s, 100 IU of vitamin D/day was considered sufficient to prevent skeletal deformities associated with rickets. Later, regulatory agencies in the USA and Europe have increased the dose and recommended that 200 IU of vitamin D per day be required for children. It was assumed that the same was true for adults. However, 32% of healthy physicians and medical residents at a Boston hospital who took a multivitamin containing 400 IU of vitamin D per day and drank a glass of vitamin D-fortified milk per day were found to be vitamin D deficient. In Europe, few foods are fortified with vitamin D and therefore both adults and children are at high risk for vitamin deficiency.

The recommended one teaspoon (or two capsules) daily of cod liver oil (5 mL) contains 10 μg (400 IU) of vitamin D_3. This is the dose recommended by the Norwegian Health Authorities to prevent diseases like rickets, in areas with little access to vitamin D-rich fatty fish and in high-latitude countries like Norway where there is no sun-induced vitamin D production during the winter. This daily supplementation is probably more logical that another supplementation providing around 100,000 IU every three months as proposed in other countries. Serum concentrations of vitamin D_3 levels decline exponentially, with a serum half-life ranging from 36 to 78 h. The distribution of vitamin D_3 into adipose tissue prolongs its total-body half-life to approximately two months as detected on experiments on submarine personnel without sun.

There was great concern about increasing the vitamin D dose for children and adults because of potential risk of vitamin D toxicity which causes hyperphosphatemia, hypercalcemia, nephrocalcinosis, and tissue calcification, all of which can contribute to risk of death. Studies have shown that adults may take 10,000 IU of vitamin D a day for five months (Fig. 11) without altering serum calcium or urinary calcium output. Vitamin D intoxication is a very rare medical condition that may be caused by intentional or inadvertent exposure to very high amounts of vitamin D attained by ingesting more than 10,000 IU of vitamin D each day for more than six months.

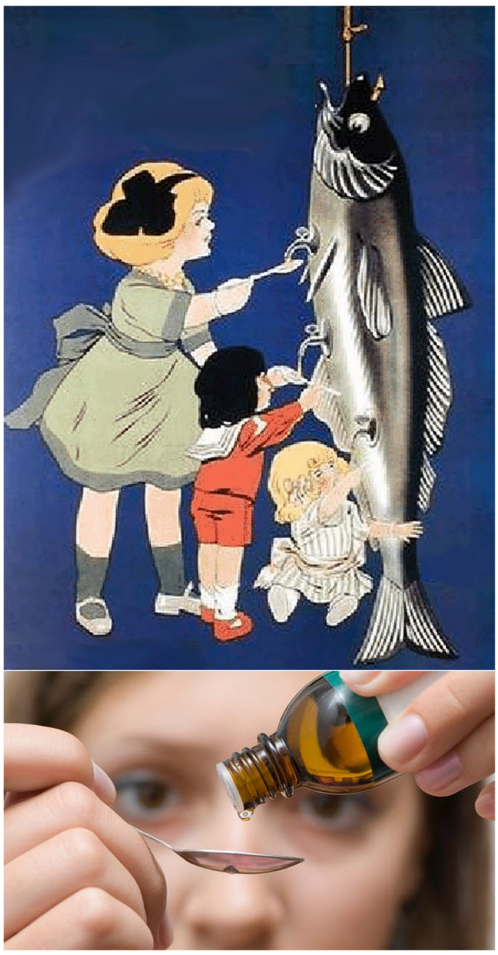

Fig. 10 It is funny to think that when supplementation is done, the "teaspoon" given for treatment of rickets remains today the best unit 100 years after the treatment of rickets and at a period when vitamin D can be dosed with the precision of the nanogram (ng)

Immunity: How vitamin D (700 million years old) can prevent prosthetic infection

Recently, our understanding of all the roles that vitamin D plays in health and disease has progressed. Long recognized for regulating calcium-phosphate homeostasis, vitamin D is now an important modulator of the immune system to infection [67]. Just as significantly, vitamin D deficiency is being

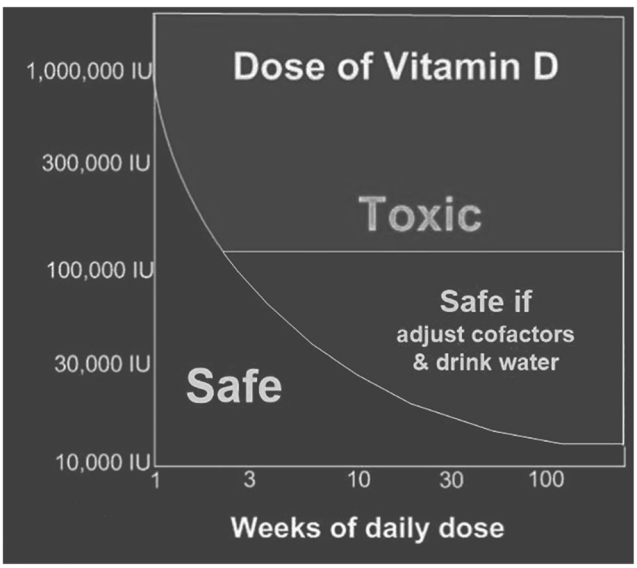

Fig. 11 How much is too much vitamin D? Very high doses of vitamin D can raise your blood calcium level, causing damage to blood vessels, heart, and kidneys. The Institute of Medicine sets the upper tolerable limit at 4000 IU of vitamin D per day. You cannot get too much vitamin D from the sun. Your body simply stops making more. But sun exposure without sunscreen can raise your risk of skin cancer

increasingly associated with a wide range of medical conditions including infectious diseases. The innate immune system is the first defense against infection; it is required to rapidly fight against invading pathogens. The innate immune system comprehends components both from the host and resident microbes (microbiota). Vitamin D is a well-known regulator of innate immunity; the first data (1949) on this topic have been generated on the treatment of diseases caused by mycobacteria, such as tuberculosis and leprosy [68]; however, the mechanisms responsible for these actions have been elucidated in more recent years (Fig. 12). 1,25(OH)2D3 enhances the production of defensin 2 and cathelicidin antimicrobial peptide (CAMP) by macrophage and monocyte keratinocytes increasing their antimicrobial activity [69–71]. Moreover, 1,25(OH)2D3 increases chemotaxis, autophagy, and phagolysosomal fusion of innate immune cells. The exposition of human monocytes to pathogens, such as *M. tuberculosis* and others, upregulates the expression of CYP27B1 and of VDR, thus enhancing the cell ability both to produce 1,25(OH)2D3 in the site of infection and to respond to this metabolite.

Maier et al. [72] demonstrated that there was a significant difference ($p < 0.001$) in serum 25D levels between patients who underwent primary total arthroplasty without infection and those who developed an infection. In addition, 64% of the patients who underwent primary arthroplasty had low levels of 25D. Taken together, these recent findings highlight the potentially important role of 25D repletion in the prevention of infection (Fig. 13). Hegde and colleagues [73] investigated 25D3 supplementation in a mouse model of joint infection. Their data reveal that intraperitoneal supplementation with 25D3 may have a prophylactic role in arthroplasty. The epidemiological observations may be directly linked to the well-established fact that vitamin D is necessary for normal macrophage activity and inflammatory responses.

25-Hydroxyvitamin D3 (25D3) holds promise as a risk modifier for two important reasons. First, epidemiologic data demonstrate that > 65% of patients undergoing arthroplasty have an insufficient or low level of total 25-hydroxyvitamin D (25D; accounts for both 25D2 and 25D3 in the serum). Second, recent epidemiologic work

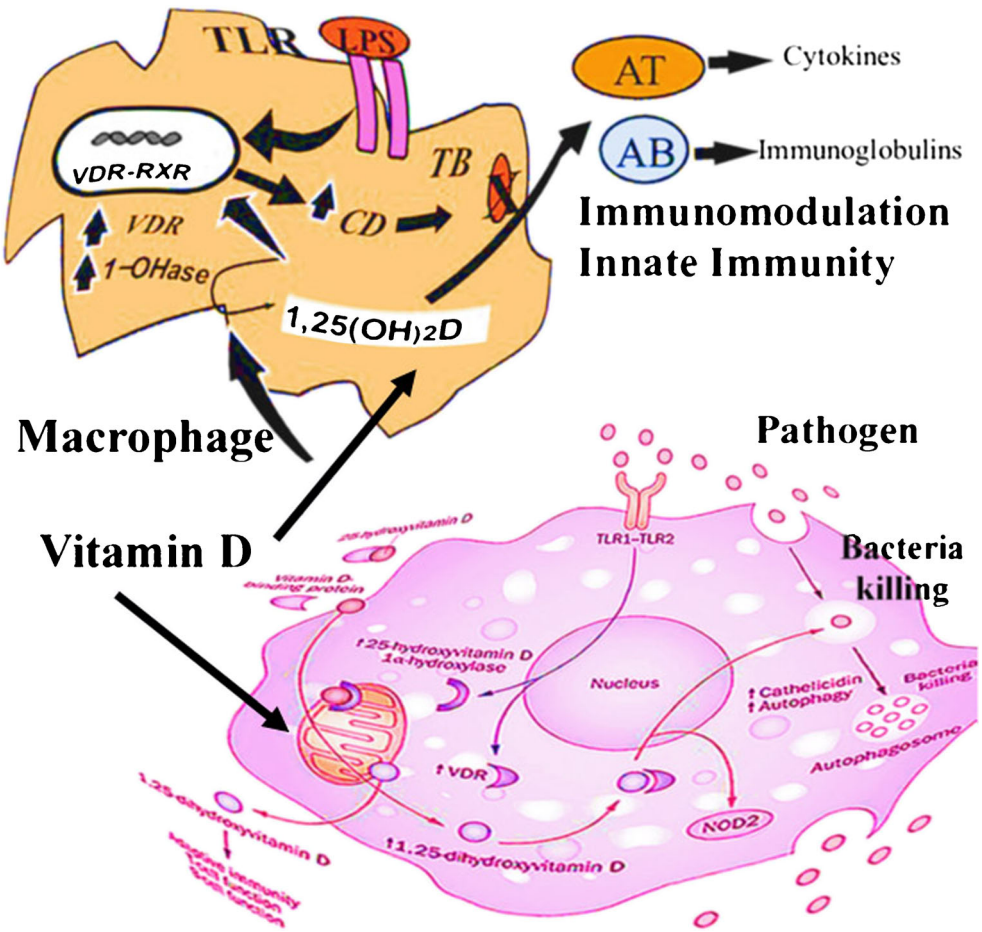

Fig. 12 Mechanisms of action of vitamin D to prevent infection

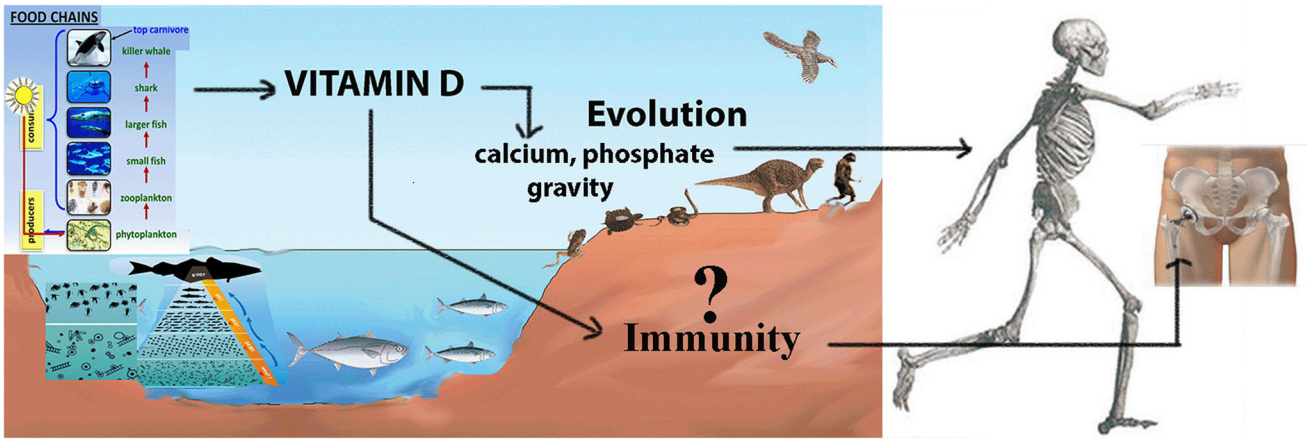

Fig. 13 Vitamin D is a substance synthetized millions years ago but became necessary for regulation of calcium when animals moved from sea to earth; the immunity function might be more ancestral

has suggested that vitamin D deficiency is directly correlated with the frequency of infection.

Conclusion

The role of vitamin D on bone metabolism is well established. It is remarkable that humans evolved in a manner whereby they depended on sunlight for an essential hormone that not only was responsible for guaranteeing skeletal health but also played a role in a large variety of other organ systems. It is truly amazing that in the twenty-first century with all of the advances of modern medicine, vitamin D deficiency has made a resurgence not only in breastfed infants but also in young, middle-aged, and older adults. Vitamin D deficiency and its consequences are extremely subtle, but may have enormous implications for human health and disease. The actual prevalence of hypovitaminosis D in the world is well established (more than 1 billion people) particularly in some geographic areas and in some specific populations. It is therefore normal to find hypovitaminosis in various orthopaedic populations including trauma and arthroplasties. These orthopaedic populations may only reflect the general prevalence of vitamin D deficiency but may also represent specific populations at risk. After the success of treatment of rickets by osteotomies and later with vitamin D, it is disappointing that we are still wondering in the twenty-first century whether supplementation of a substance synthetized millions years ago and necessary for growth of all the animals will be effective in preventing osteoporotic fracture or infection in arthroplasties, or whether supplementation may improve clinical and functional outcomes of fracture; it is funny to think that when supplementation is done, the "teaspoon" given for treatment of rickets remains today the best unit. Thus, it is important that orthopaedic surgeons design new studies on this knowledge, recognize patients with vitamin D insufficiency, and provide supplementation when necessary.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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