CHOLECALCIFEROL (VITAMIN D_3) – PHARMACOLOGICAL PROPERTIES, THERAPEUTIC UTILITY AND POTENTIAL NEW FIELDS OF CLINICAL APPLICATION

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Abstract. Vitamin D is a secosteroid produced in the skin from 7-dehydrocholesterol under the influence of ultraviolet irradiation. Vitamin D is also found in certain foods and is used to supplement dairy products. Both the natural form (vitamin D_3, cholecalciferol) and the plant-derived form (vitamin D_2, ergocalciferol) are present in the diet. These forms differ in that ergocalciferol contains a double bond (C_{22-23}) and an additional methyl group in the side chain. Ergocalciferol is less potent and has some pharmacokinetic disadvantages that have unambiguously outlined cholecalciferol as the optimal agent for vitamin D supplementation. Vitamin D is a prohormone that serves as a precursor to a number of biologically active metabolites. It is first hydroxylated in the liver to form 25-hydroxyvitamin D (25(OH)D). This metabolite is further converted in the kidney to a number of other forms, the best studied of which are 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) and 24,25-dihydroxyvitamin D ($24,25(OH)_2D$). Of the natural metabolites, only vitamin D and $1,25(OH)_2D$ (as calcitriol) are available for clinical use. Moreover, a number of analogs of $1,25(OH)_2D$ are being synthesized to extend the usefulness of this metabolite to a variety of nonclassic conditions. Calcipotriol, for instance, is being used to treat psoriasis, a hyperproliferative skin disorder. Doxercalciferol and paricalcitol have recently been approved for the treatment of secondary hyperparathyroidism in patients with chronic kidney disease. Other analogs are being investigated for the treatment of various disease states. The regulation of vitamin D metabolism is complex, involving calcium, phosphate, and a variety of hormones, the most important of which is PTH, which stimulates the production of $1,25(OH)_2D$ by the kidney. Despite the specific advantages of analogs/metabolites, cholecalciferol has been well appreciated as effective in diverse conditions due to primary or secondary vitamin D deficiency and moreover has superior safety profile and is less expensive. The present paper is focused on a concise outline of the clinically-validated therapeutic applications of cholecalciferol with a prospectus for further therapeutic utilities, based on its pleiotropic effects beyond the calcium homeostasis and bone health.

Key Words: Vitamin D, Cholecalciferol, Vitamin D status, Vitamin D deficiency, 25-hydroxyvitamin D

Introduction

Lately, the pharmacological properties of vitamin D have gathered much attention, especially in the last decades due to the delineation of its precise molecular pharmacodynamics. Vitamin D is a prohormone that is first hydroxylated in the liver to form 25-hydroxyvitamin D (25(OH)D), which is further converted in the kidney to a number of other forms, the best studied of which are 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) and 24,25-dihydroxyvitamin D ($24,25(OH)_2D$). Of the natural metabolites, only vitamin D and $1,25(OH)_2D$ (as calcitriol) are available for clinical use. Moreover, a number of analogs of $1,25(OH)_2D$ are being synthesized to extend the usefulness of this metabolite to a variety of non-classic conditions [1, 2].

Tightly bound to a carrier protein, the vitamin D-binding protein (DBP), Vitamin D and its metabolites circulate in the plasma, [3]. This α-globulin binds 25(OH)D and $24,25(OH)_2D$ with comparably high affinity, and vitamin D and $1,25(OH)_2D$ with lower affinity. The liver appears to be the principal

organ for clearance [4]. Excess vitamin D is stored in adipose tissue [5]. The metabolic clearance of calcitriol in humans indicates a rapid turnover, with a terminal half-life measured in hours [2]. Several of the synthetic $1,25(OH)_2D$ analogs are bound poorly by the vitamin D-binding protein and as a result, their clearance is very rapid, with a terminal half-life measured in minutes. Such analogs have little of the hypercalcemic, hypercalciuric effects of calcitriol, an important aspect of their use for the management of conditions such as psoriasis and hyperparathyroidism [2, 6].

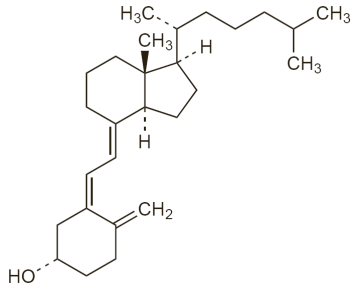
The elucidation of the exact mechanism of action of vitamin D metabolites still remains under active investigation. However, calcitriol is well established as the most potent agent with respect to stimulation of intestinal calcium and phosphate transport and bone resorption.

Calcitriol appears to act on the intestine both by induction of new protein synthesis (e.g., calcium-binding protein) and by modulation of calcium flux across the brush border and basolateral membranes by a means that does not require new protein synthesis. The molecular action of calcitriol on bone has received less attention. However, like parathyroid hormone (PTH), calcitriol can induce receptor activator of nuclear factor κB (RANKL) ligand in osteoblasts and proteins such as osteocalcin, which may regulate the mineralization process. The metabolites 25(OH)D and $24,25(OH)_2D$ are far less potent stimulators of intestinal calcium and phosphate transport or bone resorption. However, 25(OH)D appears to be more potent than $1,25(OH)_2D$ in stimulating renal reabsorption of calcium and phosphate and may be the major metabolite regulating calcium flux and contractility in muscle. Specific receptors for $1,25(OH)_2D$ exist in target tissues. However, the role and even the existence of separate receptors for 25(OH)D and $24,25(OH)_2D$ remain controversial [2].

$1,25(OH)_2D$ exerts a number of actions including regulation of parathyroid hormone secretion from the parathyroid gland, insulin secretion from the pancreas, cytokine production by macrophages and T cells and proliferation and differentiation of a large number of cells, including cancer cells. These properties are due to the fact that the receptors for $1,25(OH)_2D$ exists in a wide variety of tissues—not just bone, gut and kidney.Thus, the clinical utility of $1,25(OH)_2D$ analogs is likely to expand [7].

The effectiveness and safety of the short- and long-term management of vitamin D-deficiency states and other conditions have been confirmed and substantiated by a number of clinical trials, postmarketing surveillance and long-term studies. There is also a profound interest in the importance of vitamin D, not only in the maintenance of bone health but also in terms of its potential role in the prevention of

Table 1. *Physicochemical and pharmaceutical characteristics of cholecalcioferol*

rINN (BAN):	Cholecalciferol, Colecalciferol
ATC	A11CC05
Chemical name:	(5*Z*,7*E*)-9,10-Secocholesta-5,7,10(19)-trien-3β-ol
CAS number:	CAS: 67-97-0
Molecular formula:	$C_{27}H_{44}O$
Structural formula:	
Molecular mass:	384.6
Pharmacopoeias:	Ph. Eur., Ph. Chin., Ph. Int.; Ph. Jp., USP, and Ph. Viet.
Melting point:	84.5˚C
Polarity	Log P = 7.5
Properties	White or almost white crystals, which are sensitive to air, heat, and light. Practically insoluble in water; freely soluble in alcohol; soluble in trimethylpentane and in fatty oils. Solutions in solvents without an antioxidant are unstable and should be used immediately. A reversible summarization to pre-cholecalciferol takes place in solution, depending on temperature and time. The activity is due to both compounds. Store under nitrogen in airtight containers at a temperature of 2 degrees to 8 degrees. The contents of an opened container should be used immediately. Protect from light.

nonskeletal disorders such as auto-immune diseases, cancer, mental health problems and cardiovascular diseases, briefly outlined in the following sections of the paper.

I. Mode of action

Vitamin D_3 (Table 1) is a secosteroid prohormone that is converted to its active metabolite in a two-step biotransformation process. Vitamin D is first hydroxylated in the liver to form 25-hydroxyvitamin D (25(OH)D). This metabolite is further converted in the kidney to a number of other forms, among which 24,25-dihydroxyvitamin D (24,25$(OH)_2$D) and 1,25-dihydroxyvitamin D (1,25$(OH)_2$D; calcitriol) [2]. Calcitriol is a hormone, which performs many of its biologic functions by regulating gene transcription through a nuclear high-affinity vitamin D receptor (VDR) [8, 9]. This active metabolite of vitamin D binds to the nuclear VDR, which binds retinoic acid X receptor (RXR) to form a heterodimeric complex that binds to specific nucleotide sequences in the DNA known as vitamin D response elements [10, 11]. Once bound, a variety of transcription factors attach to this complex, resulting in either up-regulation or down-regulation of the gene's activity. There are estimated to be 200 to 2000 genes that have vitamin D response elements or that are influenced indirectly, possibly by epigenetics, to control a multitude of genes across the genome [11, 12]. A recent microarray study on the influence of vitamin D status and vitamin D_3 supplementation on genome-wide expression in white blood cells before and after vitamin D_3 supplementation found that an improved serum 25(OH)D concentration was associated with at least a 1.5-fold alteration in the expression of 291 genes. This study suggested that any improvement in vitamin D status will significantly affect the expression of genes that have a variety of biologic functions of more than 80 pathways linked to cancer, autoimmune disorders, and cardiovascular disease, which have been associated with vitamin D deficiency [7].

A major hormonal actions of vitamin D is to maintain serum calcium and phosphorus levels in a healthy physiologic range to sustain a variety of metabolic functions, transcription regulation, and bone metabolism [2]. The interaction of 1,25$(OH)_2$D with its VDR in the small intestine increases the efficiency of intestinal calcium absorption from approximately 10- 15% up to 30- 40% and intestinal phosphorus absorption from approximately 60% to 80% [10].

Although the molecular action of 1,25$(OH)_2$D on bone has received less attention, it has been found that 1,25$(OH)_2$D interacts with VDR in osteoblasts to stimulate a receptor activator of nuclear factor κB ligand (RANKL), which, in turn, interacts with receptor activator of nuclear factor κB (NFκB) on immature preosteoclasts, stimulating them to become mature bone-resorbing osteoclasts [2]. The mature osteoclast removes calcium and phosphorus from the bone to maintain blood calcium and phosphorus levels [10]. In the kidneys, 1,25$(OH)_2$D stimulates calcium reabsorption from the glomerular filtrate. The metabolites 25(OH)D and 24,25$(OH)_2$D are far less potent stimulators of intestinal calcium and phosphate transport or bone resorption. However, 25(OH)D appears to be more potent than 1,25$(OH)_2$D in stimulating renal reabsorption of calcium and phosphate and may be the major metabolite regulating calcium flux and contractility in muscle. Specific receptors for 1,25$(OH)_2$D exist in target tissues. However, the role and even the existence of separate receptors for 25(OH)D and 24,25$(OH)_2$D remain controversial [2].

The VDR is present in most tissues and cells in the body. Many of these organs and cells, including the brain, vascular smooth muscle, prostate, breast, and macrophages, not only have a VDR but also have the capacity to produce 1,25$(OH)_2$D [13-15]. This production probably depends on the availability of circulating 25(OH)D, indicating the biological importance of sufficient blood levels of this vitamin D metabolite [10]. In these "non-target" tissues, 1,25$(OH)_2$D exerts a number of actions including regulation of parathyroid hormone secretion from the parathyroid gland, insulin secretion from the pancreas, cytokine production by macrophages and T cells, and proliferation and differentiation of a large number of cells, including cancer cells [2, 14, 16].

Table 2 presents a concise synopsis of the principal actions and interplay between the chief calcium-regulating hormones – parathyroid hormone (PTH) and vitamin D on the three main target tissues—intestine, kidney, and bone.

The net effect of PTH is to raise serum calcium and reduce serum phosphate; the net effect of vitamin D is to raise both [2, 17]. Regulation of calcium and phosphate homeostasis is achieved through a variety of feedback loops. Calcium is the principal regulator of PTH secretion. It binds to a novel ion recognition site that is part of a G_q protein–coupled receptor called the calcium sensing receptor (CaR) and links changes in intracellular free calcium concentration to changes in extracellular calcium [2]. As serum calcium levels rise and bind to this receptor, intracellular calcium levels increase and inhibit PTH secretion.

Table 2. *Actions of parathyroid hormone (PTH) and vitamin D on gut, bone, and kidney [2].*

Target organs	PTH	Vitamin D
Intestine	Increased calcium and phosphate absorption (by increased $1,25(OH)_2D$ production)	Increased calcium and phosphate absorption by $1,25(OH)_2D$
Kidney	Decreased calcium excretion, increased phosphate excretion	Calcium and phosphate excretion may be decreased by 25(OH)D and $1,25(OH)_2D$[1]
Bone	Calcium and phosphate resorption increased by high doses. Low doses may increase bone formation.	Increased calcium and phosphate resorption by $1,25(OH)_2D$; bone formation may be increased by $1,25\ (OH)_2D$ and $24,25(OH)_2D$
Net effect on serum levels	Serum calcium increased, serum phosphate decreased	Serum calcium and phosphate both increased

[1]*Direct effect. Vitamin D often increases urine calcium owing to increased calcium absorption from the intestine and resulting decreased PTH.*

Phosphate regulates PTH secretion directly and indirectly by forming complexes with calcium in the serum. Because it is the ionized free concentration of calcium that is detected by the parathyroid gland, increases in serum phosphate levels reduce the ionized calcium and lead to enhanced PTH secretion. Such feedback regulation is appropriate to the net effect of PTH to raise serum calcium and reduce serum phosphate levels. Likewise, both calcium and phosphate at high levels reduce the amount of $1,25(OH)_2D$ produced by the kidney and increase the amount of $24,25(OH)_2D$ produced. The high calcium works directly and indirectly by reducing PTH secretion. The high phosphate works directly and indirectly by increasing FGF23 levels. Since $1,25(OH)_2D$ raises serum calcium and phosphate, whereas $24,25(OH)_2D$ has less effect, such feedback regulation is again appropriate. $1,25(OH)_2D$ itself directly inhibits PTH secretion (independently of its effect on serum calcium) by a direct action on PTH gene transcription. This provides yet another negative feedback loop [2]. The ability of $1,25(OH)_2D$ to inhibit PTH secretion directly is being exploited using calcitriol analogs that have less effect on serum calcium because of their lesser effect on intestinal calcium absorption. Such drugs are proving useful in the management of secondary hyperparathyroidism accompanying chronic kidney disease and may be useful in selected cases of primary hyperparathyroidism [1].

II. Effects of vitamin D beyond the calcium homeostasis and bone health

Many physiological processes are directly or indirectly regulated by vitamin D-derived hormones. Vitamin D and its metabolites have widespread physiological roles far beyond the well described effects in skeletal biology. Vitamin D deficiency is implicated in numerous disease conditions such as cancer, autoimmunity, cardiovascular diseases, diabetes and so on [7, 8, 24, 25]. The role of vitamin D deficiency in non-musculoskeletal conditions is a relatively novel field of interest. Well substantiated experimental data describe convincingly regulatory and pharmacological effects of vitamin D regarding various disease state models. These experimental data are strongly supported by epidemiological and observational human data, and more recently by clinical trials and meta-analyses thereof [7, 10].

- **Anticancer potential**

Several studies haverelatedhigher serum levels of 25(OH)D to reduced incidence of many types of cancers [7, 14, 16, 26]. It has been hypothesized that the local conversion of 25(OH)D to 1,25(OH)2D in healthy cells in the colon, breast, and prostate can help prevent malignancy by inducing cellular maturation, inducing apoptosis and inhibiting angiogenesis while enhancing the expression of genes including *involved in* controlling cellular proliferation [7, 15, 16, 21, 25]. Another vitamin D–regulated gene is *CYP3A4*, whose protein product detoxifies the bile acid lithocholic acid. Lithocholic acid is believed to damage the DNA of intestinal cells, and it may promote colon carcinogenesis. Stimulating the production of a detoxifying enzyme by 1,25(OH)2D could explain a protective role for improving vitamin D status against colon cancer. Because vitamin D regulates a gamut of physiologic processes, including immune modulation, resistance to oxidative stress, and modulation of other hormones, it is not surprising that low

vitamin D status has been associated with increased risk of several cancers and cancer mortality [7].

Adequate levels of 25(OH)D are critical for the prevention of various solid tumors, including prostate, breast, ovarian, and colon cancers, as suggested by epidemiological studies [14, 15]. A meta-analysis for the US Preventive Services Task Force regarding vitamin D supplementation concluded that each 4-ng/mL increase in blood 25(OH)D levels was associated with a 6% reduced risk of colorectal cancer but not with statistically significant dose-response relationships for prostate and breast cancer. In a large prospective study of lethal prostate cancer (1260 cases vs. 1331 controls), men with the highest quartile of plasma 25(OH)D levels had less than half the risk of lethal prostate cancer compared with men with the lowest quartile of plasma 25(OH)D levels. Another meta-analysis including 1822 colon and 868 rectal cancers reported an inverse association between circulating 25(OH)D levels and colorectal cancer, with a stronger association for rectal cancer [7].

- **Cardiovascular and metabolic effects**

The regulatory effects of vitamin D on various cardiovascular risk factors such as hypertension and diabetes mellitus has been well substantiated in several experimental data[10, 27-29]. Activation of the vitamin D receptor suppresses e.g. the renin-angiotensin system. These experimental data are strongly supported by epidemiological and observational human data that link vitamin D deficiency to the incidence, degree and prevalence of cardiovascular risk factors and disease conditions [8, 29].

25(OH)D and $1,25(OH)_2D$ levels are inversely related to coronary artery calcifications and are lower in patients with myocardial infarction. An in vitro study suggested that low 25(OH)D levels influence the activity/expression of macrophages and lymphocytes in atherosclerotic plaques, thus promoting chronic inflammation in the artery wall. Additionally, $1,25(OH)_2D_3$ inhibited foam cell formation and promoted angiogenesis in endothelial colony-forming cells in vitro, possibly due to an increase in vascular endothelial growth factor expression and pro–matrix metalloproteinase-2 activity. A short course of treatment with vitamin D (4000 IU for 5 days) effectively attenuated the increase in circulating levels of inflammatory cytokines after an acute coronary event. These findings support the anti-inflammatory effects of vitamin D on the vascular system and suggest mechanisms that mediate some of its cardioprotective properties. In addition, low 25(OH)D concentrations result in elevations in PTH levels, which have been linked to insulin resistance and significant increases in the serum levels of many acute phase proteins [7].

Observational studies indicated that a serum 25(OH)D level less than 30 ng/mL was strongly associated with hypertension and metabolic syndrome. This effect is thought to be partly mediated through regulation of the renin-angiotensin-aldosterone axis [7, 10, 29]. The Intermountain Heart Collaborative Study Group prospectively analyzed a large electronic medical records database that contained 41,504 patient records. Serum 25(OH)D levels less than 30 ng/mL were associated with highly significant increases in the prevalence of diabetes, hypertension, hyperlipidemia, and peripheral vascular disease. Serum 25(OH)D levels were also highly associated with coronary artery disease, myocardial infarction, heart failure, and stroke and with incident death, heart failure, coronary artery disease/myocardial infarction, stroke, and their composite. Black normotensive children who received 2000 IU/d of vitamin D_3 were compared with those who received 400 IU/d for 16 weeks in an RCT. Teenagers who received 400 IU/d of vitamin D_3 increased their mean ± SD plasma levels of 25(OH)D from 13.6±4.2 to 23.9±7.2 ng/mL and had no reduction in arterial wall stiffness. In contrast, teenagers who received 2000 IU/d of vitamin D_3 increased their mean ± SD plasma levels of 25(OH)D from 13.2±3.4 to 34.2±12.1 ng/mL and significantly lowered their arterial wall stiffness. This finding is supported by the observation that serum 25(OH)D levels less than 30 ng/mL were strongly associated with hypertension, elevated blood glucose, and metabolic syndrome in adolescents [7].

A large meta-analysis comprising 11 prospective studies involving 3,612 cases and 55,713 non-case participants provided the largest and most comprehensive assessment thus far of the association between circulating 25(OH)D levels and type 2 diabetes. It suggested a strong inverse association between serum 25(OH)D concentration and incidence of type 2 diabetes. The combined RR of 0.59 suggested that the risk of future diabetes may be reduced by 41% (95% CI, 33%-48%) by having a serum 25(OH)D level greater than 32 ng/mL compared with a serum 25(OH)D level less than 19.5 ng/mL at baseline [7]. The MIDSPAN family study was a prospective study of 1040 men and 1298 women from the West of Scotland recruited in 1996 and followed up for a median of 14.4 years. Plasma levels of 25(OH)D less than 15 ng/mL were not associated with a risk of cardiovascular disorders in this cohort with very low 25(OH)D

levels. The median plasma 25(OH)D level was 18.6 ng/mL, and the median vitamin D intake was 3.2 μg/d (128 IU/d). However, there was some evidence that a 25(OH)D level less than 15 ng/mL was associated with all-cause mortality [7].

However, large, high quality, and randomized controlled trials aiming primarily on cardiovascular end-points are absent. Speculations about the vitamin D usage in prevention or therapy of cardiovascular disease need to take potential drawbacks of vitamin D overdosing into account [7, 30]. The limited evidence regarding vitamin D therapy currently prevents general recommendations for vitamin D application in cardiology [7, 29].

- **Immune modulating activity/ Efficacy in autoimmune disorders**

Vitamin D has been defined as a natural immune modulator [7, 8, 24]. Epidemiologic, genetic, and basic science studies indicate a potential role of vitamin D in the pathogenesis of certain systemic and organ-specific autoimmune diseases, such as type 1 diabetes mellitus, MS, rheumatoid arthritis (RA), and Crohn disease (CD) [7, 26, 31]. Vitamin D's effects on the innate immune system are predominantly through the toll-like receptors and on the adaptive immune system through T-cell differentiation, particularly the T helper cell (T_H) type 17 response. Because T_{H17} cells are critical in the pathogenesis of RA, this has led to an interest in the effects of vitamin D deficiency in RA [7]. Vitamin D inhibits immune reactions in general, but it enhances the transcription of endogenous antibiotics, such as cathelicidin and defensins[7]. Vitamin D suppresses autoimmune disease pathology by regulating the differentiation and activity of CD4+ T cells, resulting in a more balanced T_{H1}/T_{H2} response that favors less development of self-reactive T cells and autoimmunity. The T_{H1}-dependent autoimmune diseases, including MS, type 1 diabetes, CD, and RA, are also inhibited by $1,25(OH)_2D_3$ owing to inhibition of antigen presentation, reduced polarization of T_{H0} cells to T_{H1} cells, and reduced production of cytokines from the latter cells [7].

The $1,25(OH)_2D_3$ down-regulated the proinflammatory cytokine (interleukin 1β, interleukin 6, and tumor necrosis factor) production in human activated macrophages by significantly decreasing the aromatase activity, especially in the presence of an estrogenic milieu, such as in RA synovial tissue. A prospective cohort study of 29,368 women aged 55 to 69 years without a history of RA found an inverse association between vitamin D intake and RA after 11 years of follow-up. There was a 34% reduction in the development of RA with greater vitamin D intake. Women using a multivitamin with 400 IU of vitamin D reduced their risk of RA by 40% [7].

There is a large body of evidence linking a lack of vitamin D early in life to the development of type 1diabetes [7, 10]. Vitamin D supplementation during infancy was reported to confer partial protection against β-cell autoimmunity. There is consistent evidence from observational studies for potential long-term programming effects of vitamin D supplementation on immunologic diseases, such as type 1 diabetes, MS, asthma, and allergic diseases [32]. There was a 63% decreased risk of islet cell antibodies in offspring with a single standard deviation (156 IU) increase in recalled maternal dietary vitamin D intake during pregnancy. Similarly, higher maternal cod liver oil (a source of vitamin D) intake during pregnancy was associated with a decreased risk of type 1 diabetes in offspring, and fetal exposure to vitamin D deficiency was linked to a higher metabolic and cardiovascular disease risk in adult life [7].

In animal modelsand in cultured cells, $1,25(OH)_2D_3$ has been reported to improve insulin production, modulate T- and β-cell activity, enhance phagocytic killing activity, improve vascular smooth muscle resistance, and reduce the risk of autoimmune diseases [7, 27, 28].

Evidences supporting a protective role for vitamin D in MS risk and progression continue to emerge. Notable recent findings are that high 25(OH)D levels at the time of a first demyelinating event predicts a lower MS risk and a decreased risk of MS in offspring whose mothers had high 25(OH)D levels. An American study of more than 187,000 women followed up for 10 to 20 years reported promising results with women taking at least 400 IU of supplemental vitamin D daily. The risk of MS was decreased by 41%. An epigenetic study in lymphoblastoid cell lines reported relevant insights into how vitamin D may influence the immune system and the risk of MS through VDR interactions with the chromatin state inside MS-associated genomic regions. Higher 25(OH)D levels were associated with decreased exacerbation risk in relapsing-remitting MS. However, the literature is limited by small study sizes, heterogeneity of dosing, form of vitamin D tested, and clinical outcome measures [7].

Effects in respiratory tract conditions

At the turn of the past century, children with rickets were at higher risk for upper respiratory tract in-

fections and for dying of them. Macrophages have a VDR, and when they ingest an infectious agent, such as tuberculosis bacillus, the toll-like receptors are activated, resulting in signal transduction to increase the expression of VDR and *CYP27B1*. In turn, 25(OH)D is converted to $1,25(OH)_2D$, which signals the nucleus to increase the expression of cathelicidin, a defensin protein that kills infective agents, such as tuberculosis bacillus [7, 10].

Cord blood 25(OH)D levels have been associated with tolerogenic immune regulation and fewer respiratory tract infections in newborns. Also, high 25(OH)D levels during maternity were associated with a decrease in childhood wheezing by nearly 50% compared with low maternal 25(OH)D levels. Newborns with 25(OH)D levels less than 10 ng/mL were twice as likely to develop respiratory tract infections compared with those with levels of 30 ng/mL or greater, and every 4-ng/mL increase in the cord blood 25(OH)D level lowered the cumulative risk of wheezing by age 5 years. Serum concentrations of 25(OH)D in 198 healthy adults revealed that a concentration of 38 ng/mL or higher reduced the risk of acute viral respiratory tract infections and number of days ill by 2-fold [7].

The potential role of vitamin D in reducing the risk of allergies may be related to epigenetic regulation. Misdirected epigenetic programming offered an explanation for why vitamin D deficiency in pregnancy may be associated with increased allergy rates in the offspring. The cord blood level of 25(OH)D found a U-shaped association, with a 2.4-fold odds ratio (OR) of low and a 4-fold OR of high levels of 25(OH)D to develop allergen-specific IgE. Eczema was significantly more likely in those with 25(OH)D levels less than 20 ng/mL compared with those with 25(OH)D levels of 30 ng/mL or greater (OR, 2.66; 95% CI, 1.24-5.72; *P*=.01). Noteworthy, although vitamin D can favorably influence several pathways associated with respiratory tract diseases, there are few clinical trials to support the beneficial effect of vitamin D supplementation for these patients [7]. Moreover the available studies differ on whether a higher vitamin D intake or status in pregnancy or at birth is protective against asthma and allergies. To address this uncertainty, the Vitamin D Antenatal Asthma Reduction Trial (VDAART) has been commenced. VDAART is an ongoing randomized, double-blind, placebo-controlled trial of vitamin D supplementation in pregnant women that will determine whether prenatal supplementation can prevent the development of asthma and allergies in women's offspring [32].

Meta-analyses on respiratory outcomes and recovery from tuberculosis did not report any beneficial effects of supplementation for patients with cystic fibrosis or tuberculosis, respectively [7]. In contrast, other studies provided evidence for the beneficial effects of vitamin D supplementation in cystic fibrosis in terms of anti-inflammatory effects and prevention of infectious exacerbations albeit at doses exceeding those typically applied clinically in skeletal and endocrine disorders [33].

- **Neurodevelopmental regulation, neuroprotective effects and efficacy in neurological disorders**

Over the past decades a number of studies have implicated several ways in which cholecalciferol affects the developing brain including its effects on cell differentiation, neurotrophic factor expression, cytokine regulation, neurotransmitter synthesis, intracellular calcium signaling, anti-oxidant activity, and the expression of genes/proteins involved in neuronal differentiation, structure and metabolism. Dysfunction in any of these processes could adversely affect development and it has been shown in diverse experimental models to examine the impact of the dietary absence of vitamin D *in utero* as well as in epidemiological human studies [13, 34].

The brain has a VDR and has the ability to produce $1,25(OH)_2D_3$. In vivo mouse studies found that in utero hypovitaminosis D impairs brain development and leads to persistent changes in the adult brain [13, 34]. The $1,25(OH)_2D_3$ is rapidly incorporated into embryonic hippocampal cells, moves into the nucleus, and then returns to the cytoplasm. These events delay cell proliferation and induce cell differentiation characterized by the expression of differentiation markers, modification of soma lengthening, and increase in neurite length and branching. At birth, rats with prenatal vitamin D deficiency had heavier and longer brains, enlarged lateral ventricles, and decreased cortical thickness. Evidence from human studies is scanty. One recent study found that higher maternal serum 25(OH)D levels in late pregnancy (<12 vs. >30 ng/mL) were associated with larger head circumference of offspring at 9 years old but not with measures of cognition or psychological health [7]. In addition, there may be a critical window during late gestation in which vitamin D insufficiency precipitates an altered adult behavioral phenotype. In rats, offspring of vitamin D–deficient mothers had significant impairment of latent inhibition (ability to ignore irrelevant stimuli), a feature often associated with schizophrenia, whereas those transiently deplet-

ed had subtle and discrete alterations in learning and memory. In a Finnish birth cohort study, 9114 individuals were drawn from the northern Finland 1966 birth cohort. In males, the use of at least 2000 IU of vitamin D during the first year of life was associated with a reduced risk of schizophrenia (RR, 0.23; 95% CI, 0.06-0.95) compared with those taking lower doses [7].

There is minimal evidence for an association of low maternal vitamin D status with risk of autism [35]. Children of dark-skinned mothers, particularly immigrants to locations with low ambient UV radiation may be at increased risk, but this finding has been inconsistent [7].

The $1,25(OH)_2D_3$ seems to have a neuroprotective role, inducing remyelination by endogenous progenitor cells and stimulation of amyloid-β clearance by macrophages of patients with Alzheimer disease [7, 13]. A vitamin D_3–enriched diet correlated with a decrease in the number of amyloid plaques and inflammation in the brains of AβPP mice. These observations suggest that a vitamin D_3–enriched diet may reduce the risk of Alzheimer disease as well as depression and neurocognitive disorders. An Australian study of 743 white pregnant women found that maternal vitamin D insufficiency during pregnancy is significantly associated with offspring language impairment. Vitamin D deficiency was also associated with prominent changes in behavior and brain neurochemistry in the adult mouse. In the follow-up of a British birth cohort (n=7401), current and subsequent risk of depression in middle adulthood was associated with low serum 25(OH)D levels. Although there is a strong association between risk of neurologic disorders and serum 25(OH)D concentrations, there are only a few short-term clinical trials of vitamin D in patients with MS that have not reported benefit and no clinical trials evaluating other neurologic disorders [7].

III. Safety pharmacology and toxicodynamics

Intake of vitamin D substantially greater than physiologic amounts (>250 mcg/day) is toxic because circulating vitamin D binding protein (DBP) is preoccupied and that forces the free and unbound percent of vitamin D to increase [22]. The toxic doses are associated with hypercalcaemia and hypercalciuria result from increased intestinal absorption of calcium and mobilization of calcium from the skeleton and may lead to impairment in renal function, nephrocalcinosis, nephrolithiasis, urinary tract infections, and so forth [17, 30]. Patients with intoxication are often asymptomatic, but they may have anorexia, nausea, vomiting, weight loss, polyuria, polydipsia, and alterations in mental status [30]. Children and infants often show listlessness and hypotonia. Intoxication can occur unexpectedly, possibly as a result of changes in diet, gastrointestinal absorption of calcium, or hydration status [17].

The freely circulating vitamin D, along with its metabolites, can accumulate not only in adipose but also in muscle. The average capacity of human plasma DBP to bind vitamin D and its metabolites is 4700 nmol/L, and this exceeds by 20 times the physiologic total concentration of its vitamin D-derived ligands. The majority of cases of vitamin D intoxication have involved vitamin D_2. The clinical cases involving vitamin D_3, to date, have been industrial accidents or poisonings from an unknown source. Vieth et al. have assayed blood levels of vitamin D and its metabolites by chromatography and found that despite record-high 25(OH)D concentrations in humans (2400 nmol/liter), they were still small in comparison to a large excess of vitamin D3 (17,000 nmol/liter) suggesting that the capacity of liver to hydroxylate vitamin D is limited [22].

Concentrations of $1,25(OH)_2D$ are not increased such by vitamin D intoxication. This reflects the high level of regulation of this hormone via both its synthesis and catabolism. Nonetheless, vitamin D toxicity is probably manifest by the excessive levels of "free" $1,25(OH)_2D$, displaced from its carrier protein, DBP, by the vast excess of other vitamin D metabolites [6]. This excess was confirmed by studies looking into "free" $1,25(OH)_2D$ concentrations in vitamin D intoxicated individuals. This excess of metabolite over binding capacity was also confirmed by the high total of vitamin D and 25(OH)D concentrations (19500 nmol/L) in a patient intoxicated after consuming over a million units (>25 mg) daily for many months [21].

In general 3 major theories about the mechanism of vitamin D toxicity have been coined. All involve increased concentrations of a vitamin D metabolite reaching the VDR in the nucleus of target cells and causing exaggerated gene expression. At issue is the offending vitamin D metabolite and how it becomes elevated. The 3 hypotheses to explain this are as follows: i) vitamin D intake raises plasma $1\alpha,25(OH)_2D$ concentrations, which increase cellular $1\alpha,25(OH)_2D$ concentrations; ii) Vitamin D intake raises the concentrations of many vitamin D metabolites, especially vitamin D itself and 25(OH)D. iii) Vitamin D intake raises plasma 25(OH)D to μmol/L concentrations that exceed the DBP binding capacity and "free

25(OH)D" enters the cell, where it has direct effects on gene expression; These concentrations exceed the DBP binding capacity and cause release of "free" 1α,25$(OH)_2$D, which enters target cells [6, 22].

The third toxicodynamic mechanism has been corroborated by an elegant preclinical study. Vitamin D intoxication was produced with oral doses of either vitamin D_3 or 25(OH) D_3 in CYP27B1 -/- (1α-hydroxylase knockout) and wild-type mice. These compounds were equally toxic in wild-type and the mutant mice. Since the null mutant mice are unable to produce 1,25-dihydroxyvitamin D, it is clear 1,25-dihydroxyvitamin D is not responsible for vitamin D intoxication. On the other hand, 25-hydroxyvitamin D rises to levels of 400-700 ng/ml or 1000-1750 nM in the serum of both groups of mice. Toxicity was evidenced by severe hypercalcaemia and weight loss. Measurement of 1,25-dihydroxyvitamin D_3 in serum confirmed its absence from serum of the CYP27B1 -/- mice given 25-hydroxyvitamin D_3. Since high concentrations of 25-hydroxyvitamin D can bind the vitamin D receptor and can induce transcription, 25-hydroxyvitamin D is likely responsible for toxicity of vitamin D excess [36].

Even though as suggested by in animal studies the toxicity of vitamin D has conventionally been attributed to its induction of hypercalcaemia, some toxic endpoints observed in response to hypervitaminosis D such as anorexia, lethargy, growth retardation, bone resorption, soft tissue calcification, and death can be dissociated from the hypercalcaemia that usually accompanies them. On these grounds an alternative hypothesis has been reported which proposes that vitamin D exerts toxicity by inducing a deficiency of vitamin K. According to this model, vitamin D increases the expression of proteins whose activation depends on vitamin K-mediated glutamate γ-carboxylation; as the demand for carboxylation increases, the pool of vitamin K is depleted. Since vitamin K is essential to the nervous system and plays important roles in protecting against bone loss and calcification of the peripheral soft tissues, its deficiency results in the symptoms associated with hypervitaminosis D. This notion is circumstantially supported by the observation that animals deficient in vitamin K or vitamin K-dependent proteins exhibit remarkable similarities to animals fed toxic doses of vitamin D, and the observation that vitamin D and the vitamin K-inhibitor Warfarin have similar toxicity profiles and exert toxicity synergistically when combined. The hypothesis further proposes that vitamin A protects against the toxicity of vitamin D by decreasing the expression of vitamin K-dependent proteins and thereby exerting a vitamin K-sparing effect [37].

IV. Pharmacokinetics

Nutritional and pharmacological doses of cholecalciferol are well absorbed from the gastrointestinal tract [2]. When absorbed from the gut, vitamin D enters the circulation on chylomicrons first, and it is only slowly transferred to the specific plasma binding protein (DBP). Vitamin D has relatively low affinity for DBP; reviews estimate this at between 1×10^{-5} and 1×10^{-7} mol/L.Transport of dietary vitamin D contrasts significantly with that of vitamin D_3made during skin synthesis, which is mainly bound to DBP. The consequence of chylomicron transport of dietary vitamin D is the possibility of uptake by peripheral tissues, such as adipose tissue and muscle, due to the action of lipoprotein lipase[6].

Vitamin D_3 is a lipophilic molecule similar to its closely related lipid precursor cholesterol, so it requires binding to the protein carrier DBP for solubility in plasma[3, 8, 9]. Its mono-, di-, and tri-hydroxylated metabolitesshow progressively increasing polarity, culminating in the water-soluble biliary form calcitroic acid[6]. The liver converts vitamin D into 25(OH)D, a process that some attribute to a microsomal cytochrome P450 enzyme, CYP2R1, or the mitochondrial cytochrome P450 CYP27A1; neither is subject to tight regulation. 25(OH)D quickly enters the plasma pool that constitutes the predominant pool of vitamin D in the body, with a capacity of ≈4.5 μmol/L. 25$(OH)D_3$ and 25$(OH)D_2$, its vitamin D_2counterpart, have a strong affinity for DBP at 5×10^{-8} mol/L, which is at least an order of magnitude higher than that of its vitamin D precursors. As a consequence, 25$(OH)D_3$ has a half-life of 15 d in the circulation. The normal circulating level of 25(OH)D [25$(OH)D_3$ + 25$(OH)D_2$] in the blood is only 25–200 nmol/L, indicating that ligand only occupies 2–5% of DBP in the physiologic state[6].

The dihydroxy-metabolites have widely differing affinities for DBP, with 25$(OH)D_3$-26,23-lactone binding 3–5 times as tightly as 25(OH) D_3. The inactive metabolites 24,25$(OH)_2D_3$ and 25,26$(OH)_2D_3$ bind with equal affinity as 25$(OH)D_3$, and the active form 1α,25$(OH)_2D_3$ binds DBP with an affinity of 2×10^{-7} mol/L, an order of magnitude less than its precursor. 1α,25$(OH)_2D_3$has a half-life of 10–20 h, although this depends to some extent on the state of the highly inducible catabolic machinery. The accumulation of these metabolites in the bloodstream is mainly a function of their affinity for DBP,

although rate of synthesis and degradation must also play a partial role[6].

The liver takes up the vitamin D left in the chylomicron remnant and quickly removes it from the bloodstream.The loss into tissue and liver pools thus logically explains the short distribution half-life ≈4–6 h, of physiologically relevant doses of vitamin D. Studies with radiolabeled vitamin D have shown that the whole-body half-life is ≈2 months [6].

In the liver series of 25-hydroxylases—the major ones are the microsomal CYP2R1 and the mitochondrial CYP27A1 metabolizecholecalciferol. The resulting 25-hydroxyvitamin D_3 ($25(OH)D_3$) is then carried on vitamin D binding protein (DBP) to the kidney, where the classic renal CYP27B1 1α-hydroxylates it and puts it back into circulation as its hormonally active form 1α,25-dihydroxyvitamin D_3 ($1\alpha,25(OH)_2D_3$; calcitriol) [2, 6]. Recent studies have conclusively shown that CYP24A1 is responsible for the formation of several principal accumulating metabolites, $24,25(OH)_2D_3$ and $25(OH)D_3$-26,23-lactone, as well as the catabolite calcitroic acid[6, 9]. Measurements of the plasma level show that circulating $1\alpha,25(OH)_2D_3$ is in the picomolar range, a concentration 1000 times lower than that of 25(OH) D. Molecular probes for CYP27B1 mRNA based on reverse transcriptase–polymerase chain reaction and for CYP27B1 protein based on immune localization studies have shown that this "activating" CYP is expressed not only in the kidney but also in several extrarenal tissues; in addition, it is subject to different regulation than the renal enzyme[6].

Tracer studies with vitamin D have shown a rapid clearance of the parent from the blood, due to tissue redistribution, nevertheless the whole body clearance is slow due to sequestration in adipose tissues, the terminal elimination half-life is 50-60 days [39]. The liver appears to be the principal organ for biotransformation and clearance and the kidney is important for biological activation [6], but noteworthy activated macrophages, certain lymphoma cells, and skin and bone cells also have been shown to produce 1,25-dihydroxylated vitamin D, but the physiologic importance of such locally produced activated vitamin D is not well understood. Excessive unregulated production of 1,25-dihydroxylated vitamin D by activated macrophages and lymphoma cells is responsible for the hypercalciuria associated with sarcoidosis, other chronic granulomatous disorders and the hypercalcaemia associated with lymphoma [19, 30]. Excess vitamin D is stored in adipose tissue. The metabolic clearance of the hormonally active metabolite calcitriol in humans indicates a rapid turnover, with a terminal half-life measured in hours [2, 6]. The metabolites of vitamin D analogs are excreted principally in bile and feces [4]. Although some vitamin D that is excreted in bile is reabsorbed in the small intestine, enterohepatic circulation does not appear to be an important mechanism for conservation of the vitamin [19].

- **Comparative pharmacokinetics in specific populations**

Vitamin D deficiency can result from nutritional deprivation, inadequate sunlight exposure, impaired gastrointestinal absorption, reduced synthesis of 25-hydroxyvitamin D and/or 1,25-dihydroxyvitamin D or end-organ resistance to 1,25-dihydroxyvitamin D [7, 17]. Nutritional vitamin D deficiency is rare in North America, because milk and cereals are commonly fortified with vitamin D_2, but appears far more common in Europe, especially in Northern areas and far more common elsewhere in the world [7]. Noteworthy, vitamin D deficiency can occur in alcoholics and the elderly because of their poor nutritional status and limited sunlight exposure. Data relating altered intestinal absorption of vitamin D and age are controversial. Although some evidence suggested that intestinal absorption of vitamin D may be decreased in geriatric adults, other evidence did not show clinically important age-related alterations in GI absorption of the vitamin in therapeutic doses[19]. Moreover in elderly people, the amount of body fat is usually increased and total body fluid is reduced. These changes affect drug distribution and should be considered when administering lipophilic substances such as vitamin D, which can have a higher relative distribution volume, a longer half-life, increased accumulation, and prolongation of the pharmacological effect [30].

The intestinal absorption of [^{3}H]cholecalciferol was studied in five patients with alcoholic liver disease, six patients with primary biliary cirrhosis, and 15 healthy subjects. The rate of appearance in plasma of [^{3}H]cholecalciferol after oral ingestion and the subsequent appearance of [^{3}H]polar metabolites in the alcoholic subjects were similar to those in the healthy subjects. In subjects with primary biliary cirrhosis the rate of appearance in plasma of [^{3}H] cholecalciferol was significantly reduced. The rate of appearance of labeled polar metabolites of cholecalciferol was also lower in this group, suggesting that increased removal of labeled vitamin by conversion into more polar metabolites could not account for the

reduced plasma [^{3}H]cholecalciferol response. The findings of the study indicate that intestinal absorption of cholecalciferol and its hepatic 25-hydroxylation appear to be normal in alcoholic liver disease but impaired in primary biliary cirrhosis [40].

Obese adults (BMI > 30 kg/m^2) are at high risk for vitamin D deficiency because the body fat sequesters the fat-soluble vitamin. When obese and non-obese adults were exposed to simulated sunlight or received an oral dose of 50,000 IU of vitamin D, they were able to raise their blood levels of vitamin D by no more than 50% compared with non-obese adults [5].

Patients on multiple anticonvulsant medications, glucocorticoids, or antiretroviral treatment are at increased risk for vitamin D deficiency because these medications increase the catabolism of 25(OH)D [19, 38].

Patients with one of the fat malabsorption syndromes and bariatric patients are often unable to absorb the fat-soluble vitamin D, and patients with nephrotic syndrome lose 25(OH)D bound to the vitamin D-binding protein in the urine [17, 41]. On these grounds in obese patients, patients with malabsorption syndromes, and patients on medications affecting vitamin D metabolism, the 2011 Endocrine Society Clinical Practice Guideline for evaluation, treatment, and prevention of Vitamin D Deficiency has suggested a higher dose (two to three times higher; e.g. 6000–10,000 IU/daily) of vitamin D to treat vitamin D deficiency to maintain a 25(OH)D level above 30 ng/ml, followed by maintenance therapy of 3000–6000 IU/D [5].

Patients who suffer from chronic granuloma-forming disorders including sarcoidosis, tuberculosis, and chronic fungal infections and some patients with lymphoma have activated macrophages that produce $1,25(OH)_2D$ in an unregulated fashion, non-dependent on the normal biotransformation in the kidney [19, 30]. This results in an increase in the efficiency of intestinal calcium absorption and mobilization of calcium from the skeleton that can cause hypercalciuria and hypercalcaemia. These patients may require vitamin D treatment to raise their blood level of 25(OH)D to approximately 20–30 ng/ml to prevent vitamin D-deficiency metabolic bone disease while mitigating hypercalciuria and hypercalcaemia[10].

Gastrointestinal disease is now the predominant cause of vitamin D deficiency. Intestinal malabsorption syndromes that affect the small intestine (especially the duodenum and jejunum) are all associated with impaired vitamin D absorption as a result of either rapid transit or enhanced fecal loss of 25-hydroxyvitamin D because of impaired enterohepatic circulation [41]. Similarly, patients with chronic severe parenchymal and cholestatic liver disease frequently exhibit vitamin D deficiency resulting from the associated malabsorption syndrome combined with a decreased hepatic capacity to convert vitamin D to 25-hydroxyvitamin D [17]. Those patients who take phenobarbital, phenytoin and other CYP450 inducers have increased activity of microsomal hydroxylases in the liver, and consequent accelerated metabolism of vitamin D can lead to functional vitamin D deficiency [5, 30, 38].

In the absence of PTH (idiopathic or surgical hypoparathyroidism) or an abnormal target tissue response to PTH (pseudohypoparathyroidism), serum calcium falls and serum phosphate rises [17, 18, 46]. In such patients, $1,25(OH)_2D$ levels are usually low, presumably reflecting the lack of stimulation by PTH of $1,25(OH)_2D$ production. The skeletons of patients with idiopathic or surgical hypoparathyroidism are normal except for a slow turnover rate. A number of patients with pseudohypoparathyroidism appear to have *osteitis fibrosa*, suggesting that the normal or high PTH levels found in such patients are capable of acting on bone but not on the kidney. The distinction between pseudohypoparathyroidism and idiopathic hypoparathyroidism is made on the basis of normal or high PTH levels but deficient renal response (i.e., diminished excretion of cAMP or phosphate) in patients with pseudohypoparathyroidism. In hypoparathyroidism the principal therapeutic concern is to restore normocalcemia and normophosphatemia. Under most circumstances, vitamin D and dietary calcium supplements suffice [17, 46].

Renal disease is one of the most common causes of vitamin D deficiency, and requirements for supplementation therapy. As kidney function declines, 1α-hydroxylase activity decreases, leading to reductions in circulating levels of 1,25-dihydroxyvitamin D and, consequently, impaired gastrointestinal calcium absorption. Additionally, phosphate excretion falls as renal function declines [17, 47]. Hyperphosphatemia then results in further decreases in the serum calcium level by chelating ionized calcium and inhibiting any remaining 1α -hydroxylase enzyme activity [17].

In rare instances, inborn errors of vitamin D metabolism can result in hypocalcemia. Patients with these disorders generally present early in life with hypocalcemia and skeletal abnormalities despite adequate vitamin D intake [17]. Vitamin D-dependent rickets type I is an autosomal recessive disorder that stems from impaired renal 1a-hydroxylase ac-

tivity. Affected patients have low concentrations of 1,25-dihydroxyvitamin D but respond to treatment with physiological doses of 1,25-dihydroxyvitamin D. In contrast, patients with vitamin D-dependent rickets type II have a variety of mutations in the vitamin D receptor, exhibit dramatically increased circulating concentrations of 1,25dihydroxyvitamin D (as a consequence of their secondary hyperparathyroidism), and respond poorly even to pharmacological doses of 1,25-dihydroxyvitamin D. Patients with the more severe form of this disease frequently have alopecia [17, 47].

The clinical manifestations of vitamin D deficiency can include myotonia, muscle weakness, and, in severe cases, hypocalcemia and tetany [17, 47]. Additionally, skeletal abnormalities are also frequently present. The characteristic skeletal disturbance in vitamin D-deficient states is osteomalacia and rickets in children [17, 48, 49]. The malacic bone results from impaired mineralization and is subject to distortion in shape and to fracture. When osteomalacia develops in young, actively growing children, it is referred to as rickets. If vitamin D deficiency is present during the first year of life, the characteristic features of rachitic bone can include widened cranial sutures, frontal bossing, posterior flattening of the skull, bulging of the costochondral junctions, indentation of the ribs at the diaphragmatic insertions, and enlargement of the wrists. After the first year of life, the deformities resulting from vitamin D deficiency are most severe in the long bones because of their rapid growth and weight-bearing function. The bony shafts of the long bones can be deformed (bowed) and subject to fracture. The ends of the long bones become enlarged and bowleg or knock-knee deformities progressively worsen. In long-standing disease, there may be *coxa vara* and rachitic saber shins. Moderate deformities occurring before age of 4 may resolve with adequate vitamin D treatment, but those occurring later usually result in lasting deformity, compromised adult height or both [17].

The clinical features associated with osteomalacia in adults are subtler than those with rickets in children. In the mature, fully grown skeleton, bone turnover is less than 5% per year. Thus, a mineralization defect in adults must be present for several years to produce clinical manifestations. The characteristic symptom, if any, is pain when weight or pressure is applied to the affected bones. Low backache relieved by recumbency is one of the earlier complaints, but the pain may include other portions of the spine, ribs, and feet. Significant osteomalacia may be present without radiographic manifestations, but there is often a generalized decrease in bone mineral density. The most characteristic feature of adult osteomalacia is the pseudofracture (Looser's zone, Milkman's syndrome), a straight, transverse band localized, often symmetrically, at the concave ends of the shafts of long bones, ribs, scapulae, and pubic rami. The origin of these radiographic abnormalities is unknown, but they may arise from the pressure of overlying pulsating arteries. Skeletal fractures (sometimes superimposed on pseudofractures) can occur and usually unite very slowly. Long-standing disease can lead to bowing of long bones and distortion of the pelvic outlet (assuming a triangular appearance on standard anteroposterior views). Loss of vertebral height as a result of typical biconcave deformities can lead to kyphosis as a late manifestation. The skeletal deformities may be associated with other features of malnutrition and/or secondary hyperparathyroidism (e.g., subperiosteal resorption). Associated proximal weakness can contribute to a waddling gait or severe crippling [17].

The goals of treatment in states of vitamin D deficiency are to (1) correct hypocalcemia, alleviate related symptoms, and prevent grand mal seizures and cataracts, (2) prevent the skeletal deformities of rickets and the occurrence of fragility fractures, (3) prevent toxicity (hypercalcaemia, hypercalciuria, and their consequences), and (4) promote normal growth and development in children [17]. The choice of the vitamin D preparation used for therapy depends on the cost of the medication and associated illnesses that may influence vitamin D metabolism [1, 47].

The precursor molecules, ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3), are the least expensive. These compounds and 25-hydroxyvitamin D_3 (calcifediol) have the theoretical advantage that they are precursors of the other vitamin D metabolites, so that physiologic regulation may avert toxicity. A disadvantage of vitamin D_2 and D_3 is that they tend to be chemically unstable and lose activity during storage. They also tend to accumulate in fat and muscle during long-term administration so that the effect becomes cumulative. With both compounds, the therapeutic dose approaches the toxic dose, a long period is required for optimal biologic effect, and activity may persist after cessation of administration, a disadvantage in the event of intoxication. The advantages of dihydrotachysterol (DHT) and calcitriol (1,25-dihydroxyvitamin D_3) are that the onset of maximum biological activity is short and their effects last only a short period after cessation of

treatment. Both are effective when 1α-hydroxylation of 25-hydroxyvitamin D is defective. However, these drugs are relatively expensive, and hypercalcaemia can occur episodically in patients on long-term treatment. The hypercalcaemia is easily managed by stopping the drug and it is prevented by reducing the dose [17, 21, 22].

Optimal management of vitamin D deficiency is dependent on its underlying cause and treatment must be individualized with regard to pathogenesis and severity of the disorder. For cases of nutritional vitamin D deficiency, ergocalciferol or cholecalciferol are reasonable initial choices: 400–800 IU of vitamin D will achieve normal circuiting levels of 25-hydroxyvitamin D in most patients [17].

In patients with more severe deficiency (associated with symptoms) high dose regimens of cholecalciferol is frequently used at the beginning of therapy to more rapidly increase systemic vitamin D levels. After that time, maintenance dosages should be reduced and titrated to maintain 25-hydroxyvitamin D levels of at least 30 ng/mL. The cause of vitamin D deficiency should provide guidance for the amounts of vitamin D supplementation required [5, 17].

Deficiency states associated with malabsorption may require pharmacological amounts of vitamin D (up to 10–100,000 IU/d) [10, 17]. In the presence of malabsorption, concurrent magnesium depletion must always be considered. Vitamin D_2 or D_3 may not be appropriate in some circumstances. If vitamin D deficiency is secondary to intestinal resection, malabsorption, or impaired enterohepatic circulation, calcitriol (0.5–2.0 μg/d) may be absorbed more readily. In the setting of defective renal hydroxylation of 25-hydroxyvitamin D (e.g., end-stage renal disease, type I vitamin D-dependent rickets), therapy with either DHT or calcitriol is preferred [17, 19], albeit clinical evidence suggest that vitamin D precursors are still of value in these circumstances [50-53]. In general, states of vitamin D deficiency in which renal 1α-hydroxylation is intact should not be treated with analogs such as DHT or calcitriol. These compounds bypass the renal site of feedback control of 1,25-dihydroxyvitamin D biosynthesis and thus carry a greater risk of inducing hypercalcaemia[17].

In most patients with vitamin D deficiency (those with adequate renal function and 1a-hydroxylase activity) the object is to restore and maintain optimal serum 25(OH) vitamin D concentrations. Monitoring serum levels after vitamin D supplementation begins should provide an adequate guide for dose adjustments. Measures at 1- to 2-monthly intervals until the desired levels are obtained should be sufficient, and periodic measures thereafter will ensure stable vitamin D nutrition has been achieved. Intoxication can be a problem with vitamin D or any of its metabolites [17].

- **Definition of sufficiency, insufficiency and deficiency – Vitamin D status**

There is limited evidence for when to monitor response to therapy, but the aims are to detect: i) those who remain deficient after loading or during maintenance ii) those patients in whom vitamin D therapy uncovers sub-clinical primary hyperparathyroidism [20].

Monitoring the levels of 25(OH)D is the paradigm for vitamin D replacement therapy assessment [7, 54]. There is considerable variability between the results of studies examining the dose response to vitamin D supplementation, but it appears that much of this inconsistency results from the confounding effects of UV exposure in the summer months [55]. When consideration is confined to the results of studies that examined the effect of supplementation on winter 25(OH)D levels, the results are more consistent: a daily supplement of 20 to 25 μg (800 to 1000 IU) calciferol will cause an increase in 25(OH)D of 24 to 29 nmol/L. Most of these studies have suggested that a new steady-state 25(OH)D level is reached by about 3 months. While this is in line with what would be expected given the elimination half-life of 25(OH)D, more recent data has found that the steady-state levels are not obtained until after 6 months of treatment [20].

Accordingly, it is useless and unnecessary to measure vitamin D levels too soon after the therapy has started. A minimum of 3 months treatment must be given and it may be more prudent to wait until 6 months have passed. Noteworthy routine monitoring of serum 25(OH)D is unnecessary but may be appropriate in patients with symptomatic vitamin D deficiency or malabsorption and where poor compliance with medication is suspected. Based on the pharmacokinetics of 25(OH)D, assessment of adjusted serum calcium levels within 1 month after the administration of the last loading dose should be undertaken to detect those with primary hyperparathyroidism. The presence of hypercalcemia should lead to cessation of further vitamin D supplementation prior to investigation of the hypercalcaemia. Adjusted serum calcium should be checked 1 month after completing the loading regimen or after starting vitamin D supplementation in case primary hyperparathyroidism has been unmasked [20].

It is well known that vitamin D treatment can unmask previously undiagnosed primary hyperparathyroidism, which has to be taken into account. Although the dosing regimen is unlikely to result in toxicity, it should be recognized that certain groups may be at increased risk of this or adverse side effects and they should be monitored by measuring adjusted serum calcium levels. Such vulnerable patients with increased sensitivity to vitamin D therapy include individuals with genetic abnormalities in vitamin D metabolism, co-morbidities such as chronic kidney disease, granuloma-forming diseases or hyperparathyroidism should be identified and treated with lower dosing [5, 20, 30].

V. Clinical application of vitamin D

- **Prophylaxis of rickets and osteomalacia in children and adults/ Prophylaxis of rickets in preterm newborns/ Treatment of rickets and osteomalacia in children and adults;**

Vitamin D deficiency results in inadequate absorption of Ca^{2+} and phosphate. The consequent decrease of plasma Ca^{2+} concentration stimulates PTH secretion, which acts to restore plasma Ca^{2+} at the expense of bone. Plasma concentrations of phosphate remain subnormal because of the phosphaturic effect of increased circulating PTH [2, 17]. In adults, vitamin D deficiency results in osteomalacia, a disease characterized by generalized accumulation of undermineralized bone matrix. Severe osteomalacia may be associated with extreme bone pain and tenderness. Muscle weakness, particularly of large proximal muscles, is typical and may reflect both hypophosphatemia and inadequate vitamin D action on muscle. Gross deformity of bone occurs only in advanced stages of the disease. Circulating 25(OH) D concentrations below 8 ng/ml are highly predictive of osteomalacia[17].

In children, the result is a failure to mineralize newly formed bone and cartilage matrix, causing the defect in growth known as rickets [48, 49]. As a consequence of inadequate calcification, bones of individuals with rickets are soft, and the stress of weight bearing gives rise to bowing of the long bones [17]. The decreased resistance to deformation associated with the forces of normal muscle activities during weight-bearing, such as crawling and walking. In children, rickets is also associated with osteomalacia, associated with a delay in mineralization of preformed osteoid at the cortical and trabecular bone surfaces and is typically associated with a marked increase in osteoid surface and thickness with prolongation of the mineralization lag time and reduction in the mineral apposition rate on bone histomorphometry. Osteomalacia in association with secondary hyperparathyroidism predisposes the affected child to fragility fractures and possibly to an increase in deformation of the long bones [17, 48, 49, 56].

The therapeutic efficacy of cholecalciferol in the prophylaxis and therapy of rickets and osteomalacia is based on many decades of medical experience as well as on numerous controlled and uncontrolled clinical trials, and is clearly proven [48, 49, 56, 57]. Vitamin D_3 therapy of rickets and osteomalacia leads to an improvement of clinical symptoms, a normalization of laboratory values (vitamin D, PTH, calcium and phosphate) and an improvement of bone deformities [57].

Rickets can still be observed among children and adolescents living in Europe, and a significant proportion of healthy children and adolescents presents serum 25(OH)D values below the threshold indicating an insufficient vitamin D status. Optimal serum levels of 25(OH)D. There is no consensus on optimal levels of vitamin D as measured in serum. Severe vitamin D deficiency is defined by most experts as a 25(OH)D level of less than 20 ng/ml (50 nmol/l) and light deficiency (insufficiency) as a 25-hydroxyvitamin D level of less than 30ng/ml (75 nmol/l). A recent consensus panel suggested that 25ng/ml (62.5 nmol/l) may be the minimal acceptable 25(OH)D level in children [42].

- **Definition of Vitamin D deficiency and monitoring guidelines**

Lips has classified vitamin D insufficiency into mild (serum 25(OH)D 25–50 nmol/L), moderate (12.5–25 nmol/L) and severe (<12.5 nmol/L) insufficiency, which are broadly associated with <15%, 15–30% and >30% increases in PTH, respectively [20]. In contrast, North American experts and authorities have suggested that the optimal serum 25(OH) D concentration may be as high as 80–100 nmol/L [5, 10, 21, 22].

As mentioned above, the main manifestation of vitamin D deficiency is osteomalacia in adults and rickets in children [47-49], which are generally associated with a serum 25 hydroxyvitamin D (25(OH) D) concentration of less than 20 nmol/L[20]. Less severe vitamin D deficiency, sometimes termed vitamin D insufficiency, may lead to secondary hyperparathyroidism, bone loss, muscle weakness, falls and fragility fractures in older people [17, 20].

Vitamin D status is currently best assessed by measurement of serum 25(OH)D. As there is a broad inverse relationship between serum 25(OH)D and parathyroid hormone (PTH), the threshold serum 25(OH)D concentration below which PTH increases above the normal range has been used to define biochemical criteria for vitamin D insufficiency. However, the inverse relationship between serum 25(OH) D and PTH may be influenced by age, calcium intake, physical inactivity, renal function, ethnicity, magnesium status and vitamin D binding protein. Furthermore, the use of different assays for25(OH)D and PTH may also influence the apparent threshold 25(OH)D concentration at which secondary hyperparathyroidism occurs. As a result, there is no clear consensus on the biochemical criteria that define vitamin D deficiency and insufficiency [20].

The US-Institute of Medicine (IOM) report on Dietary Reference Intakes for Calcium and Vitamin D investigated the relationship between vitamin D status and bone health, using evidence from two systematic reviews commissioned by the Agency for Healthcare Research and Quality (AHRQ), from the University of Ottawa and the Tufts Evidence-Based Practice Centre [20, 23]. These examined the relationship between serum 25(OH)D as a marker of vitamin D status and PTH, calcium absorption, calcium balance, bone mineral density (BMD), fracture risk and rickets/osteomalacia as potential indicators of bone health. The two AHRQ groups also investigated the relationship between vitamin D status and physical performance, including falls. From these analyses, the IOM highlighted that studies have demonstrated different threshold serum 25(OH)D concentrations above which PTH reaches a plateau, ranging from <30 nmol/L to 100–125 nmol/L. The IOM also suggested that most people with a serum 25(OH)D between 30 and 50 nmol/L have adequate calcium absorption [20].

- **Effect of Vitamin Dsupplementation on absolute change in 25(OH)D concentrations.**

An exhaustive systematic review of Cranney commissioned by the Institute of Medicine (IOM) has analyzed a total of 74 RCTs in 81 published reports that evaluated the effect of vitamin D supplementation on circulating 25(OH)D concentrations [23].

Seven trials included infants, but few of these studies used vitamin D_3. One trial suggested that 200 IU of vitamin D_2may not be enough to prevent vitamin D deficiency, in some infants residing at northern latitudes. A dose-response was noted in this same trial (100, 200, 400 IU/day). Consistent responses to vitamin D supplementation were noted across the seven trials, and some trials suggested that infants who are vitamin D deficient, may respond differently and require higher doses of vitamin D [23].

There were four trials that examined the effect of vitamin D on 25(OH)D in children or adolescents with doses ranging from 200 to 2,000 IU of vitamin D_3/ day and 400 IU of vitamin D_2. In these investigations there were consistent increases in 25(OH)D concentrations ranging from 8 nmol/L (200 IU), 16.5 (with 600 IU D3) to 60 nmol/L (2,000 IU of vitamin D_3) [23].

The systematic review identified and analyzed six small trials of vitamin D supplementation in pregnant or lactating women. No randomized trials studied the effect of 400 IU vitamin D_3. Three trials used 1,000 IU of vitamin $D_2$and one trial used 1,000 IU of vitamin D_3. Treatment with 1,000-3,600 IU/day of vitamin D_2 and 1,000 IU/day of vitamin D_3resulted in significant increases in serum 25(OH)D concentrations in lactating mothers and in cord blood. One trial found that supplementation of lactating mothers with 1,000 IU of vitamin D_2during winter months did not increase serum 25(OH)D concentrations in the infants [23].

Ten small trials included premenopausal women and younger males. Three trials compared vitamin D_2 to vitamin D_3 in healthy young adults. Of these, one trial analyzed content of the tablets. Doses of vitamin D_3 ranged from 600 to 10,000 IU/day and vitamin D_2 (4,000 IU/day or 50,000 to 100,000 for one dose). Three trials found that vitamin D_2 and D_3 in healthy adults may have different effects on serum 25(OH)D concentrations. Vitamin D_2 appeared to have a smaller effect on serum 25(OH)D, which may have been due to more rapid clearance and/or different metabolism than vitamin D_3. One trial compared 100,000 IU vitamin D_2 orally versus injection and found a greater variability in response with the intramuscular preparation. A dose-response effect was noted in those trials that used multiple doses of vitamin D_3 [23].

This systematic reviewalso analyzed 44 trials that were conducted exclusively in postmenopausal women and older men, with 14 of these in elderly populations living in long-term care or nursing homes. One trial was in early postmenopausal women. Doses of vitamin D_3 ranged from 100 to 4000 IU/day and 9,000 IU vitamin D. One trial was conducted in African American women. One trial found that wintertime declines in serum 25(OH)D were prevented with 500 IU of vitamin D_3 daily. A dose response with increasing doses

of vitamin D_3 was noted although there was a variability in response to similar doses across trials that may have been due to differences in serum 25(OH)D assays or baseline 25(OH)D status. Although some trials suggested a greater response to vitamin D in populations that were vitamin D deficient at baseline compared to those who were not, this was difficult to assess due to heterogeneity of assays [23].

Of the total of 44 RCTs investigated the effect of oral vitamin D_3 supplementation (± calcium) versus no treatment, placebo or calcium on serum 25(OH) D sixteen trials presented sufficient data to combine results of the absolute change in serum 25(OH)D Concentrations and perform an meta-analysis. Combining the 16 trials with a random effects model demonstrated large heterogeneity of treatment effect, but nevertheless the point estimates for each trial consistently favored vitamin D_3 treatment [23].

- **Vitamin D deficiency in chronic kidney disease**

In chronic kidney disease as renal function worsens, low circulating 1,25-dihyrdoxyvitamin D levels, low calcium levels, and high serum phosphate levels lead to secondary hyperparathyroidism (SHPT). SHPT, identified by elevated parathyroid hormone (PTH) levels, is associated with both bone disease (renal osteodystrophy) and, in epidemiologic studies, poor outcomes in dialysis patients [47]. Noteworthy, low 25(OH)D levels in patients with chronic kidney disease (CKD) including end-stage renal disease (ESRD) have been associated with a higher risk of all-cause mortality and a faster progression of kidney disease. In the general population, low 25(OH)D levels have also been associated with all-cause mortality, cardiovascular events, peripheral vascular disease, hypertension, congestive heart failure, and the later need for renal replacement therapy. Low $1,25(OH)_2D$ levels have been associated with all-cause mortality [51, 53].

Before the advent and clinical introduction of calcitriol, patients with end stage renal disease (ESRD) were treated with high doses of nutritional vitamin D (ergocalciferol or cholecalciferol) to treat SHPT. Unfortunately, most of the studies of its effects during that time are small and observational in nature [53]. Once calcitriol was introduced and subjected to more rigorous, although small, randomized controlled trials, it quickly became the mainstay of therapy for SHPT [53]. Nevertheless clinical evidence clearly indicates that cholecalciferol has a role in renal patients with vitamin D deficiency (see below).

Recent studies including two Cochrane reviews confirm that in both dialysis and predialysis CKD patients (4 studies, 153 patients), calcitriol and vitamin D compounds decrease PTH (−196 pg/ml [95% CI, −298 to −94] in dialysis patients; −49 pg/ml [95% CI, −86 to −13] in predialysis patients) but increase serum phosphate and calcium levels. Not enough data exist from randomized clinical trials to draw conclusions about patient-level outcomes such as fractures, mortality, or need for dialysis in predialysis patients [53].

Observational studies of vitamin D in CKD

A plethora of observational studies have shown an association between the use of active vitamin D therapy in patients on dialysis and with CKD and improved survival. These range from larger studies from databases of dialysis providers to smaller cohort studies [51, 53].

Active vitamin D therapy has been associated with slower progression to ESRD. There are a few studies in the literature in which an association between activated vitamin D use and improved survival was not found. One of these studies showed a mortality benefit for vitamin D (combining both oral and intravenous vitamin D analogs) using traditional models and marginal structural models but not when using a more complicated modeling system called instrumental variable models. The other study did show an association with improved all-cause mortality but not with specific causes of mortality such as cardiovascular or infection, suggesting the possibility that perhaps all of these specific causes are influenced by vitamin D and thereby diluting the effect on any individual one [53].

Randomized clinical trials

There have been few recent trials of nutritional vitamin D in CKD, which are outlined in **Table 3**. In summary, the findings from these trials and meta-analyses thereof indicate that both nutritional and active vitamin D therapies have been shown to lower PTH levels, the primary indication for their use. The evidence is stronger, with larger, better designed, clinical trials for active vitamin D analogs [51, 53]. Nevertheless a small recent trial comparing doxercalciferol and cholecalciferol showed no difference in end-of-treatment PTH levels between active and nutritional vitamin D compounds [52].

Cholecalciferol vs. active (non-pro-drug) vitamin D analogs

The available clinical evidence suggests an important role of vitamin D in patients with CKD and ESRD and potentially in the general population [47,

Table 3. *Synopsis of randomized trials of cholecalciferol in CKD patients [50, 51, 53].*

Study	Design	Sample Size	Control Group	Results	Limitations
Chandra et al.	Double-blind, placebo-controlled, randomized controlled pilot study	20	Yes	Among cholecalciferol-treated participants, serum 25(OH)D concentration increased on average from 17.3 ng/ml (95% CI, 11.8–25.2) at baseline to 49.4 ng/ml (95% CI, 33.9–-72.0) at week 12. As-treated analysis indicated a trend toward lower PTH levels among cholecalciferol-treated participants (P=0.07)	Small study Short follow-up period
Dogan et al.	Randomized	40	Yes	Administration of depot oral cholecalciferol (300,000 IU vitamin D_3) resulted in a significant increase in calcidiol (6.8±3.5 to 17.8±21.4 ng/ml, P<0.001), significant decrease in iPTH (368±274 to 279±179 pg/ml, P<0.001). No statistically significant change in Ca, P, Ca × P, and urinary calcium creatinine rate was observed	Small study Short follow-up period Methodology does not specify whether investigators were blinded to the intervention
Oksa et al.	Randomized	87	No	Vitamin D insufficiency/deficiency in CKD significantly improved after the 12-mo cholecalciferol treatment, with more significant improvement with higher dose (20,000 IU/wk) being more effective and equally safe	Lack of a placebo control The inclusion of a subgroup of patients who received calcium carbonate for correction of metabolic acidosis is a potential confounder
Kovesdy et al.	Randomized, not blinded	80	Active	80 CKD patients randomized to ergocalciferol versus paricalcitol. Paricalcitol group showed lower PTH levels than ergocalciferol group	Not blinded Differential initiation of phosphate binders in the two groups

95% CI, confidence interval; PTH, parathyroid hormone.

51]. There are different roles for nutritional and active vitamin D compounds (**Table 4**). Nutritional vitamin D (e.g. cholecalciferol) may play more of a role in infections, whereas active vitamin D compounds - in albuminuria and mortality. Both nutritional and active vitamin D eventually affect the same vitamin D receptor; however, nutritional vitamin D has to undergo additional activation in the body, potentially at sites distant from the kidney [53].

Active vitamin D analogs has been shown to decrease albuminuria, blood pressure and modify glomerular filtration in patients with diabetic kidney disease. There are current ongoing studies to test these outcomes with nutritional vitamin D compounds as well. It is important to mention that there are very few data about combining therapy with both nutritional and active vitamin D compounds; thus, caution should be used in clinical practice be-

Table 4. *Clinical role of cholecalciferol vs. active vitamin D analogs in CKD patients [53].*

Outcome	Active vitamin D analog (A)[1] or cholecalciferol (D)	Proposed Mechanism	Level of Evidence
• PTH SUPPRESSION	A, possibly D	Direct suppression of parathyroid gland	A: RCT
			N: limited RCTs
• **Reduction of albuminuria**	A, possibly D	Suppression of renin-angiotensin system	A: RCT
			N: limited RCTs
• **Reduced risk of infections**	D	Increase in cathelicidin levels	Observational studies in general population only
• **Reduction of BP**	A, possibly D	Suppression of renin-angiotensin system	A: RCT
• **Progression of kidney disease**	A, possibly D	Suppression of renin-angiotensin system	A: Observational studies only
			N: low levels associated with outcome
• **Cardiovascular effects (left ventricular hypertrophy and vascular calcification)**	A	Suppression of renin-angiotensin system, possibly direct effects on myocytes	Animal studies
• **Mortality**	A	Likely multiple mechanisms	Observational studies only

[1]Active vitamin D analogs: calcitriol (D_3 analog); paricalcitol (D_2 analog); doxercalciferol (D_2 analog); maxacalcitol (D_3 analog); 1-alfa-calcidiol (D_3 analog); 22-oxacalcitriol (D_3 analog)

cause of worry about possible vitamin D intoxication, manifested by hypercalcaemia and possibly vascular calcifications [51, 53].

The fact that the 1α-hydroxylase enzyme has been found in parts of the body outside the kidney suggests that there is a scope for cholecalciferol use in patients with kidney disease. A recent study in hemodialysis patients showed that 1,25-dihydroxvitamin D levels increased after supplementation with nutritional vitamin D, suggesting that even in ESRD there is enough extrarenal 1α-hydroxylase activity to influence serum levels. This is supported by the clinical findings cohort study of 158 hemodialysis patients who received cholecalciferol supplementation in a nonrandomized study showed higher 25(OH)D, 1,25-dihydroxyvitamin D, and albumin levels, while at the same time reducing serum calcium, PTH, brain natriuretic peptide, left ventricular mass index, and erythropoietin stimulating agent and active vitamin D doses [53].

A study of seven hemodialysis patients who underwent cholecalciferol supplementation reported that after supplementation, there were lower levels of proinflammatory cytokines, IL-8, IL-6, and TNF and differences in circulating monocyte proteins. These studies are small and are not randomized clinical trials; however, their results suggest that nutritional vitamin D may be needed in patients with kidney disease [53].

Vitamin D insufficiency is common in women of childbearing age and increasing evidence suggests that the risk of osteoporotic fracture in adulthood could be determined partly by environmental factors during intrauterine and early postnatal life [7, 62]. Noteworthy maternal vitamin D insufficiency is common during pregnancy and is associated with reduced bone-mineral accrual in the offspring during childhood; this association is mediated partly through the concentration of umbilical venous calcium. A large

body of evidence suggest that vitamin D supplementation during pregnancy is safe at supplemental doses [64], although recent evidence suggests that even pharmacological doses are well tolerated and devoid of adverse effects [65].

In a very recent comprehensive meta-analysis of 74 studies of vitamin D supplementation during pregnancy, Harvey et al. identified positive relationships between maternal 25(OH)D and: 1) offspring birth weight (in meta-analysis of three observational studies) (2) offspring cord blood or postnatal calcium concentrations in a meta-analysis of six intervention studies; and (3) offspring bone mass (in observational studies judged to be of good quality) [63].

A longitudinal study has been allocated that studied 198 children born in 1991-92 in a hospital in Southampton, UK; the body build, nutrition, and vitamin D status of their mothers had been characterized during pregnancy. The children were followed up at age 9 years to relate these maternal characteristics to their body size and bone mass. It was found that 49 (31%) mothers had insufficient and 28 (18%) had deficient circulating concentrations of 25(OH)-vitamin D during late pregnancy. Reduced concentration of 25(OH)-vitamin D in mothers during late pregnancy was associated with reduced whole-body ($r=0.21$, $p=0.0088$) and lumbar-spine ($r=0.17$, $p=0.03$) bone-mineral content in children at age 9 years. Both the estimated exposure to ultraviolet B radiation during late pregnancy and the maternal use of vitamin D supplements predicted maternal 25(OH)-vitamin D concentration ($p<0.0001$ and $p=0.0110$, respectively) and childhood bone mass ($p=0.0267$). Reduced concentration of umbilical-venous calcium also predicted reduced childhood bone mass ($p=0.0286$). These findings imply that vitamin D supplementation of pregnant women, especially during winter months, could lead to long-lasting reductions in the risk of osteoporotic fracture in their offspring [61].

The dose-response, safety and effectiveness of vitamin D supplementation during pregnancy have been evaluated in a randomized, controlled trial. The study enrolled women with a singleton pregnancy at 12 to 16 weeks' gestation, which received 400, 2000, or 4000 IU of vitamin D_3 per day until delivery. The primary outcome was maternal/neonatal circulating 25-hydroxyvitamin D concentration at delivery, with secondary outcomes of a 25(OH)D concentration of 80 nmol/L or greater achieved and the 25(OH)D concentration required to achieve maximal 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) production. Of the 494 women enrolled, 350 women continued until delivery: mean 25(OH)D concentrations by group at delivery and 1 month before delivery were significantly different ($p < 0.0001$), and the percent who achieved sufficiency was significantly different by group, greatest in 4000-IU group ($p < 0.0001$). The relative risk (RR) for achieving a concentration of 80 nmol/L or greater within 1 month of delivery was significantly different between the 2000- and the 400-IU groups (RR = 1.52), the 4000- and the 400-IU groups (RR = 1.60) but not between the high dose 4000- and 2000-IU groups (RR = 1.06). Circulating 25(OH)D had a direct influence on circulating $1,25(OH)_2D_3$concentrations throughout pregnancy ($p < 0.0001$), with maximal production of $1,25(OH)_2D_3$ in all strata in the 4000-IU group. There were no differences between groups on any safety measure. Not a single adverse event was attributed to vitamin D supplementation or circulating 25(OH)D levels. These findings indicate that vitamin D supplementation of 4000 IU/d for pregnant women is safe and most effective in achieving sufficiency in all women and their neonates regardless of race, whereas the current estimated average requirement is comparatively ineffective at achieving adequate circulating 25(OH)D concentrations, especially in African Americans [65].

In addition to pediatric bone outcomes, there is evidence to suggest that a low vitamin D in pregnancy may affect other obstetric outcomes for the mother that impact on fetal and infant health. For example, in a nested case-control study, mothers who developed severe pre-eclampsia had a low 25(OH)D in mid-gestation [66]. This supports other evidence in the literature that vitamin D deficient mothers may be at greater risk of pre-eclampsia[56].

- **Other deficiency states**

Zaniew & Jarmolinski have assessed the vitamin D status and bone density in steroid-treated children with glomerulopathies and to evaluate the effect of prophylactic vitamin D and calcium supplementation[67]. To address this issues a retrospective analysis was performed on 55 children aged 4-18 yrs with glomerulopathies. The following data were analyzed: antropometrical parameters, bone densitometries, parathormone, 25(OH)D, urinary calcium excretion and medications received for prevention of low bone mass. A significant number of children (38%) had decreased spinal bone mineral density (BMD z-score < -2.0) and the majority of them (89%) had hypovitaminosis D (25(OH)D< 30 ng/ml), 75% were vitamin D insufficient (25(OH)D < 20 ng/ml) and 16%

were vitamin D deficient (25(OH)D < 10 ng/ml). The mean serum 25(OH)D concentration was comparable to that of controls). Nearly all patients (82%) were receiving preparations of calcium and/or vitamin D to improve bone health. Patients on cholecalciferol had higher mean concentration of 25(OH)D compared to those who were not receiving it (p=0.027) and to the controls (p=0.047). In 23 children on vitamin D and calcium supplementation for an average 6-month time, an increase in the mean BMD values (p=0.004) was observed, however, mean BMD z-score and 25-OHD concentrations did not significantly change over time. These findings imply that vitamin D and bone density deficits are remarkably common in steroid-treated children with glomerulopathies, despite vitamin D and calcium repletion, advocating close monitoring and dose tailoring [67].

Intestinal malabsorption syndromes (e.g., short bowel syndrome, pancreatitis, inflammatory bowel disease, amyloidosis, celiac sprue, and malabsorptive bariatric surgery procedures) are all associated with impaired vitamin D absorption as a result of either rapid transit or enhanced fecal loss of 25-hydroxyvitamin D because of impaired enterohepatic circulation [68]. Similarly, patients with chronic severe parenchymal and cholestatic liver disease frequently exhibit vitamin D deficiency resulting from the associated malabsorption syndrome combined with a decreased hepatic capacity to convert vitamin D to 25-hydroxyvitamin D [17]. Deficiency states associated with malabsorption may require pharmacological amounts of vitamin D (10–100,000 IU/d). For example, patients with malabsorptive gastric bypass procedures may require 50,000-100,000 IU of vitamin D_3maintenance dosing from once weekly to as frequently as daily to maintain sufficiency [17, 68]. In extreme malabsorptive states, UVB exposure (i.e., sunlight or phototherapy) can be effective for those who do not respond to large oral doses.Vitamin D for intramuscular administration is a possible alternative in cases of severe malabsorption. In the presence of malabsorption, concurrent magnesium depletion must always be considered [68].

No randomized clinical trials have been allocated.

- **Treatment of hypoparathyroidism and pseudohypoparathyroidism in adults;**

Hypoparathyroidism is a deficiency of effective parathyroid hormone [47]. The condition can arise from a failure of the glands to secrete the hormone or a failure of the tissues to respond to it (pseudohypoparathyroidism) [46]. The most common cause of hypoparathyroidism is surgical excision of or damage to the parathyroid glands as a result of total thyroidectomy, radical neck dissection, or repeated operations for primary hyperparathyroidism. The frequency of impaired parathyroid function following such operations is generally related to the amount of tissue resected and the experience and skill of the surgeon. In some cases, autotransplantation of parathyroid tissue at the time of neck surgery is indicated to prevent hypoparathyroidism. The nonsurgical causes of parathyroid gland destruction are relatively uncommon [17].

The major goal of therapy in all hypoparathyroid states is to restore serum calcium and phosphorus to satisfactory levels so as to prevent symptoms of hypocalcemia and progression of long-term complications. Although lowering of serum phosphate levels with diets low in phosphate (e.g., restricting dairy products and meat) and oral non-absorbable aluminum hydroxide gels (to bind intestinal phosphate) might be expected to increase the conversion of 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D, such treatment has received little attention. Rather, treatment with supplemental calcium and vitamin D has been the mainstay of therapy [17, 18, 46].

Generally, at least 1 g/day of elemental calcium is required in hypoparathyroid patients under 40 yr of age and 2 g/d in patients over 40 yr. The amount of supplemental calcium necessary for adequate control may vary from 1 g/d (even in the absence of vitamin D supplements) in mild, partial hypoparathyroidism to 5–10 g/d (plus supplemental vitamin D) in more severe cases. Supplements can be provided by administering calcium as the gluconate, lactate, carbonate, or citrate salt [17].

In patients with less severe parathyroid insufficiency, oral calcium supplementation alone may be adequate. However, in most patients, therapy with vitamin D or one of its analogs is also required to prevent hypocalcemia. Noteworthy the efficacy of vitamin D precursors (vitamin D_2 and D_3) has been evaluated in clinical trials, as reviewed elsewhere [18] (**Table 4**) and are the least expensive form of therapy [17]. Many patients with hypoparathyroidism can be effectively (and inexpensively) treated with vitamin D supplements. Doses of 50,000 IU to 100,000 daily may be required, depending on the severity of hypoparathyroidism, the individual response, and the dose of calcium supplement. Generally, the vitamin D dose can be a stable foundation of therapy, and titration of the calcium supplement can be used to respond to the inevitable fluctuations in calcium control. It is ideal

to identify a dose of vitamin D that allows the patient a modest requirement for calcium supplementation, as a need for large daily calcium doses soon becomes onerous [17].

Importantly, the onset of the actions of vitamin D and the waning of its effects after a reduction in dose are usually quite delayed. Vitamin D is highly stored in adipose tissue and this depot is slow to accumulate and release its stores. This means that dose adjustments must be performed at 1- to 2-mo intervals, with careful assessments of biological response (serum calcium levels) before the next adjustment is made. These decisions can be challenging because of the variability in serum calcium levels in this situation. The careful identification of trends in serum calcium levels is required. The delayed onset of action and the delay in the reduction in action can be disadvantageous [17]. For these reasons, analogs with shorter half-lives and no requirement for renal 1-α hydroxylation (such as dihydrotachysterol and calcitriol) are preferred in some patients [17, 47]. The peculiarities of vitamin D substances and analogs for the pharmacotherapy of hypoparathyroidism states are summarized in **Table 5**.

- **Vitamin D and osteoporosis**

Persistent, mild insufficiency of vitamin D has been well-appreciated as one cause of osteoporosis and much evidence indicates that muscle function in elderly people is adversely affected by vitamin D insufficiency [7, 10, 20, 31, 41]. Vitamin D deficiency is common in patients with osteoporotic fractures, with two studies showing 95-97 percent of fracture patients being classified as vitamin D deficient [39]. In an exemplary study Nurmi et al. have analyzed the serum 25(OH)D status in 223 patients with an acute hip fracture in southeastern Finland (61°N). Severe vitamin D deficiency, defined as 25(OH)D<37.5nmo-

Table 5. *Vitamin D precursors and analogs for treatment of hypoparathyroidism states – dosage and monitoring [17, 18, 46]*

Vitamin D compounds	Dosage	Comments	Safety issues
Vitamin D_2 (ergocalciferol) or vitamin D_3 (cholecalciferol)	25,000–100,000 IU once daily; time to onset of action, 10–14 days; time to offset of action, 14–75 days	Vitamin D_3 is more potent than D_2 but may be more difficult to obtain at the doses needed. Because of the long half-life of vitamin D_2 and D_3, dosing can be adjusted and serum levels of calcium, albumin, phosphorus, and creatinine determined every 4 weeks, once symptoms have been controlled.	Vitamin D toxicity is an important concern and may occur at any time. Manifestations may include altered mental status, fatigue, thirst, dehydration, reduced renal function, nephrolithiasis, and constipation. Treatment involves discontinuation of the vitamin D preparation and the calcium salt. Depending on the severity, and especially if the toxic effects are from vitamin D metabolites with long half-lives, intravenous saline hydration and possibly oral glucocorticoids may be warranted to antagonize vitamin D action and more rapidly restore normocalcemia. Levels of 25-hydroxyvitamin D should be monitored, even in patients receiving calcitrol and alfacalcidiol to prevent vitamin D insufficiency. The target 25-hydroxyvitamin D level is 30 ng/ml or more.
1,25-dihydroxyvitamin D_3 (calcitriol)	0.25–1.0 μg once or twice daily; time to onset of action, 1–2 days; time to offset of action, 2–3 days	Most active metabolite of vitamin D at the vitamin D receptor in vivo	
1α-hydroxyvitamin D_3 (alfacalcidiol)	0.5–3. 0 μg occasionally up to 5. 0 μg) daily; time to onset of action, 1–2 days; time to offset of action, 5–7 days	This metabolite is rapidly converted to 1,25-dihydroxyvitamin D_3 in vivo; its duration of action closely resembles that of 1,25-dihydroxyvitamin D	
Dihydrotachysterol	0.2–1.0 mg once daily; time to onset of action, 4–7 days; time to offset of action, 7–21 days	Synthetic analogue	

l/l, was found in 53% of the patients. Half (50%) of the patients living in their own homes, 55% of those in residential homes, and 61% of institutionalized elderly had vitamin D deficiency. Similarly another study as shown an 25(OH)D level < 39 ng/ml in more than 97% of all patients hospitalized for fracture [69].

To assess the therapeutic efficacy of cholecalciferol in osteoporosis, randomized placebo-controlled studies were conducted and have been analyzed in comprehensive systematic reviews and meta-analyses [7, 20, 41]. There is international consensus that a patient suffering from osteoporosis should receive calcium therapy. Most studies have investigated, therefore, a combination of vitamin D + calcium versus placebo. Therapy-induced reduction of fracture incidence was regarded as the most important primary target parameter. Bone mineral density (BMD) is often determined as a surrogate parameter, in many of the controlled studies, while in some trials the number of falls was considered [23]. The available meta-analyses of vitamin D efficacy in end-points relevant to osteoporosis are summarized in **Table 5**, as compiled elsewhere [7].

Effects on bone mineral density (BMD)

In a comprehensive systematic review of vitamin D supplementation effects on bone health, prepared for the Agency for Healthcare Research and Quality U.S. Department of Health and Human Services, Cranney et al. analyzed seventeen RCTs that focused on the effect of supplemental vitamin D_2 or D_3 on BMD, predominantly in populations of late menopausal women. Only one small trial included premenopausal women. Most trials had small sample sizes, were two to three years in duration and used vitamin D doses of ≤ 800 IU daily. Most trials used vitamin D_3 and also included calcium > 500 mg as a co-intervention. Combined results of trials of vitamin D_3 plus calcium versus placebo were consistent with a small effect on lumbar spine, femoral neck and total body BMD. In one of the studies conducted by the Women Health Initiative a significant benefit of vitamin D_3 400 IU plus 1,000 mg of calcium on total hip BMD was found. Nevertheless, in combined trials of vitamin D_3 plus calcium versus calcium, a significant increase in BMD was not observed, suggesting vitamin D_3 may be of less benefit in calcium replete postmenopausal women. Vitamin D_3 alone versus placebo did not show significant increases in BMD, except in one trial that noted an increase in femoral neck BMD. Only a few trials reported the impact of baseline serum 25(OH)D concentrations on BMD and in all of these trials, baseline 25(OH)D was not associated with increased BMD. Overall, there is good evidence that vitamin D_3 plus calcium results in small increases in BMD of the spine, total body, femoral neck and total hip. Based on included trials, it was less certain if vitamin D_3 alone has a significant effect on BMD [23].

Effects on rates of falls and fractures

Chapui et al. have studied the effects of supplementation with vitamin D_3 (cholecalciferol) and calcium on the frequency of hip fractures and other nonvertebral fractures, identified radiologically, in 3270 healthy ambulatory women (mean age, 84 years). Each day for 18 months, 1634 women received tricalcium phosphate (containing 1.2 g of elemental calcium) and 20 micrograms (800 IU) of vitamin D_3, and 1636 women received a double placebo. The authors measured serial serum parathyroid hormone and 25-hydroxyvitamin D (25(OH)D) concentrations in 142 women and determined the femoral bone mineral density at base line and after 18 months in 56 women. Among the women who completed the 18-month study, the number of hip fractures was 43 percent lower ($p = 0.043$) and the total number of nonvertebral fractures was 32 percent lower ($p = 0.015$) among the women treated with vitamin D_3 and calcium than among those who received placebo. The results of analyses according to active treatment and according to intention to treat were similar. In the vitamin D_3-calcium group, the mean serum parathyroid hormone concentration had decreased by 44 percent from the base-line value at 18 months ($p < 0.001$) and the serum 25(OH)D concentration had increased by 162 percent over the base-line value ($p < 0.001$). The bone density of the proximal femur increased 2.7 percent in the vitamin D_3-calcium group and decreased 4.6 percent in the placebo group ($p < 0.001$). These findings indicate that supplementation with vitamin D_3 and calcium reduces the risk of hip fractures and other nonvertebral fractures among elderly women [70].

Trivedi et al. sought to determine the effect of four monthly vitamin D supplementations on the rate of fractures in men and women aged 65 years and over living in the community. Randomized double blind controlled trial of 100 000 IU oral vitamin D_3 (cholecalciferol) supplementation or matching placebo every four months over five years. 2,686 people (2,037 men and 649 women) aged 65-85 years living in the general community, recruited from the British doctors register and a general practice regis-

ter in Suffolk. After five years 268 men and women had incident fractures, of which 147 had fractures in common osteoporotic sites (hip, wrist or forearm, or vertebrae). Relative risks in the vitamin D group compared with the placebo group were 0.78 (95% confidence interval 0.61 to 0.99, p=0.04) for any first fracture and 0.67 (0.48 to 0.93, p=0.02) for first hip, wrist or forearm, or vertebral fracture. 471 participants died. The relative risk for total mortality in the vitamin D group compared with the placebo group was 0.88 (0.74 to 1.06, p=0.18). Findings were consistent in men and women and in doctors and the general practice population [71].

The relative contributions of vitamin D and calcium for reducing fracture risk remain unclear because improving calcium intake is also associated with suppression of PTH levels independent of vitamin D status [7]. Noteworthy as proximal muscle weakness is a prominent clinical feature of vitamin D deficiency the supportive treatment in osteoporosis also decreases the incidence of falls and of fractures respectively [26, 31]. Noteworthy a comprehensive review and meta-analysis of 6 RCT and one controlled trial has shown that vitamin D supplementation significantly increased muscle strength in the experimental group for upper and lower limbs [72].

A meta-analysis of data from RCTs found a dose-response relationship between a higher vitamin D dose and higher achieved serum 25(OH)D levels, with prevention of falls and fractures. The greatest benefit was observed at 700 to 1000 IU/d or a mean serum 25(OH)D concentration of 30 to 44 ng/mL. Similar results were reported in a more recent meta-analysis of pooled participant-level data from 11 double-blind RCTs of oral vitamin D supplementation, with or without calcium, compared with placebo or calcium alone in persons 65 years or older. Reduction in the risk of fracture occurred only at the highest vitamin D intake level (median, 800 IU/d; range, 792-2000 IU/d), with a 30% reduction in the risk of hip fracture and a 14% reduction in the risk of any nonvertebral fracture. This reduction was independent of the assigned treatment dose of vitamin D, age group, sex, type of dwelling, and study [7]

The aforementioned comprehensive systematic review of Cranney et al. included fifteen RCTs evaluated the effect of vitamin D_2 or D_3 (with or without calcium supplementation) on fractures in postmenopausal women and elderly men. The majority of the trials used vitamin D_3 preparations (300 - 800 IU daily). Ten trials were of higher quality although high losses to follow-up and inadequate reporting of allocation concealment were limitations of a number of trials. Vertebral fractures were not included as an outcome in most trials. Vitamin D_3 (700 - 800 IU daily) combined with calcium supplements (500 - 1200 mg) significantly reduced non-vertebral and hip fractures although the benefit was predominantly in elderly subjects living in institutionalized settings (hip fractures, OR 0.69, 95% CI 0.53-0.90) [23].

Recommendations regarding Vitamin D status monitoring in osteoporosis patients

Recent recommendations of the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO) for the optimal management of elderly and postmenopausal women regarding vitamin D supplementation have also indicated that patients with serum 25(OH)D levels less than 20 ng/mL have increased bone turnover, bone loss, and, possibly, mineralization defects compared with patients with serum 25(OH)D levels of 20 ng/mL or greater. Similar relationships have been reported for frailty, nonvertebral and hip fracture, and all-cause mortality, with poorer outcomes at less than 20 ng/mL. Thus, ESCEO recommended that 20 ng/mL be the minimal serum 25(OH)D concentration at the population level and in patients with osteoporosis to ensure optimal bone health. Also, in fragile elderly individuals who are at elevated risk for falls and fractures, ESCEO recommended a minimal serum 25(OH)D level of 30 ng/mL for the greatest effect on fracture [7]. These consideration well correlate to the recommendations of other authorities incl. The UK National Osteoporosis Society and Institute of Medicine (IOM) and the Canadian Agency for Healthcare Research and Quality (AHRQ) [20].

The European Menopause and Andropause Society (EMAS) has published a position paper with expert consensus on vitamin D and postmenopausal health. In this consensus it has been recommended that postmenopausal women with risk factors for low vitamin D status should be screened for serum 25(OH)D status and adequately treated. The marker should be measured at 2–3 months intervals until its level is stabilized in the normal range. Women with serum 25(OH)D levels below 20 ng/mL (50 nmol/L) may need treatment with 4000–10,000 IU/day to achieve adequate levels. If individual risk factors are not modifiable, the appropriate dose should be maintained with an annual serum 25(OH)D measurement. Women with morbid obesity (pre- and post gastrointestinal bypass surgery), malabsorption syndromes, and hepatic or renal diseases require specific tailored

doses of vitamin D supplements. Women with vitamin D deficiency related to osteoporosis and/or previous incidental fractures should receive adequate amounts of vitamin D (800–1200 IU/day if there are no risk factors for low serum vitamin D) and specific bone conserving therapies [41].

- **Other studies and applications**

Muscle weakness is a prominent feature of the clinical syndrome of severe vitamin D deficiency. Clinical findings in vitamin D-deficiency myopathy include proximal muscle weakness, diffuse muscle pain, and gait impairments such as a waddling way of walking. Double-blind RCT demonstrated that 800 IU/d vitamin D_3resulted in a 4–11% gain in lower extremity strength or function, and up to 28% improvement in body sway and up to 72% reduction in the rate of falling in adults older than 65 yr after 5 months of treatment [10]. A recent systematic review with meta-analysis has investigated the effects of vitamin D supplementation on muscle strength in healthy individuals. Six randomized controlled trials and one controlled trial were identified and quality assessment showed all seven trials were of 'good quality'. Data was extracted from 310 adults, 67% female, with mean ages ranging from 21.5 to 31.5 years. Trials lasted from 4 weeks to 6 months and dosages differed from 4,000 IU per day to 60,000IU per week. Upper and lower limb muscle strength had a standardized mean difference of 0.32 (95% CI=0.10, 0.54) and 0.32 (95% CI=0.01, 0.63) respectively, suggesting vitamin D supplementation significantly increased muscle strength in the experimental group for upper (P=0.005) and lower limbs (P=0.04) [72].

Very recently the first randomized trial on the efficacy of vitamin D in fibromyalgia has been published. Thirty women with fibromyalgia according to the 1990 and 2010 American College of Rheumatology criteria, with serum 25(OH)D levels <32 ng/mL (80 nmol/L), were randomized to treatment group or control group. The goal was to achieve serum 25(OH)D levels between 32 and 48 ng/mL for 20 weeks via oral supplementation with cholecalciferol. The control group received placebo medication. Re-evaluation was performed in both groups after a further 24 weeks without cholecalciferol supplementation. The main hypothesis was that high levels of serum 25(OH)D should result in a reduction of pain (visual analog scale score). Additional variables were evaluated using the Short Form Health Survey 36, the Hospital Anxiety and Depression Scale, the Fibromyalgia Impact Questionnaire, and the Somatization subscale of Symptom Checklist-90-Revised. A marked reduction in pain was noted over the treatment period in the treatment group. This also was correlated with scores on the physical role functioning scale of the Short Form Health Survey 36. Thus the optimization of 25(OH)D levels in fibromyalgia syndrome proved to have a positive effect on the perception of pain [73].

Vitamin D deficiency is associated with an increased risk of total mortality [7, 71]. Most, but not all, studies documented increased mortality rates in patients with low 25(OH)D concentrations. In a study of 247,574 individuals from the primary care sector, a reverse J-shaped relation was reported between serum level of 25(OH)D and all-cause mortality, with the lowest mortality rate at 20 to 24 ng/mL. This finding underscores the importance of not only including the very low (4 ng/mL) but also the higher (56 ng/mL) levels of 25(OH)D in the analysis [7].

A meta-analysis of prospective cohort studies including 5,562 deaths of 62,548 participants suggested a nonlinear decrease in mortality risk as circulating 25(OH)D concentration increases, with optimal outcomes occurring at concentrations of approximately 30 to 35 ng/mL. In a similar meta-analysis, vitamin D intake and blood 25(OH)D levels were inversely associated with risk of colorectal cancer, and a 10-ng/mL increase in blood 25(OH)D levels conferred a risk rate (RR) of 0.74. A meta-analysis of prospective studies of 6853 patients with chronic kidney disease found that the mortality risk decreased by 14% per 10 ng/mL increase in 25(OH)D levels. The major cause of mortality was cardiovascular disease [7].

In a recent meta-analysis with 70,528 randomized participants (86.8% females) with a median age of 70 years, vitamin D supplementation with or without calcium reduced mortality by 7%. However, vitamin D supplementation alone did not affect mortality, but risk of death was reduced if vitamin D was given with calcium [7].

There is growing evidence regarding the effects of vitamin D deficiency on various adverse pregnancy outcomes regarding the offspring and the maternal functions [34, 35, 56, 61, 63, 65, 66]. A recent meta-analysis of data from 24 studies found that women with circulating 25(OH)D levels less than 20 ng/mL in pregnancy experienced an increased risk of preeclampsia (OR, 2.09; 95% CI, 1.50-2.90), gestational diabetes mellitus (OR, 1.38; 95% CI, 1.12-1.70), preterm birth (OR, 1.58; 95% CI, 1.08-2.31), and small-for-gestational-age (OR, 1.52; 95% CI, 1.08-2.15) [7]. However, many of these outcomes are rare and require a large sample size to study, representing

Table 6. *Synopsis of meta-analyses of vitamin D on outcomes beyond the scope of the proposed indications, as reviewed elsewhere [7].*

Source Meta-analysis	Included studies	(No.)	Dose/duration	End-points	Effects
Chung et al,2011	19 RCTs (3 for cancer and 16 for fracture outcomes) 28 observational studies (for cancer outcomes)	NA	Vitamin D with or without calcium (limited data from RCTs assessed high-dose vitamin D [1000 IU/d])	Benefits and harms of vitamin D with or without calcium supplementation on clinical outcomes of cancer and fractures	Positive effect of high-dose vitamin D on reduced risk of total cancer Positive effect on fracture Negative effect on renal and urinary tract stones
Buttigliero et al,2011	25 studies (3 randomized trials involving patients with advanced prostate cancer explored the prognostic role of vitamin D supplementation)	1273	1 trial: doxercalciferol 2 trials: calcitriol Duration: 11.7-18.32 mo	Influence of hypovitaminosis D on prognosis of cancer Improvement outcome of vitamin D supplementation	No effect on survival
Bjelakovic et al,2011	50 randomized trials	94,148	Supplemental vitamin D (vitamin D_3 [cholecalciferol] or vitamin D_2[ergocalciferol]) or an active form of vitamin D (1α-hydroxyvitamin D [alfacalcidol] or 1,25-dihydroxyvitamin D [calcitriol]) at any dose, duration, and route of administration vs. placebo or no intervention	Beneficial and harmful effects of vitamin D for prevention of mortality	Positive effect of vitamin D_3 on mortality Negative effect of vitamin D_3combined with calcium on nephrolithiasis Negative effect on hypercalcaemia
Irlam et al,2010	16 additional trials (only 1 trial was single supplements of vitamin D)	22,120 participants in the trials	NA	Reducing mortality and morbidity	No effect

Source Meta-analysis	Included studies	(No.)	Dose/duration	End-points	Effects
Autier et al,2012	76 trials	6207	Doses of 5-250 μg/d (median, 20 μg/d)	Circulating 25(OH)D level	Positive effect of vitamin D_3intake without calcium on serum 25(OH)D concentrations No effect of concomitant use of calcium supplementation and high 25(OH)D concentration at baseline
Bjorkman et al,2009	52 clinical trials	6290	Vitamin D supplementation	Responses of parathyroid hormone	Positive effect in chronically immobile patients on 25(OH)D levels but a slight effect on PTH decrease
Tripkovic et al,2012	17 studies	1016	Varying dosages and treatment periods	Compared the effects of vitamin D_2 and vitamin D_3on serum 25(OH)D concentrations	Positive effect of vitamin D3compared with vitamin D_2 in the raising of serum 25(OH)D concentrations
Kandula et al,2011	22 studies	264	Ergocalciferol or cholecalciferol	Benefits and harms of vitamin D supplementation	Positive effect on 25(OH)D and PTH levels
Song et al,2013	21 prospective studies	81,216	Circulating 25(OH)D	Association between blood levels of 25(OH)D and risk of incident type 2 diabetes	Inverse and significant association between circulating 25(OH)D levels and risk of type 2 diabetes

Source Meta-analysis	Included studies	(No.)	Dose/duration	End-points	Effects
George et al,2012	15 trials	NA	Vitamin D or analogues	Glycemia, insulin resistance, progression to diabetes, and complications of diabetes	No effect on fasting glucose, hemoglobin A1c, or insulin resistance Small positive effect on fasting glucose and insulin resistance in patients with diabetes or impaired glucose tolerance No effect on glycated hemoglobin in diabetic patients
Bath-Hextall et al,2012	11 studies	596	Vitamin D vs. vitamin E	Treating established atopic eczema/dermatitis	Negative effect at high doses
Muir et al, 2011	13 trials	NA	Vitamin D (800-1000 IU/d)	Muscle strength, gait, and balance	Positive effect on balance and muscle strength
Annweiler et al,2009	16 trials	24-33,067	NA	Muscle, balance, and gait performance	No significant effect on balance and gait Positive/no effect on muscle strength No effect on sit-to-stand test
Wang et al,2010	18 trials	NA	NA	Reduce the risk of cardiovascular events	No effect on cardiovascular disease risk
Pittas et al,2010	18 trials	37,162	Vitamin D (400-8571 IU/d) with or without calcium	Cardiometabolic outcomes	No effect on glycemia or incident diabetes, blood pressure, and cardiovascular outcomes

Source Meta-analysis	Included studies	(No.)	Dose/duration	End-points	Effects
Ferguson and Chang,2009	3 double-blind randomized crossover trials	41	800 and 1600 IU of vitamin D alone with or without 1 g of calcium	Respiratory outcomes	No adequate evidence of benefit or harm
Abba et al,2008	12 trials	3393	Several vitamins and minerals and diets	Promote the recovery of tuberculosis	No effect on number of deaths or number of participants with positive sputum test results
Autier and Gandini,2007	18 independent RCTs	57,311	Vitamin D supplements varied from 300 to 2000 IU/d	Any health condition	Not enough evidence for effective decision

a challenge for cohorts with a limited number of preserved samples. Experimental studies have provided evidence of disrupted vitamin D metabolic homeostasis in the preeclamptic placenta and have suggested that increased oxidative stress could be a causative factor of altered vitamin D metabolism in preeclamptic placentas [7].

A meta-analysis of 3 trials involving 463 women suggested that women who received vitamin D supplements during pregnancy less frequently had a baby with a birth weight less than 2500 g than did those who received no treatment or placebo; the statistical significance was borderline.89 In terms of other conditions, there were no significant differences in adverse effects, including nephrotic syndrome, stillbirths, and neonatal deaths, between women who received vitamin D supplements and women who received no treatment or placebo.Hossein-nezhad&Holick indicated a significant inverse relation between serum 25(OH)D level and the incidence of gestational diabetes mellitus. Overall, vitamin D deficiency (25(OH)D<20 ng/mL) in pregnancy was significantly related to the incidence of gestational diabetes mellitus, with an OR of 1.61 [7].

Vitamin D and its metabolites have wide-spread physiological roles far beyond the well described effects in skeletal biology. Many physiological processes are directly or indirectly regulated by vitamin D and in consequence, vitamin D deficiency is implicated in numerous disease conditions. Conversely observational studies have found a decreased risk of many disorders, including certain types of cancer [14, 15, 21, 25], mental disorders [13, 34, 35], infectious disease and inflammation [16, 33], cardiovascular disease [29], type 2 diabetes mellitus [28, 41], and autoimmune disorders [8, 24], associated with serum 25(OH)D levels greater than 28 to 32 ng/mL [7]. It has, therefore, been argued that 25(OH)D levels should be in the range of 28 to 40 ng/mL to maximize these nonskeletal benefits. The results of some clinical trials provide further evidence confirming the results of observational and association studies, whereas others do not [7].

Many of the clinical findings for these non-classical end-points have been discussed in the context of the corresponding in vitro and in vivo preclinical data in the secondary pharmacodynamics Section. **Table 6** summarizes the most important meta-analyses on vitamin D supplementation, comparing the nonbeneficial effects of vitamin D supplementation in randomized trials for nonskeletal outcomes, as comprehensively reviewed elsewhere [7].

VI. SAFETY PROFILE – ADVERSE EFFECTS AND TOXICITY

The risk of minor adverse effects from vitamin D supplementation is considered low even in high dose regimens [5, 7, 23, 30, 31]. Overt vitamin D toxicity manifests itself through chronic hypercalcaemia. It is rarely seen unless the vitamin D dose is very high,

either through inappropriate high-dose treatment or accidental overdosing [23, 30].

The Food and Nutrition Board of the UK Institute of Medicine (IOM) has summarized the evidence from a number of supplementation studies of vitamin D, which covered a range of doses (800 to 300,000 IU/day) and duration (months to years). They concluded that vitamin D below 10,000 IU/day is not usually associated with toxicity, whereas doses equal to or above 50,000 IU/day for several weeks or months are frequently associated with toxicity, including documented hypercalcaemia. Although the IOM report states that toxicity as defined by hypercalcaemia is not seen with a 25(OH)D below 500 nmol/L, there are cases where serious symptoms have been associated with lower 25(OH)D. The European Food Safety Authority (EFSA) has recently reviewed evidence and concluded that an upper limit of 4000 IU (100 μg) a day is safe for adults and children over 11 years of age. Less severe symptoms include hypercalciuria and renal stones. Studies that have shown a reverse-J-shaped curve for the relationship between mortality and 25(OH)D show a beneficial effect as 25(OH)D concentrations increase to 30 nmol/L, with lowest mortality at 50 nmol/L and then increased risk above 75 nmol/L. Yearly high-dose vitamin D is ineffective and may cause increased risk of fracture [20].

High intakes of vitamin D_3, or application to vulnerable patients can cause hypercalcaemia. The high serum calcium potentially leads to soft tissue calcification and resultant renal and cardiovascular damage. Patients with granulomatous disease are at risk of hypercalcaemia because of increased 1α-hydroxylase activity (which converts 25(OH)D to active $1,25(OH)_2$ D). Toxicity has been reported during vitamin D treatment of tuberculosis and in patients with active sarcoidosis [20, 30]. Specialist advice should be sought before starting these patients on vitamin D therapy. Noteworthy, in normal subjects, overall higher serum calcium concentrations were seen with vitamin D treatment at 2400 IU per day and 3800 IU per day compared to the lower doses tested, but only in the higher dose group (3800 IU per day) did this exceed normal limits (10 mg/dL, 2.63 mmol/L) [20].

Clinical evidence suggests an increased incidence of renal stones in those taking vitamin D with calcium supplements. Observational studies have shown that there is increased risk of renal stones with supplemental calcium intake, whereas dietary calcium intake may protect against this. There is no strong evidence that correcting vitamin D deficiency with vitamin D alone will increase the risk of renal stones. However patients with active nephrolithiasis should be managed on a case by case basis [20].

- **Mineral balancedisorders - hypercalemia, hypercalciuria and associated sequelae:**

Vitamin D use especially in overdose or in vulnerable patients is characterized by hypercalcaemia and hypercalciuria as a result both of excessive gastrointestinal calcium absorption and of enhanced mobilization of calcium from bone [30]. These in turn are associated with ectopic calcification and/or nephrocalciniosis. A single high dose of vitamin D can usually be given without ill effects, even though some workers have described subsequent characteristic changes in the concentrations of phosphorus and calcium in the blood. However, long-term administration, especially with intoxication, is characterized by hypercalcaemia and hypercalciuria as a result both of excessive gastrointestinal calcium absorption and of enhanced mobilization of calcium from bone [30].

Hypercalcaemia is not necessarily accompanied by clinical symptoms and disappears within 48 hours when the drug is withdrawn. In patients with hypoparathyroidism or chronic renal insufficiency, the rate of reversal of hypercalcaemia after stopping treatment with the vitamin D analogues is slower than in healthy subjects. In these diseases, frequent control of blood biochemistry is recommended during treatment with vitamin D analogues [30].

An exemplary case of severe hypercalcaemia in a child receiving vitamin D was associated with hyperlipidemia has been allocated. An 11-month-old girl developed polyuria, polydipsia, and vomiting. She had been given vitamin D 400 IU/day for just 1 month. A misdiagnosis of rickets was made, and 1 month before admission she was given bolus vitamin D 600 000 IU in two doses 15 days apart. Her serum calcium concentration was 5.5 mmol/l; total cholesterol, high density lipoprotein cholesterol, very low density lipoprotein cholesterol, low density lipoprotein cholesterol, and triglyceride concentrations were respectively: 6.37 mmol/l, 0.77 mmol/l, 1.37 mmol/l, 4.1 mmol/l, and 3.0 mmol/l. After 16 days of therapy with intravenous fluids, furosemide, glucocorticoids, calcitonin, magnesium sulfate, and phosphorus, her serum calcium concentration fell below 3 mmol/l. Her hyperlipidemia resolved gradually [30].

Metastatic calcification is observed in various tissues, but arterial calcification is the most usual. In some patients undergoing dialysis, calcification of the blood vessels has been so extensive that cannulation could not be performed. Some individuals devel-

op hypertension in response to vitamin D, which in some of them may be directly related to hypercalcaemia and which may be reversible when renal function is normalized [30].

Calcification of the lung from vitamin D is rare, but has been reported in an infant after ingestion of toxic doses [30]. High doses of vitamin D can affect dental development. In one case, caused by incorrectly fortified milk, there was dental hypoplasia and focal pulp calcification [30]. In severe hypervitaminosis D signs of hypomineralization and demineralization of the long bones and calcification of the soft tissues can be seen radiologically. Some cases of hypervitaminosis D with hypercalcaemia and acute renal insufficiency have been reported in patients with osteoporosis or osteomalacia who have taken pharmacological doses of vitamin D for extended periods of time [30].

Metastatic calcification of the pancreatic ducts has also been reported. Importantly, in patients with pancreatitis associated with hypercalcaemia of unclear origin, vitamin D poisoning can be responsible, especially when episodes are recurrent [30].

Calcium deposits in the cornea and the conjunctiva as well as band keratopathy have been reported in patients taking vitamin D. Tympanic membrane calcification can lead to severe, irreversible conductive deafness [30].

Daily doses of more than 40 micrograms of vitamin D can cause calcium deposition in the kidneys [30, 76]. Renal damage is largely due to this. Tubular function is impaired first, glomerular function later. Medullary nephrocalciniosis can develop as a result of therapy with high doses of vitamin D combined with calcium; this is mainly seen in infants and schoolchildren. Ultrasound screening of children treated with high doses of vitamin D and calcium is therefore recommended. Early high-dosage prophylaxis or treatment can cause nephrocalciniosis and hypercalciuria in children [30].

There have been increasing numbers of reports of nephrocalcinosis diagnosed by renal ultrasound in patients with X-linked hypophosphatemia treated with vitamin D and phosphate, and a study of a small group of treated and untreated patients pointed to an association between this therapy and nephrocalcinosis [30].

In a French analysis of 22 510 urinary calculi performed by infrared spectroscopy, drug-induced urolithiasis was divided into two categories: first, stones with drugs physically embedded (1.0%), notably indinavir monohydrate (53%), followed by triamterene (18%), sulfonamides (12%), and amorphous silica (10%); secondly, metabolic nephrolithiasis induced by drugs (0.6%), involving mainly calcium/vitamin D supplementation (40%) and carbonic anhydrase inhibitors (24%) [30].

Hypersensitivity reactions such as pruritus, rash, urticaria have been documented with the drug [19, 30]. Vitamin D_3 has been reported to cause skin hyperreactivity in a patient with pre-existing pseudoxanthoma elasticum. A 68-year-old woman with pseudoxanthoma elasticum was given oral vitamin D_3 0.25 micrograms/day. After 2 weeks she developed new yellow papules on pre-existing plaques on the neck and abdomen, without itching or pain. Biopsy showed a thicker epidermis and more abundant calcium deposition than in a biopsy before treatment. Electron microscopy showed electrondense deposits between the degenerated elastic fibers, which had been surrounded by normal collagen fibers before treatment. After treatment with vitamin D_3 there were lucent areas that suggested unusual mineralization in association with electron-dense deposits. Serum concentrations of calcium were within the reference range throughout [30].

- **Drug Interactions**

Bile acid sequestrant (e.g. cholestyramine or colestipol hydrochloride) administration may result in decreased intestinal absorption of vitamin D analogs; patients taking cholestyramine or colestipol hydrochloride should be instructed to allow as long a time interval as possible between the ingestion of vitamin D analogs and the resin, or these patients may require IM ergocalciferol supplements. Orlistat may result in decreased GI absorption of fat-soluble vitamins such as vitamin D analogs. At least 2 hours should elapse between (before or after) any orlistat dose and vitamin D analog administration; administering fat-soluble vitamins at bedtime may be a convenient time. Although the manufacturer of orlistat recommends that a vitamin supplement containing fat-soluble vitamins (A, D, E, and K) be used during orlistat therapy,such vitamin concentrations in clinical studies with the drug remained within the normal range for most patients despite decreases, and vitamin supplementation was only occasionally needed [19].

Anticonvulsants (barbiturates, primidone, carbamazepine and phenytoin) and other drugs that induce liver enzymes (such as rifampicin, and antiretrovirals) can accelerate the hepatic catabolism of vitamin D and can lead to reduced serum concentrations of calcifediol and osteomalacia. Prophylac-

tic vitamin D treatment of patients taking long-term enzyme inducers can be helpful and should be given at least in cases of anticonvulsant-drug-induced osteomalacia [5, 30].

Isoniazid may reduce the effectiveness of vitamin D_3 due to inhibition of the metabolic activation of vitamin D to hormonally active metabolites [42].

If Vitamin D_3 is combined with metabolites or analogues of vitamin D, careful monitoring of serum calcium levels is recommended [43].

Patients treated with cardiac glycosides may be susceptible to high calcium levels and should have ECG parameters and calcium and possibly digitalis levels monitored [19].

Concomitant administration of thiazide diuretics and pharmacologically related drugs (e.g. clopamide, indapamide) increases the risk of hypercalcaemia because they reduce the urinary excretion of calcium. Clinical evidence has shown that concurrent administration of thiazide diuretics and pharmacological doses of vitamin D analogs in patients with hypoparathyroidism may result in hypercalcaemia, which may be transient and self-limited or may require discontinuance of vitamin D analogs. Thiazide-induced hypercalcaemia in hypoparathyroid patients is probably caused by increased release of calcium from bone. Noteworthy this unusual susceptibility to hypercalcemic effects has been attributed from an interaction with calcium carbonate which had been taken simultaneously [19].

Concomitant administration ofglucocorticoidsmay reducethe effect ofvitamin D_3 [19], whereas anabolic steroids and danazol could augment the hypercalcemic effects of vitamin D [30, 38].

- **Overdose**

For ethical reasons, no systematic studies have examined vitamin intoxication in humans. But numerous anecdotal reports over the years have described accidental vitamin D intoxication with either vitamin D_3 or vitamin D_2;reviewedelsewhere[6, 30, 39].

Symptomatic vitamin D intoxication is extremely rare but can be caused by inadvertent or intentional ingestion of excessively high doses. Doses of more than 50,000 IU per day raise levels of 25-hydroxyvitamin D to more than 150 ng per milliliter (374 nmol per liter) and are associated with hypercalcaemia and hyperphosphatemia. Nevertheless doses of 10,000 IU of vitamin D3 per day for up to 5 months, have been proven to be devoid of adverse effects and toxicity [10].

Noteworthy, patients with chronic granulomatous disorders are more sensitive to serum 25-hydroxyvitamin D levels above 30 ng per milliliter because of macrophage production of 1,25-dihydroxyvitamin D, which causes hypercalciuria and hypercalcaemia[5, 30, 39]. In these patients, however, 25-hydroxyvitamin D levels need to be maintained at approximately 20 to 30 ng per milliliter to prevent vitamin D deficiency and secondary hyperparathyroidism [10].

The initial symptoms of overdose D include weakness, fatigue, lassitude, headache, nausea, vomiting, and diarrhea. Renal function can be impaired at an early stage, with polyuria, polydipsia, nocturia, reduced urinary concentrating ability, and proteinuria [19].

There have been very occasional reports of mental disturbances, including depression, cerebellar ataxia, peripheral facial paresis, apathy, lack of interest, and (in cases of acute poisoning) even stupor. Mental changes can occur before the appearance of any somatic symptoms. There may be a correlation between the seriousness of hypervitaminosis D and the extent of electroencephalographic changes [30].

The characteristic features of chronic intoxication are deposition of calcium salts in various tissues. A follow-up study of 24 children with vitamin D intoxication (13 900–200 000 IU/day) for up to 13 years showed that 23% of the patients had permanent damage. The severity of renal, neurological, and digestive symptoms was related to the daily dose, but the final consequences depended on the duration of overdosage. Vitamin D intoxication has also been seen after so-called ‘‘Stoss’’ prophylaxis in children, that is the use of single very high doses of vitamin D, and it has therefore been suggested that this procedure should be avoided [30].

Because most of the toxicity studiesreported 25(OH)D and sometimes $1\alpha,25(OH)_2D$, it is worth mentioning the vitamin D metabolite values reported with overt toxicity symptoms to draw some dose-response data and monitoring recommendations.Although an occasional report did find evidence of modest elevations of $1\alpha,25(OH)_2D$, all reported that 25(OH)D concentrations were well above the normal range at 710–1587 nmol/L, with several patients exhibiting values consistently around 750 nmol/L [6]. In addition, several reports have documented clusters of patients with vitamin D intoxication due to vitamin D–contaminated food sources. In a study of 35 hypervitaminotic patients with hypercalcaemia resulting from chronic ingestion of overfortified milk, the average 25(OH)D concentration was 560 nmol/L (range: 140–1490 nmol/L). In an extended family group accidentally intoxicated with a veterinary vi-

tamin D concentrate (peanut oil solution containing 2×10^6 IU cholecalciferol), it has been shown that 25(OH)D concentrations ranged from 847 to 1652 nmol/L in intoxicated family members, whereas plasma 1α,25$(OH)_2$D concentrations were within the normal range in 8 of 11 patients [6]. A perusal of these data and the anecdotal reports support the notion of R.Vieth; namely, that hypercalcaemia only results when 25$(OH)D_3$ concentrations have consistently been above 375–500 nmol/L [21, 22].

A case of accidental ingestion of a veterinary vitamin D concentrate (cholecalciferol in peanut oil; 2 million IU/g) as a cooking oil sheds some light on the mechanism of pathogenesis in hypercalcaemia in vitamin D toxicity. Serum concentrations of calcifediol in nine of the cases were 8–15 times greater than the upper limit of the reference range, while total calcitriol concentrations were increased in only three patients. The percentage of unbound calcitriol was increased in all patients, as was the total calcitriol concentration. Increased unbound calcitriol concentrations might play a role in the pathogenesis of hypercalcaemia in vitamin D toxicity [30].

Hypercalcaemia has been associated with the ingestion of an over-the-counter vitamin D supplement. A 42-year-old man who for 2 years had taken a supplement containing vitamin D_2 had a serum calcium concentration of 3.75 mmol/l and a serum 25(OH)D concentration of 487 ng/ml (reference range 9–47). He had normal concentrations of calcitriol, parathyroid hormone, angiotensin-converting enzyme, and thyroid hormone, and had normal bone marrow, radiography, and CT of the chest, neck, and abdomen. Vitamin D_2 was withdrawn and he was rehydrated. He was advised to wear sunscreen at all times when outdoors. Three months after discharge the results of all blood tests were in the reference ranges, including serum concentrations of calcium and 25(OH)D. Examination of three lots of the over-the-counter formulations the patient had used showed mean vitamin D contents of 1.3, 13, and 22 mg/g of powder. The patient had taken 3 g/day, or 156 000–2 604 000 IU/day of vitamin D_2, which is about 80–1300 times the generally recommended safe upper limit of 2000 IU/day [30].

- **Vitamin D in Pregnancy and Lactation**

Vitamin D is classified as USA Food and Drug Agency (FDA) Pregnancy Risk Category: A[1] agent.

[1] Risk Factor D if used in pharmacological doses.

High doses of vitamin D are known to be teratogenic in experimental animals [76, 78], but direct convincing evidence for this is lacking in humans [30, 64]. Because of its action to raise calcium levels, vitamin D has been suspected in the pathogenesis of the supravalvular aortic stenosis syndrome, which is often associated with idiopathic hypercalcaemia of infancy. The full features of this rare condition are characteristic elfin facies, mental and growth retardation, strabismus, enamel defects, craniosynostosis, supravalvular aortic and pulmonary stenosis, inguinal hernia, cryptorchidism in males, and early development of secondary sexual characteristics in females [30, 64].

Excessive intake or retention of vitamin D during pregnancy by mothers of infants who develop supravalvular aortic stenosis syndrome has not been consistently found. Although the exact cause is unknown, it is possible that the syndrome results from abnormal vitamin D metabolism in the mother, the fetus, or both [64].

Very high levels of vitamin D have been used to treat maternal hypoparathyroidism during pregnancy without ill effects [30]. In two studies, 15 mothers were treated with doses averaging 107,000 IU/day throughout their pregnancies to maintain maternal calcium levels within the normal range. All of the 27 children were normal at birth and during follow-up examinations ranging up to 16 years. The active metabolite calcitriol, in doses up to 3 μg/day, was used to treat another mother with hypoparathyroidism. The high dose was required in the latter half of pregnancy to prevent hypocalcemia. The infant had no apparent adverse effects from this exposure. In a similar case, a mother received 100,000 IU/day throughout gestation, resulting in a healthy, full-term infant [64]. In contrast, a 1965 case report described a woman who received 600,000 IU of vitamin D and 40,000 IU of vitamin A daily for 1 month early in pregnancy. The resulting infant had a defect of the urogenital system, but this was most probably caused by ingestion of excessive vitamin A, considering the well established-dysmorphogenic potential of the latter [64].

The excellent safety profile of cholecalciferol has been further corroborated in a recent trial of nutritional and pharmacological vitamin D supplementation during pregnancy. In this randomized, controlled trial, women with a singleton pregnancy at 12 to 16 weeks' gestation received 400, 2000 or 4000 IU of cholecalciferol per day until delivery. The primary outcome was maternal/neonatal circulating 25-hydroxyvitamin D (25(OH)D) concentration

at delivery, with secondary outcomes of a 25(OH) D concentration of 80 nmol/L or greater achieved and the 25(OH)D concentration required to achieve maximal calcitriol production. Of the 494 women enrolled, 350 women continued until delivery: Mean 25(OH)D concentrations by group at delivery and 1 month before delivery were significantly different ($p < 0.0001$), and the percent who achieved sufficiency was significantly different by group, greatest in 4000-IU group ($p < 0.0001$). The relative risk (RR) for achieving a concentration of 80 nmol/L or greater within 1 month of delivery was significantly different between the 2000- and the 400-IU groups but not between the pharmacological doses 4000- and 2000-IU groups. Circulating 25(OH)D had a direct influence on circulating $1,25(OH)_2D_3$ concentrations throughout pregnancy ($p < 0.0001$), with maximal production of the active metabolite in all strata in the 4000-IU group. There were no differences between groups on any safety measure. Not a single adverse event was attributed to vitamin D supplementation or circulating 25(OH)D levels. This findings indicate that vitamin D supplementation of 4000 IU/d for pregnant women is safe and most effective in achieving sufficiency in all women and their neonates regardless of race, whereas the current estimated average requirement (400 IU in UK, USA and elsewhere) is comparatively ineffective at achieving adequate circulating 25(OH)D concentrations, especially in African Americans [65].

During pregnancy, maternal serum concentrations of 25-hydroxyvitamin D, the circulating form of vitamin D, correlate with dietary vitamin D intake. Maternal serum concentrations of 1,25-dihydroxyvitamin D, the hormonal circulating and active form of vitamin D, are elevated during pregnancy; 1,25-dihydroxyvitamin D is synthesized mainly by the decidual cells of the placenta and allows for increased calcium absorption. The fetus is entirely dependent on the mother for its supply of 25-hydroxyvitamin D, which is believed to cross the placenta. Hypocalcemia and increased parathyroid hormone secretion induce synthesis of 1,25-dihydroxyvitamin D after birth in both full-term and preterm neonates. Nevertheless, serum concentrations of 25-hydroxyvitamin D are a rate-limiting factor in the synthesis of 1,25-dihydroxyvitamin D. In vitamin D-replete infants, circulating 1,25-dihydroxyvitamin D concentrations are higher than those observed in older infants. In countries where dairy products are not routinely supplemented with vitamin D, maternal vitamin D supplementation during pregnancy is necessary. However, there is no indication for the use of pharmacological doses of vitamin D or its metabolites in the perinatal period [62].

A number of investigators have measured vitamin D levels in the mother during pregnancy and in the newborn. Although not universal, most studies have found a significant correlation between maternal serum and cord blood levels. In one study, a close association between both of the transport vitamin D forms in maternal and cord serum was discovered. No significant correlation could be demonstrated, however, between the two biologically active forms in maternal and cord blood [64].

Using a perfused human placenta, a 1984 report confirmed that calcifediol and calcitriol were transferred from the mother to the fetus, although at a very slow rate.

Binding to vitamin D3–binding protein was a major rate-limiting factor, especially for calcifediol, the transport form of vitamin D3. The researchers concluded that placental metabolism of calcifediol was not a major source of fetal calcitriol [64].

Maternal levels at term are usually higher than those in the newborn because the fetus has no need for intestinal calcium absorption. Maternal levels are elevated in early pregnancy and continue to increase throughout pregnancy. During the winter months a weak correlation may exist between maternal vitamin D intake and serum levels, with exposure to ultraviolet light the main determinant of maternal concentrations. A Norwegian study, however, was able to increase maternal concentrations of active vitamin D significantly during all seasons with daily supplementation of 400 IU [64].

Vitamin D is excreted into breast milk in limited amounts [19, 64]. A direct relationship exists between maternal serum levels of vitamin D and the concentration in breast milk. Chronic maternal ingestion of large doses may lead to greater than normal vitamin D activity in the milk and resulting hypercalcaemia in the infant. In the lactating woman who is not receiving supplements, there is considerable controversy about whether her milk contains sufficient vitamin D to protect the infant from vitamin deficiency. Several studies have supported the need for infant supplementation during breast feeding. Other investigators have concluded that supplementation is not necessary, if maternal vitamin D stores are adequate [64].

A study published in 1977 measured high levels of a vitamin D metabolite in the aqueous phase of milk. Although two other studies supported these

findings, the conclusions were in direct opposition to previous measurements and have been vigorously disputed. The argument that human milk is low in vitamin D is supported by clinical reports of vitamin D deficiency-induced rickets and decreased bone mineralization in breast-fed infants. Moreover, one investigation measured the vitamin D activity of human milk and failed to find any evidence for significant activity of water-soluble vitamin D metabolites. Vitamin D activity in the milk was 40–50 IU/L, with 90% of this accounted for by the usual fat-soluble components [64].

The US National Academy of Sciences' RDA for vitamin D in the lactating woman is 400 IU. The Committee on Nutrition, American Academy of Pediatrics, recommends vitamin D supplements for breast-fed infants if maternal vitamin D nutrition is inadequate or if the infant lacks sufficient exposure to ultraviolet light. A second committee of the American Academy of Pediatrics considers maternal consumption of vitamin D to be compatible with breast feeding. However, the serum calcium levels of the infant should be monitored if the mother is receiving pharmacological doses [64].

VII. Conclusion and synopsis

Medicinal products and food supplements based on cholecalciferol (vitamin D_3) constitute the most-important source of vitamin D supplementation or pharmacological intervention in deficiency states. Itself cholecalciferol exhibit little in the way of biologic activity. It undergoes hydroxylation in the liver to form 25-hydroxyvitamin D by action of the enzyme vitamin D-25 hydroxylase. 25-Hydroxyvitamin D serves as the primary storage form of vitamin D hormone. To become biologically active, 25-hydroxyvitamin D requires further hydroxylation to form the active calcium and phosphorus-regulating hormone 1,25-dihydroxyvitamin D by action of the enzyme 25-hydroxyvitamin D-1a-hydroxylase. This hydroxylation step takes place primarily in the kidney and is tightly regulated by circulating PTH, phosphate, and calcium levels. Thus, vitamin D deficiency can result from nutritional deprivation, inadequate sunlight exposure, impaired gastrointestinal absorption, reduced synthesis of 25-hydroxyvitamin D and/or 1,25-dihydroxyvitamin D or end-organ resistance to 1,25-dihydroxyvitamin D.

Optimal management of vitamin D deficiency is dependent on its underlying cause and treatment must be individualized with regard to pathogenesis and severity of the disorder. Deficiency states associated with malabsorption may require pharmacological amounts of vitamin D. In most patients with vitamin D deficiency (those with adequate renal function and 1α-hydroxylase activity) the object is to restore and maintain optimal serum 25(OH) vitamin D concentrations. Monitoring serum levels after vitamin D supplementation begins, should provide an adequate guide for dose adjustments.

Therapeutic doses of cholecalciferol are usually well tolerated, but aggressive dosing, especially in populations at risk (e.g. patients with sarcoidosis, and other chronic granulomatous conditions) poses risk of hypercalcaemia and hypercalciuria. The toxic effects of vitamin D excess are primarily related to the role of free $1,25(OH)_2D$ in the regulation of plasma calcium. Excessive production of $1,25(OH)_2D$ or greatly increased plasma 25(OH)D (which may displace $1,25(OH)_2$ D from DBP) may lead to elevated level of plasma calcium due partly to over stimulated intestinal absorption and partly to excessive calcium mobilization from bone. There is also limited evidence that high concentrations of vitamin D directly affect various organ systems such as kidney, bone, the central nervous system and the cardiovascular system.

The ubiquitous expression of vitamin D receptors in various tissues and the compelling evidence from both observational studies and RCTs have well expanded the horizon for its possible utilities beyond the musculoskeletal system. Nevertheless, further studies are needed to clearly elucidate these potential new roles of vitamin D beyond calcium homeostasis, because in general these adjunctive effects require significantly higher levels, as compared to those generally regarded as sufficient and safe.

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