Endocrine (2015) 50:12–26
DOI 10.1007/s12020-015-0606-x

POSITION STATEMENT/GUIDELINE

The clinical use of vitamin D metabolites and their potential developments: a position statement from the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO) and the International Osteoporosis Foundation (IOF)

Luisella Cianferotti[1] · Claudio Cricelli[2] · John A. Kanis[3] · Ranuccio Nuti[4] · Jean-Y. Reginster[5] · Johann D. Ringe[6] · Rene Rizzoli[7] · Maria Luisa Brandi[1]

Received: 14 January 2015 / Accepted: 13 April 2015 / Published online: 1 May 2015

Abstract Several compounds are produced along the complex pathways of vitamin D_3 metabolism, and synthetic analogs have been generated to improve kinetics and/or vitamin D receptor activation. These metabolites display different chemical properties with respect to the parental or native vitamin D_3, i.e., cholecalciferol, which has been, so far, the supplement most employed in the treatment of vitamin D inadequacy. Hydrophilic properties of vitamin D_3 derivatives facilitate their intestinal absorption and their manageability in the case of intoxication because of the shorter half-life. Calcidiol is a more hydrophilic compound than parental vitamin D_3. Active vitamin D analogs, capable of binding the vitamin D receptor evoking vitamin D-related biological effects, are mandatorily employed in hypoparathyroidism and kidney failure with impaired 1α-hydroxylation. They have been shown to increase BMD, supposedly ameliorating calcium absorption and/or directly affecting bone cells, although their use in these conditions is jeopardized by the development of hypercalciuria and mild hypercalcemia. Further studies are needed to assess their overall safety and effectiveness in the long-term and new intermittent regimens, especially when combined with the most effective antifracture agents.

Keywords Calcidiol · Calcifediol · Cholecalciferol · Calcitriol · Alfacalcidol · Eldecalcitol

✉ Maria Luisa Brandi
marialuisa.brandi@unifi.it

Luisella Cianferotti
luisella.cianferotti@unifi.it

Claudio Cricelli
cricelli@dada.it

John A. Kanis
jakanis@outlook.com

Ranuccio Nuti
ranuccio.nuti@unisi.it

Jean-Y. Reginster
jyreginster@ulg.ac.be

Johann D. Ringe
ringe@klinikum-lev.de

Rene Rizzoli
rene.rizzoli@unige.ch

[1] Bone Metabolic Diseases Unit, Department of Surgery and Translational Medicine, University of Florence, Viale Pieraccini 6, 50139 Florence, Italy

[2] Italian College of General Practitioners, Florence, Italy

[3] Centre for Metabolic Bone Diseases, University of Sheffield Medical School, Sheffield, UK

[4] Department of Medicine, Surgery and Neuroscience, University of Siena, Siena, Italy

[5] Department of Public Health, Epidemiology and Health Economics, University of Liège, CHU Sart-Tilman, Liège, Belgium

[6] Department of General Internal Medicine, Klinikum Leverkusen University of Cologne, Cologne, Germany

[7] Service of Bone Diseases, Geneva University Hospitals and Faculty of Medicine, Geneva, Switzerland

Introduction

Vitamin D metabolism comprises several activating and catabolic pathways, which generate a number of compounds culminating with the production of the secosteroid hormone calcitriol [i.e., 1,25-dihydroxyvitamin D_3, $1,25(OH)_2D_3$, and others]. Following the endogenous production of native vitamin D_3 (i.e., cholecalciferol) from 7-dehydrocholesterol in the skin, and the dietary intake of vitamin D_3 and vitamin D_2 (ergocalciferol), these compounds are quickly conveyed to the liver where they are rendered more hydrophilic by the addition of a hydroxyl group in position 25 by a complex of 25-hydroxylases (mainly CYP2R1 and CYP27A1) to form $25(OH)D_3$ [also referred to as 25-hydroxyvitamin D, calcidiol, and others] and $25(OH)D_2$, overall indicated as 25(OH)D [1] (Fig. 1). The addition of a second OH group in position 1 by CYP27B1 (i.e., 1α-hydroxylase) in the kidney activates $25(OH)D_3$ to calcitriol [also referred to as 1,25-dihydroxyvitamin D_3 or $1,25(OH)_2D_3$], the biologically active form of vitamin D_3, capable of binding the specific receptor (vitamin D receptor, VDR) acting as a transcription factor. While the renal 1α-hydroxylase is activated by PTH and is then abolished in hypoparathyroidism, hepatic 25-hydroxylase activation depends on individual synthetic potential and the presence of the substrate, i.e., vitamin D_3. Moreover, the activity of vitamin D 25-hydroxylases can be impaired during treatment with antiretroviral and antiepileptic drugs and calcitriol [1].

The modifications of parental vitamin D_3 along the metabolic pathway described above strongly modify the chemical properties of the derived compounds, which become less lipophilic, their binding to transport proteins and, therefore, their kinetics, storage, and catabolism. Indeed, more polar metabolites are likely to display smaller volumes of distribution and less trapping by the adipose tissue.

Although 25(OH)D has little biological activity *per se*, it comprises the most abundant of all circulating vitamin D metabolites. Indeed, its high affinity for the vitamin D binding protein (VDBP) makes 25(OH)D the main storage form of vitamin D in the body [2]. Thus, serum/plasma concentration of total 25(OH)D [$25(OH)D_2 + 25(OH)D_3$] is considered the single most reliable biological marker of clinical vitamin D status, representing both the endogenous UV-dependent production and the exogenous introduction of vitamin D from dietary sources or supplements [3, 4]. Nonetheless, serum/plasma 25(OH)D does not reflect the storage of vitamin D within the tissues, which can be converted to calcitriol by tissutal 1α-hydroxylase, and the whole vitamin D bioavailability, which also depends on the presence of catabolic metabolites and protein transport in the blood [5]. Recent studies have demonstrated a direct uptake of 25(OH)D by skeletal myocytes, which would internally retain 25(OH)D binded to VDBP [6]. This

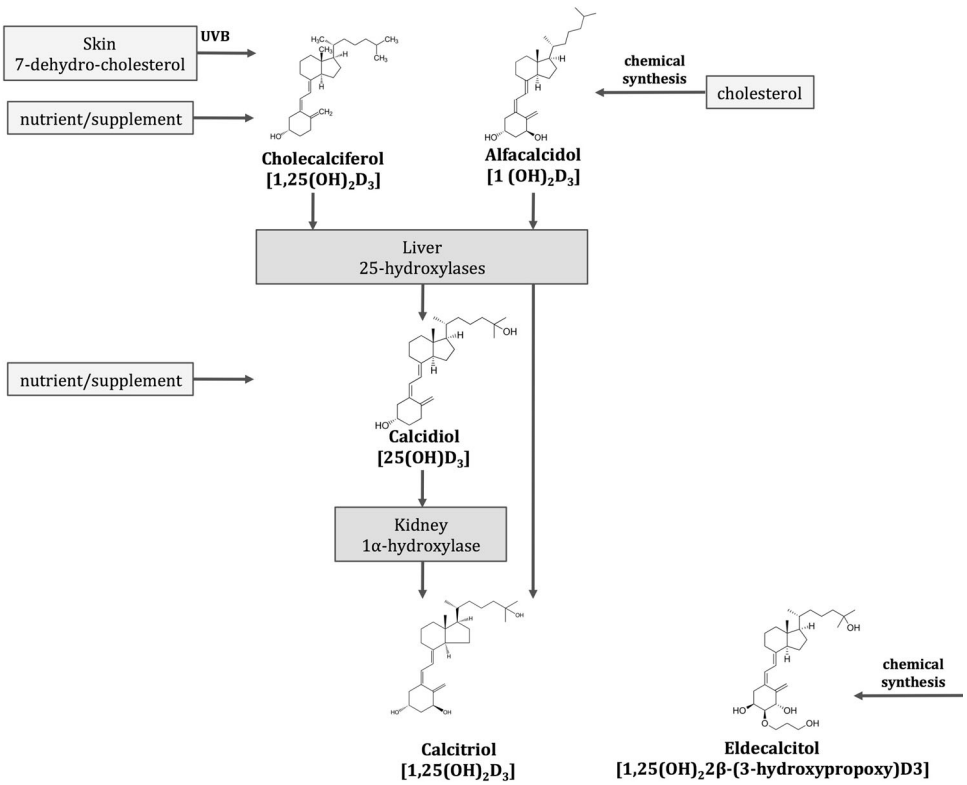

Fig. 1 Vitamin D metabolism and main synthetic metabolites available as drugs for clinical use. Cholecalciferol (vitamin D_3), synthesized in the skin from 7-dehydrocholesterol by UVB or introduced as a nutrient or a supplement, is hydroxylated in position 25 in the liver producing calcidiol [$25(OH)D_3$], which is activated in the kidney by 1α-hydroxylase producing calcitriol [$1,25(OH)_2D_3$]. Alfacalcidol [$1\alpha(OH)D_3$] is chemically synthesized by cholesterol and needs only the hepatic 25-hydroxylation to become fully activated into calcitriol. Eldecalcitol [$1,25(OH)_22\beta$(3-hydroxypropoxy)D_3] does not need any hydroxylation to exert its biologic action; the 3-hydroxypropoxy chain increases VDBP binding and the half-life

supports the idea that skeletal muscle would work as a site of extravascular storage of calcidiol.

While the hydroxylation of the side chain of 25(OH)D leads to the production of inactive metabolites, alternative modifications form metabolites, such as the epimer 25(OH)-3-epi-D, capable of binding and activating the VDR [7]. Several metabolites along the vitamin D pathway or artificially synthesized mono- or dihydroxylated compounds can be employed in clinical practice in conditions of pro-hormone or hormone deficiency (i.e., rickets, osteomalacia, hypoparathyroidism) and to address specific conditions such as osteoporosis by ameliorating intestinal calcium absorption.

Vitamin D inadequacy (insufficiency/deficiency) has been estimated to occur in more than one billion people worldwide and is associated with secondary hyperparathyroidism, rickets, osteomalacia, altered bone turnover, osteoporosis, impaired muscular performance, and increased risk of falls and fractures [8–11]. Numerous cross-sectional studies have found an association between low serum 25(OH)D concentrations and various pathologic conditions, including cardiovascular, autoimmune, infectious, proliferative diseases, and dysmetabolic syndrome [12]. Indeed, a number of non-classical effects not classically related to the maintenance of mineral and skeletal homeostasis are now attributed to the biologically active hormone/cytokine $1,25(OH)_2D_3$, sustained by the ubiquitous expression of VDR and extrarenal production of vitamin D metabolites [13, 14]. Indeed, the autocrine/paracrine vitamin D system has acquired an important role because of the presence of 25-hydroxylases and 1α-hydroxylase in several tissues other than the liver and the kidneys, so that circulating vitamin D and 25(OH)D can serve as substrates for these enzymes [15]. The affinity for VDBP would be crucial in this sense. While vitamin D_3 displays lower affinity for VDBP making it more readily accessible for internalization, in tissues classically related to the maintenance of mineral homeostasis, such as the kidneys and the parathyroids, the endocytic receptors megalin and cubilin are devoted to the uptake of vitamin D metabolites, such as 25(OH)D, avidily bound to VDBP [16].

So far, vitamin D_2 and, mostly, vitamin D_3, administered in daily doses of 800–4000 IU, have been the most common forms of dietary supplements employed in vitamin D insufficiency/deficiency. However, while it is relatively easy to reach the serum $25(OH)D_3$ target of 20 ng/ml by vitamin D_3 supplementation, it is more difficult, especially in obese individuals, to reach and maintain the target of 30 ng/ml [17], which is considered by the International Osteoporosis Foundation (IOF), the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO), and the Endocrine Society (ES), and also confirmed by recent meta-analyses, the threshold for musculoskeletal health and the prevention of falls and fractures [18–21]. Moreover, concerns have arisen in administering high doses (>100,000 IU) of vitamin D_3, especially when given at non-physiologic intervals of time, because an increased risk of fracture in the first 3 months after dosing may be explained by classic (hypercalciuria and hypercalcemia) and non-classic toxic effects, such as the increase of bone turnover markers or rapid non-genomic responses not yet identified [22–24]. As an alternative approach, some vitamin D metabolites have been employed to ameliorate vitamin D status, in order to facilitate the attainment of a given threshold and to avoid intoxication, taking advantage of the different chemical properties and related pharmacokinetics.

In January 2014, ESCEO and IOF Representatives convened a meeting to discuss and clarify the possible use of vitamin D metabolites available for clinical practice and in different pathologic conditions, in which the attainment of optimal vitamin D status with the parental compounds (i.e., vitamin D_2 and vitamin D_3) can be jeopardized.

Herein, the aim of this document is to summarize the available evidence on the clinical use of vitamin D derivatives other than vitamin D_3 and D_2 to correct vitamin D inadequacy, and to optimize mineral metabolism, in order to decrease the risk for fall and fracture (not taking into account studies on vitamin D metabolites such as doxercalciferol and paracalcitol specifically employed in hyperparathyroidism secondary to chronic kidney disease-mineral bone disorder).

Discovery and development of main vitamin D metabolites and synthetic derivatives

Since the isolation of vitamin D_2 and vitamin D_3 and the discovery of their antirachitic properties at the beginning of the twentieth century, multiple metabolites along the vitamin D endocrine pathway had been discovered [25] (Fig. 1).

Indeed, in vitro and in vivo evidence led to the hypothesis that the parental vitamin had to be metabolized to more polar metabolites in order to function. In the 1960s, it become evident that a more polar metabolite than vitamin D, still biologically active, could be isolated in the serum after administration of radioactive vitamin D_3 in animals and humans [26, 27]. Hence, a compound with more rapid biological action in enhancing intestinal calcium absorption was isolated and identified as $25(OH)D_3$ by sensible spectroscopic techniques [28, 29]. Soon after this discovery, this vitamin D analog was rapidly synthesized and its biological effectiveness confirmed [30]. In parallel, it was demonstrated that the exclusion of the liver from the circulation abolished the synthesis of $25(OH)D_3$ in rats, thus demonstrating that this organ is the physiologic site where

the 25-hydroxylation occurs in vivo [31]. Afterwards, it became clear that $25(OH)D_3$ *per se* was not biologically active but needed another hydroxylation to exert its actions on mineral metabolism [32]. These experiments ultimately led to the identification of $1,25(OH)_2D_3$, synthesized in the kidney as the full biologically active form of vitamin D in vertebrates [33–35]. With the development of a specific competitive protein binding assay in 1971, which became widely available afterwards, $25(OH)D_3$ began to be quantified in blood [36, 37]. Since then, the determination of $25(OH)D_3$ has been considered the best diagnostic marker of vitamin D status and supply. The procedure to synthesize the antirachitic $25(OH)D_3$ was patented by DeLuca (Wisconsin Alumni Research Foundation, WARF) in 1973 (US patent 3,772,361). The development of serum $25(OH)D_3$ assay allowed the pharmacokinetics of the prohormones to be evaluated and compared[both vitamin D_3 and $25(OH)D_3$] (see next paragraph).

In the same years, a 25-hydroxylated vitamin D derivative, different from $25(OH)D_3$, was isolated, purified from chick intestines, and identified as $1,25(OH)_2D_3$ [38, 39], the biologically active form of vitamin D, ultimately activated in the kidney [40, 41]. Soon after these reports, it became evident that parathyroid hormone was indispensable for the hydroxylation of $25(OH)D_3$ in the kidney for the production of metabolically active $1,25(OH)_2D_3$. Indeed, an abrogation or a decrease in the renal production of $1,25(OH)_2D_3$ was demonstrated in thyroparathyroidectomized vertebrates and uremic animals, respectively [42, 43]. Moreover, the existence of inhibitory feedback by $1,25(OH)_2D_3$ on PTH secretion was shown [44]. Calcitriol was able to directly elicit intestinal absorption of calcium and phosphate in vivo and was shown to be a potent stimulator of bone resorption and calcium mobilization from skeletal compartments to extracellular milieu [45–48]. The full structure of $1,25(OH)_2D_3$ and, in particular, the configuration of the hydroxyl group, was completely deduced when the compound was biochemically synthesized by DeLuca's group. Kinetic studies with the radiolabeled compound established the short half-life of calcitriol (5–8 h in adults), with rapid disappearance from the blood and ensuing rapid catabolism [49]. Compartmental analyses allowed the estimation of endogenous production of $1,25(OH)_2D_3$ in 0.3–1 mcg/day [49]. In parallel, an immunoassay for $1,25(OH)_2D_3$ was developed [50], making pharmacokinetic studies with the different compounds possible.

After these landmark discoveries in vitamin D metabolism, several scientists and medical companies, under license to several patents held mainly by the WARF (Wisconsin, USA) and the Research Institute for Medicine and Chemistry (Cambridge, UK), started specific research to develop and test analogs of $1,25(OH)_2D_3$, in particular for the treatment of hypoparathyroidism, renal osteodistrophy, and associated secondary hyperparathyroidism. In parallel, osteoporosis began to be recognized as a metabolic bone disease, and vitamin D analogs began to be considered for the treatment of this disorder. In 1993, the international symposium "Osteoporosis: therapy with vitamin D_3 metabolites and analogs" was held in Hong Kong, and basic and clinical research on vitamin D analogs (calcitriol and alfacalcidol) was presented. It is worth noting that in this occasion, the first description of osteoporosis as a disease of the elderly population was included, together with the efficacy of D-hormone analogs in this condition [51].

Similar to other steroid hormone receptor modulators, $1,25(OH)_2D_3$ analogs continued to be further developed and patented, with the aim of modulating VDR-mediated effects in a tissue-selective way, with minor calcemic effects, maintaining or enhancing the effects on bone (see "Clinical use of the 1α-hydroxylated compounds (calcitriol, alfacalcidol and eldecalcitol)" section). Among different synthesized analogs, 1α-hydroxylated derivatives, such as alfacalcidol, a direct precursor of calcitriol but also capable to bind the VDR, and eldecalcitol, have been tested in vitro, then in vivo in animals and humans.

Alfacalcidol (i.e., the 1α-hydroxyderivative of vitamin D_3) was one of the first analogs to be synthesized, and was shown to display a comparable biological activity in terms of intestinal calcium absorption and calcium mobilization from bone with respect to calcitriol [52]. Since alfacalcidol was synthesized from cholesterol, its synthesis was simpler and less expensive. In vivo animal studies demonstrated that alfacalcidol biopharmacological activity was similar to calcitriol, but with a wider therapeutic window [53]. Afterwards, alfacalcidol was tested in clinical trials and was the first vitamin D analog with the indication of osteoporosis.

Among the different analogs bearing a hydroxyalkoxy group at 2β position, which directly increases the affinity of these compounds for VDBP, eldecalcitol [1α,25-dihydroxy-2β-(3-hydroxypropoxy) vitamin D_3, ED-71] showed the greatest capacity to strongly inhibit bone resorption, modestly enhance formation and thus to prevent bone loss in ovariectomized or old normal rats, and prednisolone-treated rats [54–56]. This occurred with only a minimal enhancement in intestinal calcium absorption and consequent mild suppression of PTH levels [54]. In a further study comparing the efficacy of eldecalcitol and alfacalcidol in the same animal model (estrogen-deficient rat), eldecalcitol was found to increase trabecular bone mass to a greater extent than alfacalcidol [57]. This result was due to a marked inhibition of bone resorption, as demonstrated by decreased specific biochemical and histological markers, while preserving bone formation. The catabolic/

anabolic effect of eldecalcitol was found to be independent from calcium absorption and/or PTH suppression. Recently, performing experiments in rats immediately after ovariectomy, eldecalcitol promoted focal bone formation, a process also referred to as minimodeling [58]. Ensuing experiments in the mouse have found that the expression of the receptor activator of nuclear factor kappa-B ligand (RANKL) in mouse osteoblasts is reduced by eldecalcitol [59].

Rationale for the clinical use of vitamin D derivatives

Age- or disease-related decline in hepatic and renal function could affect the 25- or 1α-hydroxylating capacity, so that the correction of mineral metabolism by means of native vitamin D can be hampered. In this view, hydroxylated vitamin D analogs may overcome this problem.

Indeed, four types of vitamin D deficiency can be associated with the aging process itself and/or several diseases and with the development of a negative calcium balance, secondary hyperparathyroidism, and osteoporosis: (1) deficiency of the parental compound vitamin D_3, (2) deficiency of 25(OH)D_3 because of impaired hepatic hydroxylation, (3) deficiency of 1,25$(OH)_2D_3$ due to impaired renal 1α-hydroxylation, and (4) deficiency in the responsiveness to 1,25$(OH)_2D_3$ in target tissues mainly due to decreased expression of the VDR [60, 61].

While 25-monohydroxylated vitamin D (calcidiol) may be used to optimize vitamin D status especially when the activity of hepatic hydroxylases is diminished [62], 1α-hydroxylated vitamin D analogs can be employed when renal hydroxylation is impaired, such as in the case of kidney failure or hypoparathyroidism, in order to favor calcium absorption and/or overcome the age-related decreased VDR expression [60, 61, 63].

The primary goal of the vitamin D endocrine system is the promotion of a positive calcium balance in order to maintain an optimal mineral homeostasis, thus favoring mineralization of the skeleton of vertebrates. This is indirectly achieved through the action of 1,25$(OH)_2D_3$ in the intestine leading to an increase in calcium and phosphate absorption. Direct effects of 1,25$(OH)_2D_3$ on bone, through actions on osteoblasts/osteocytes, have been questioned, since contradictory results have ensued from experiments in transgenic mice [64]. Direct effects of 1,25$(OH)_2D_3$ in bone are mediated by the osteoblasts. Calcitriol is a potent inducer of osteoclastogenesis by means of inducing RANKL. The main objective of this osteoblast-mediated enhancement of bone resorption by calcitriol, together with the inhibition of mineralization, is to maintain extracellular calcium concentration within a narrow range, avoiding a negative calcium balance. These catabolic effects on bone would be achieved in the presence of high 1,25$(OH)_2D_3$ concentration, occurring in physiology as a consequence of extremely low calcium supply. When a positive calcium balance has been regained, the promotion of later stages of osteoblast differentiation, which probably occurs at more physiologic levels of 1,25$(OH)_2D_3$, favors mineralization.

Indeed, active 1α-hydroxylated vitamin D analogs, such as calcitriol, alfacalcidol, and eldecalcitol, have been employed in the treatment of primary and secondary osteoporosis for their positive effect on BMD and the decrease in fracture risk. In general, the use of 1α-hydroxylated forms is hampered by the higher risk of developing hypercalciuria and hypercalcemia (Table 1).

Clinical use of the 25-hydroxylated compound calcidiol

Calcidiol, which exists as a pharmaceutical compound, a supplement or nutritional ingredient (as a normal food constituent) [65], may represent a useful approach in the treatment and prevention of vitamin D deficiency. Contrary to cholecalciferol, a conversion to IU has not been established for calcidiol, which is therefore expressed in mcg when administered as a nutritional supplement.

Calcidiol can subsist in its free form or as a crystalline substance [25(OH)D_3 monohydrate]. Calcidiol is a more polar metabolite than vitamin D and, for this reason, it is more soluble in organic solvents. This property influences its intestinal absorption, its protein transport in the blood by VDBP, and the whole body distribution of the oral administered calcidiol, which displays a much shorter half-life (10–13 days) than the parental compound cholecalciferol [66–69]. The greater affinity for VDBP may make 25(OH)D more available for internalization in tissues devoted to the control of mineral homeostasis such as the kidneys and the parathyroids expressing the megalin–cubilin system of endocytic receptors [16]. As shown in a seminal study by Haddad et al. in the early 1970s, the administration of a single dose of calcidiol produced a more rapid and significant increase in serum 25(OH)D concentration, compared to a single oral dose of cholecalciferol, which produced a very slow increase of serum 25(OH)D levels because of the intermediate hepatic 25-hydroxylation [68]. Indeed, after the administration of 1.5–10 μg/Kg of calcidiol in a single dose in normal subjects, the increase in serum 25(OH)D levels began as early as 2 h, peaked within 4–8 h after dispensation, and a 33 % decrease of peak levels was observed not before 1 week. These results and, in particular, the promptness and predictability of achieved serum 25(OH)D levels and the shorter half-life supported an advantage of calcidiol over

Table 1 Major vitamin D derivatives used in clinical practice

Vitamin D analog	Synonyms	Half-life	Brand name® (Company)[a]	Form
Calcidiol [25(OH)D_3]	(3β,5Z,7E)-9,10-secocholesta-5,7,10(19)-triene-3,25-diol	10-13 days	Calderol® (Upjohn)	Oral, capsule, 20–50 mcg
			Caldiol® (Medica)	Oral, capsule, 20 mcg
	25-hydroxycholecalciferol		Dedrogyl® (Desma)[b]	Oral, solution (drops), 1.5 mg/10 ml
			Didrogyl® (Bruno Farmaceutici)[b]	Oral, solution (drops), 1.5 mg/10 ml
	25-Hydroxyvitamin D3 Calcifediol		Dédrogyl® (DB Pharma)[b]	Oral, solution (drops), 1.5 mg/10 ml
	Calcifediol Anhydrous Calcifediolum		Hidroferol® (Faes)[b]	Oral, solution (vial), 1.5 ml
Calcitriol [1,25$(OH)_2D_3$]	1,25-DHCC	5-8 h	Rocaltrol® (Roche)[b]	Oral, capsule, 0.25–0.50 mcg
	1,25-dihydroxycholecalciferol 1α,25(OH)2D3		Calcitriol Oral Solution® (Roxane)	Oral, solution, 1 mcg/ml
	1α,25-dihydroxycholecalciferol		Decostriol® (Mibe Jena, Germany; Jesalis, Hong Kong and Thailand)	Oral, capsule, 0.25 mcg
	1α,25-dihydroxyvitamin D3		Calcijex® (Abbott)[b]	Intravenous, 1–2 mcg/ml
Alfacalcidol [1α(OH)D_3]	1-hydroxycholecalciferol 1α-hydroxycholecalciferol	3 h	Alsiodol® Alfarol®	Oral, capsule, 0.25–0.50 mcg
			One Alpha®[b]	Oral, capsule, 3 mcg
				Oral, capsule, 0.25–0.50 mcg
	1α-hydroxyvitamin D3			Oral, solution (drops), 2 mcg/ml
Eldecalcitol [1,25$(OH)_2$2β(3hydroxypropoxy)D_3]	ED-71 1α,25-dihydroxy-2β-(3-hydroxypropoxy) vitamin D3	53 h	Edirol®, Chugai Pharmaceutical/Roche	Oral, capsule, 0.25–0.50 mcg

[a] Main company which registered pharmaceutical product, excluding manufacturers and packaging companies

[b] Available in Europe

cholecalciferol in correcting vitamin D deficiency and a good manageability in case of intoxication [70, 71]. Long-term treatment of vitamin D deficiency with oral calcidiol over a period of 5 years produced a sustained increase in plasma 25(OH)D levels. Indeed, a ten times lower dose of calcidiol was required to produce equivalent plasma 25(OH)D concentrations, confirming the superior therapeutic potency of calcitriol and the possible limits in endogenous hepatic 25-hydroxylation of parental vitamin D_3 [72]. This was further confirmed in an experiment comparing the efficacy in increasing serum 25(OH)D_3 levels of an intramuscular injection of calcidiol and cholecalciferol in non-lactating, pregnant dairy cows [73]. The parenteral administration of 25 mg calcidiol rapidly produced an increase in plasma levels of 25(OH)D, directly translated into an increase in plasma calcium and phosphate, while an 8-day delay in the increase of plasma 25(OH)D was observed after injection of 15 × 106 IU of cholecalciferol [73]. In an additional systematic study, the short-term effects of graded oral dosing of calcidiol on serum 25(OH)D levels in healthy men were assessed [74]. Calcidiol at 20 mcg/day was proven to be effective in raising serum 25(OH)D levels by 94 nmol/L in a 4-week time frame [74]. A recent study carried out in young healthy Gambian men to asses the effect on serum 25(OH)D after administration of a single dose of calcidiol

33 mcg in 810 mcg of olive oil has demonstrated that the peak occurs in 4.4 ± 1.8 h, while the disappearance takes place from day 6, with a median half-life of 13.4 ± 2.7 days [71]. The modifications in 25(OH)D concentration happened in the absence of any change in plasma and urinary calcium and phosphate [69]. The long-term pharmacokinetics of calcidiol versus cholecalciferol have recently been further refined and assessed when administered at a dosage of 20 mcg daily, 140 mcg weekly (total of 15 weeks), or 140 mcg in single bolus in healthy females aged 50–70 years in a randomized, double-blind trial [75]. Considering the area under the concentration–time curve as the measurement for exposure, this study demonstrated that calcidiol was 2–3 times more potent than cholecalciferol in determining the increase in plasma 25(OH)D levels (by 28 and 123 %, respectively, after the first and last daily–weekly dose, and by 114 % when administered in single bolus). All women under calcidiol supplementation reached optimal levels of serum $25(OH)D_3$ (i.e., >30 ng/ml) after just 16.8 days, while only 70 % of women receiving cholecalciferol reached this threshold within a longer period of time (mean 68.4 days) [75]. The differences in the entity of the higher therapeutic potency of calcidiol over cholecalciferol in terms of achieved plasma 25(OH)D levels between this latter study and previously published papers [72, 76] may rely on several factors: primarily on the comparison of full 24-h concentration–time curves instead of single plasma 25(OH)D measurements, which are indeed more susceptible of sampling and processing errors; on different study designs; on different assays employed (the most reliable selective assay being based on liquid chromatography coupled to tandem mass spectrometry detection, i.e., HPLC–MS/MS); on the fact that vitamin D_2 or vitamin D_3 had been indifferently administered for comparison with calcidiol [72, 75, 76].

Recent animal studies have shown that calcidiol is better absorbed in the intestine than cholecalciferol when added to animal feed (i.e., nutrient and/or supplement). This is further underlined by greater effects of calcidiol in reproductive performance, immune protection against infections, and skeletal turnover markers as compared to parental vitamin D3 in vivo in animals [77–79]. Recently, calcidiol supplementation in pregnant gilts, in addition to the control diet containing cholecalciferol, has been shown to improve feral skeletal muscle development and myoblast activity, likely improving postnatal muscle growth [80].

Regarding the expected biological effect in humans, several studies have confirmed that oral intake of calcidiol is highly effective in raising serum 25(OH)D levels and intestinal calcium absorption, suppressing serum PTH levels and blunting consequent increased bone turnover, independently from the regimen of administration (daily, weekly, or monthly) [74, 81–83]. In a pilot study in healthy adults, the efficacy of a monthly dose of calcidiol (500 μg) in raising and maintaining serum 25(OH)D levels significantly higher with respect to baseline values was shown, with an important reduction in serum PTH values ($p < 0.001$ versus baseline) and in the absence of significant episodes of hypercalcemia and hypercalciuria [83]. In a randomized, double-blind, placebo-controlled trial, supplementation with $25(OH)D_3$ 15 mcg/day administered over a period of 4 years in elderly women was demonstrated to reduce bone loss at appendicular sites, especially at low calcium intakes [84]. Although other studies have confirmed the increase in BMD at the hip [85], no studies are available on the effect of long-term calcidiol therapy on the rate of fracture at variance of vitamin D3 and D2 that showed an effect on prevention of fractures if associated with calcium.

A recent randomized study has compared the efficacy of calcidiol versus cholecalciferol (20 mcg/day, each) in the attainment of the desired serum 25(OH)D levels in healthy postmenopausal women with vitamin D deficiency. Both drugs had been formulated as spray-dried powders and administered in hard gelatin capsules in a double-blind manner over a 4-month period [86]. Supplementation with calcidiol resulted in a prompt, safe, and sustained increase in serum 25(OH)D levels, reaching 30 ng/ml after just 2 weeks, then going up to 69.5 ng/ml at the end of the intervention. Conversely, the rise in serum 25(OH)D levels determined by cholecalciferol was slow, plateauing at 31 ng/ml after 11 weeks. As secondary objectives and because of higher serum 25(OH)D levels attained, calcidiol was more effective than cholecalciferol in suppressing PTH levels and in ameliorating non-skeletal endpoints, such as reducing systolic blood pressure, maintaining or improving lower extremity function, dampening down several markers of innate immunity, as assessed by specific measurements at the end of the study period [86]. In young patients with cystic fibrosis, calcidiol plus calcium has proven to be effective in increasing BMD by 5 % and more after just one year of treatment in 25 % of patients, with less than 3 % experiencing hypercalciuria [87]. All in all, these results indicate that it is easy and overall safe to obtain and maintain serum 25(OH)D levels over 30 ng/ml that have been linked to vitamin D-induced extra-skeletal effects. Nonetheless, definitive conclusions on higher biologic potency of calcidiol cannot by drawn, yet, since comparative studies using equipotent doses are lacking, so far.

Regarding the means of administration of calcidiol, while daily and monthly appear to be safe, some concerns have arisen for the monthly administration, which would use larger amounts of the drug overcoming the VDBP binding at non-physiologic intervals of time largely exceeding its half-life [80]. It is possible that free calcidiol would enhance on one side calcitriol production, therefore

stimulating osteoclast activity, and on the other side the catabolic pathways leading to reduction of the tissutal calcidiol.

Thus far, the advantages of calcidiol daily and weekly supplementation appear to be related to the rapidity in correcting profound vitamin D deficiency with osteomalacia, without employing megadoses of parental vitamin D_3, and without risk of vitamin D intoxication (namely hypercalciuria, hypercalcemia) at the usual regimens employed [70, 86, 88], even though the physiologic autoregulation of the hepatic 25-hydroxylation is overcome. In obese individuals, where massive doses of cholecalciferol must be employed to reach at least the threshold of 30 ng/ml [85], calcidiol might be preferable to cholecalciferol for the smaller volume of distribution. Because of the linear kinetics conferred by the more hydrophilic properties, on one side, higher levels of serum 25(OH)D may be linked to vitamin D-related non-skeletal benefits, which therefore can be easily achieved, while on the other, a re-adjustment of the dose might be required in the long term. In addition, pre-formed $25(OH)D_3$ would access more easily to tissutal 1α-hydroxylase. Indeed, recent studies in monocytes exposed to *free* $25(OH)D_3$, in a serum from mice devoid of VDBP as compared to control serum, have shown an higher response in terms of cathelicidin production, indicating that bioavailable free calcidiol could be important in extra-skeletal effects such as the induction and maintenance of innate immunity [16].

In advanced liver failure, in conditions where the activity of cytochrome enzymes might be blunted (e.g., anticonvulsants, corticosteroids, and antiretroviral and antitubercular agents, alcohol abuse), and in the rare cases of inactivating mutations of genes encoding hepatic 25-hydroxylases (*CYP2R1*) and extrahepatic biliary atresia, the advantage of the clinical use of 25 hydroxylated derivative is straightforward [1, 89–93]. Some trials have also demonstrated an advantage of calcidiol in augmenting BMD in patients after kidney or heart transplantation, most probably bypassing the inhibition of 25 hydroxylases by chronic corticosteroid therapy [94, 95].

In the case of malabsorption, supplementation with oral forms of parental vitamin D_3 and derivatives can be difficult due to abnormal enterohepatic circulation, but to a greater extent with cholecalciferol with respect to calcidiol, which is then the preferred supplement in these patients [96–98].

In chronic kidney disease with secondary hyperparathyroidism, calcidiol has been shown to offer benefits, given that a decreased function of liver cytochromes has been demonstrated [99, 100]. In nephrotic syndrome or diabetes mellitus, in which the renal loss of proteins also incudes the complex $25(OH)D_3$/VDBP, supplementation with calcidiol can be advised [101, 102]. It is worth noting that calcidiol was introduced as Calderol in the U.S. Pharmacopeia with indication for renal osteodystrophy.

Finally, the finding that hypogonadism is characterized by low levels of serum 25(OH)D, and 25-hydroxylase is highly expressed in the testis opens new fields for therapeutic applications for calcifediol [103].

Clinical use of the 1α-hydroxylated compounds (calcitriol, alfacalcidol, and eldecalcitol)

The use of vitamin D derivatives hydroxylated in position 1 is mandatory in conditions characterized by absent or greatly impaired renal 1α-hydroxylation (i.e., hypoparathyroidism and kidney failure) (Fig. 1). These compounds have also been employed in diseases such as osteomalacia and osteoporosis in order to correct calcium malabsorption and/or overcome resistance to $1,25(OH)_2D$ due to age-related decline in the expression of the VDR, thus ameliorating mineral and skeletal homeostasis, especially in postmenopausal women. In addition, they may display direct protective effects in bone and skeletal muscle through binding the VDR, increasing the BMD and muscle performance, thus decreasing the incidence of falls and fractures [63]. Active vitamin D metabolites are also called D-hormones since their effects occur independently of an effect on vitamin D status. In addition, therapeutic effects are independent from renal function. Overall, these "hormonal" analogs have a narrow therapeutic window and therefore must be appropriately supervised to avoid the risk of hypercalciuria, hypercalcemia, and nephrolithiasis/calcinosis.

Calcitriol

Calcitriol is the natural ligand for the nuclear VDR, which, upon ligand binding, acts as a transcription factor in multiple tissues including bone. Since its discovery in the 1970s, several studies have examined the effects of calcitriol on bone metabolism, as assessed by markers of bone turnover, and the efficacy in preventing bone loss in healthy individuals (both sexes) and in subjects with primary and secondary osteoporosis.

Since menopause itself is accompanied by a decrease in calcium absorption *per se*, mainly because of a resistance to calcitriol and a decrease of calcitriol levels, calcitriol has been employed in several studies in order to optimize mineral and skeletal homeostasis and decrease fracture rate in women with postmenopausal osteoporosis, as a monotherapy or in combination with other antifracture agents [59] (reviewed in [63]). In these trials, calcitriol was administered daily, at a dose ranging from 0.25 to 1 mcg/day, with the majority of studies employing 0.5 mcg/day.

Regarding the effect on bone turnover markers, calcitriol as a monotherapy has been proven to be effective both in the short-term (1–4 weeks) and in the long-term in stimulating bone formation markers and blunting serum PTH levels and bone resorption markers in a dose-dependent manner, when compared to placebo or, in some cases, to calcium alone.

As reported in a systematic review, calcitriol has been proven to be effective in decreasing or even reversing the rate of bone loss in a variety of vulnerable populations in trials lasting up to 8 years, especially when administered together with bone antiresorptive agents [63]. As expected, the prolonged use of calcitriol yields the common side effects of hypercalciuria and mild hypercalcemia, usually in the mild range, without any report of deaths or life-threatening hypercalcemic episodes.

The efficacy of calcitriol in fracture risk reduction as a primary endpoint was assessed only in a few studies. The long-term effect on fracture rate of calcitriol was first assessed in an open-label study in osteoporotic postmenopausal women [104, 105]. Calcitriol (1 mcg, administered in two daily doses) was associated with an increase in BMD from 18 to 24 months of treatment, and a decrease in fracture risk over the 8 years of observation. However, this treatment was linked to the development of severe hypercalciuria, a few cases of nephrolithiasis (this latter effect also due to concomitant hyperoxaluria) and mild hypercalcemia. Thus, the following studies tested the effect of a lower dose of calcitriol. A prospective, single-blind study was carried out in osteoporotic postmenopausal women with one or more vertebral compression fractures, given calcitriol at a daily dose of 0.5 mcg, refracted in two daily doses, for 3 years. The treatment with calcitriol was able to reduce the number of new vertebral and peripheral fractures as compared to supplemental calcium, 1 g/day (12 fracture events per 100 patient-years in calcitriol group versus 44 fracture events per 100 patient-years, at 3 years of follow-up, $p < 0.05$), without significant side effects [106]. The antifracture efficacy of treatment with calcitriol lasting more than 1 year was also confirmed in patients after cardiac and lung transplantation. One study tested the efficacy of calcitriol in transplant patients (one fracture-event in calcium + calcitriol group vs. 22 fracture events in the calcium-only group) [107].

Some trials have examined the efficacy of calcitriol in combination with other agents in preserving bone mass, with study durations ranging from 6 months to 3 years [63]. As expected, these studies have demonstrated that calcitriol employed in combination with other antifracture drugs is more effective than calcitriol alone, with increases of 2–7 %/year in the groups of patients receiving both compounds. Unfortunately, no trials have considered fracture prevention as a primary endpoint. One study assessed the effect of calcitriol, estrogens, or their combination to prevent age-related bone loss in postmenopausal women with normal baseline bone mass [108]. In this group of women, estrogen replacement therapy and calcitriol when administered together produced a greater increase in BMD as compared to estrogen therapy alone, the difference being more evident at the level of appendicular bone (total hip and trochanter) [108]. Further study showed that in elderly women with a reduction in renal function (creatinine clearance less than 60 ml/min), calcitriol (0.25 mcg twice a day), alone or in combination with estrogens, decreased the risk of falling by 53 % [95 % CI −71 % to −22 %; $p = 0.003$] and by 61 % (95 % CI −76 % to −37 %; $p = 0.001$), respectively [109]. The effect on bone metabolism of an association of calcitriol and alendronate (Maxmarvil®, Yuyu Pharma, Inc., Korea) has been evaluated in Korean postmenopausal women with osteoporosis [110]. Combined treatment with calcitriol and alendronate (0.5 mcg and 5 mg daily, respectively) produced a net increase in lumbar spine BMD after 6 and 12 months as compared to alfacalcidol (2.42 ± 0.5 and 0.28 ± 0.5 % vs. baseline, respectively), with fewer episodes of hypercalciuria and overall good compliance, as also demonstrated in a postmarketing experience [110, 111].

All in all, these studies suggest that calcitriol may be used alone or in combination with other agents to limit and revert bone loss and, eventually, reduce fracture risk [63]. The incidence of side effects of hypercalciuria and hypercalcemia seems to be reduced when calcitriol is administered once a day or, better, intermittently (three consecutive days a week or one a week, in a high dose, i.e., ≥30 mcg/week), as assessed in studies employing calcitriol as an antineoplastic agent, although these findings are still not supported by studies in terms of efficacy [112].

Alfacalcidol

Alfacalcidol (calcitriol prodrug) is a 1α-hydroxylated derivative, which bypasses the strongly regulated, PTH-dependent activation in the kidney, and is converted to biologically active vitamin D by the non-saturable 25 hydroxylation in the liver. Indeed, renal 1α-hydroxylase is inhibited when $1,25(OH)_2D$ levels are normal due to the negative feedback on the parathyroids or in the case of high fibroblast growth factor 23 (FGF23) levels. Alfacalcidol is able to increase the levels of $1,25(OH)_2D$ even to slightly over-physiological levels, independent of whether the subject is replete or deplete, and independent of renal insufficiency.

In terms of bioequivalency, studies have demonstrated that calcitriol administered in a mean daily dose of 0.5 (refracted in two daily doses of 0.25 mcg) is equipotent to a mean daily dose of 1 mcg of alfacalcidol. Since

alfacalcidol after oral intake and intestinal absorption requires 25-hydroxylation in the liver for activation, it displays a retarded plasma curve with respect to calcitriol, with a consequent lower risk of developing hypercalcemia and hypercalciuria. Indeed, calcitriol after ingestion is immediately absorbed producing a plasma peak, with higher risk of calcium metabolism disturbances. Another advantage of alfacalcidol over calcitriol is the possibility to take advantage of direct intratissutal activation by locally expressed 25-hydroxylase.

Several clinical trials assessing the effect of alfacalcidol in humans were conducted in Japan in the 1980s. These initial reports showed that this compound (dose range 0.5–1 mcg/day) was effective in raising BMD and decreasing vertebral and hip fractures (reviewed in [113]), with low risk of adverse events (1.1 %), causing alfacalcidiol to become a major drug for the treatment of osteoporosis in Japan. Ensuing studies in an international setting confirmed the superior efficacy of alfacalcidol (0.5–1 mcg/day) in increasing intestinal calcium absorption, decreasing PTH, and increasing lumbar BMD more than parental vitamin D_3 alone or vitamin D_3 plus calcium [114, 115]. In a prophylactic study, alfacalcidol (1 mcg/day) was also shown to be able to fully prevent corticosteroid-induced bone loss observed under placebo [116]. A study comparing alfacalcidol and plain vitamin D_3 in glucocorticoid-induced osteoporosis demonstrated a modest increase (2.4 %) in the alfacalcidol-treated patients versus a 0.8 % loss in the vitamin D-treated ones in a 3-year treatment period [117].

Alfacalcidol has been shown to prevent falls and fractures in women with postmenopausal osteoporosis and also in elders of both sexes, especially in the case of age-related reduction in renal function [61, 113, 118, 119]. Recently, a study has demonstrated the efficacy of alfacalcidol (1 mcg + 500 mg of calcium daily) in reducing the risk of falls in men with established primary and secondary osteoporosis as compared to plain vitamin D (1000 IU + 1000 mg of calcium daily) in a prospective, 2 year-long, open-label study [120]. Treatment with alfacalcidol produced a higher increase in lumbar BMD (+3.2 vs. +0.8 %) and total hip BMD (+1.9 vs. −0.9 %) in the alfacalcidol- and vitamin D-treated groups, respectively, with a 50 % decrease in the rate of falls and and 48 % decrease in vertebral and non-vertebral fractures in the alfacalcidol-treated versus vitamin D-treated subjects [120]. The effect of calcidiol was more pronounced on the reduction of non-vertebral fractures in older men with a creatinine clearance less than 60 ml/min. These results were consistent with the reduction in falls observed in previous studies with calcitriol and alfacalcidol treatment, alone or in combination with bisphosphonates [118, 121, 122].

Eldecalcitol

Eldecalcitol, a vitamin D analog with reduced hypercalcemic effect but net antiresorbing and mild proformative properties as demonstrated in animal models (see "Discovery and development of main vitamin D metabolites and synthetic derivatives" section), has been successfully tested in humans and is available in Japan for the treatment of osteoporosis and fracture prevention.

In a randomized, double-blind, placebo-controlled trial, a 1-year treatment with oral eldecalcitol at 0.5, 0.75, or 1 mcg/day in osteoporotic patients improved lumbar BMD by 2.9, 3.4, and 3.8 % versus placebo, respectively, with transient yet mild hypercalcemia in 7–5–23 % of the treated subjects and with a parallel 20 % reduction in markers of bone turnover [123]. This occurred independently of baseline vitamin D status, as demonstrated in a post hoc analysis [124]. Thus, it is conceivable that eldecalcitol would not increase bone mass through merely ameliorating vitamin D status.

In a phase III clinical trial designed to test the antifracture efficacy of eldecalcitol (0.75 mcg/day) against alfacalcidol (1 mcg/day) over the course of 3 years, eldecalcitol was proven to be more effective than alfacalcidol in preventing vertebral and wrist fracture in vitamin D sufficient osteoporotic patients. The incidence of vertebral fractures was lower in the eldecalcitol group after 36 months of treatment (vertebral fractures at 3 years: 13.4 vs. 17.5 %; hazard ratio 0.74; 90 % CI 0.56–0.97) [125]. The increase in BMD at all sites and the decrease in bone turnover markers were more striking for eldecalcitol. In addition, eldecalcitol has been demonstrated to be more efficacious in preventing lower and severe vertebral fractures, with an overall improving in quality of life [126].

Vitamin D metabolites: the European scenario

The main vitamin D metabolites used in clinical practice are listed in Table 1.

While in the U.S., calcidiol is registered for use in renal osteodistrophy and is formulated as an injectable solution (Calderol®), in Europe, calcidiol, existing mainly as a propylene glycol solution administered as oral drops (5 mcg/drop), has been registered with different indications in the various EU countries. In France, the drug was originally employed in spasmophilia [127].

Calcidiol (Didrogyl®, Dedrogyl®, Dédrogyl®) is registered for rickets due to vitamin D deficiency in infants and children, and for osteomalacia (nutritional or due to malabsorption), treatment of vitamin D deficiency and spasmophilia in adults. Other indications in some countries

include hypocalcemia in chronic liver disease with osteomalacia, bone disease due to chronic long-term antiepileptic drug intake, osteomalacia after gastrectomy or small gut resection, neonatal hypocalcemia, hypoparathyroidism, prevention of calcium disorders secondary to corticosteroid or anticonvulsant therapy, and the prevention of vitamin D deficiency in renal failure in both children and adults. Calcidiol as solution in vials (Hidroferol®) is registered for renal osteodystrophy and in corticosteroid-induced osteoporosis.

The usual regimens are daily, weekly, or monthly administration, with preference for the daily (mean daily dose: 15–20 mcg, i.e., 3–4 drops/day) and weekly administration (mean weekly dose: 105–140 mcg, 24–28 drops/week), intervals appropriately contained within calcidiol half-life.

The optimal kinetics of calcidiol, due to prompt intestinal absorption and the relative rapid clearance from the blood, make it a good choice for vitamin D supplementation when administered in daily and weekly doses. Indeed, the use of calcidiol prevents the risk of megadoses of vitamin D_3 (>100,000 IU) used to quickly replenish vitamin D stores, which have been linked to non-classical toxic effects (Rossini 2012)(Sanders 2010), and allows better management of cases of toxicity given the shorter half-life with respect to parental vitamin D_3. Its use is advisable when an impaired 25-hydroxylation is hypothesized, as discussed above. Even if the shorter half-life and the smaller volume of distribution of calcidiol as compared to cholecalciferol might increase the risk of vitamin D inadequate response in the case of low compliance to treatment, this has not been demonstrated in long-term analyses on the adherence to these two different drugs and related outcomes, yet.

Europe, France, Italy, and Spain are the countries where calcidiol is most employed. In 2013, 3 million boxes of calcidiol were sold, for a total of 14.5 millions of euros, registering a 15 % increase with respect to 2012 (unpublished data). Interestingly, the use of calcidiol has intensified after its kinetic properties with respect to vitamin D_3 has been disclosed [86].

Among the active vitamin D analogs, calcitriol and alfacalcidol are registered in Europe for postmenopausal osteoporosis and renal bone disease, although data have shown their efficacy also in glucocorticoids-induced and in male osteoporosis. For these compounds, daily administration is recommended (mostly used regimens: 0.5 μg/day for calcitriol, 1 μg/day for alfacalcidol). The different doses employed are equivalent (i.e., same biological activity in terms of VDR activation). Eldecalcitol is not yet available in Europe.

Conclusions and research agenda

Vitamin D analogs, both natural derivatives and synthetic compounds, have been employed to ameliorate mineral and skeletal homestasis. In particular, calcidiol represents a valid alternative to cholecalciferol in terms of better kinetic properties, in the absence of the risk of toxic effects at the usual regimens employed, at least in the short term, thus representing a big advantage for the treatment of vitamin D deficiency. In addition, its use could be advisable in case of impaired 25-hydroxylation, decreased bioavailability of vitamin D_3 (e.g., obesity), malabsorption, and hypogonadism.

No conclusions can be drawn at this time regarding long-term supplementation and effectiveness on supposed classical and, especially, non-classical effects. No definitive conclusions have been drawn for the parental compound vitamin D with respect to the prevention of falls and fractures [128]. The few randomized studies available so far have compared same amounts (μg) of calcidiol and cholecalciferol, not equipotency. While it would be relatively easy to perform dose–response studies in different conditions with a single compound, it is difficult to compare these two compounds side by side with very different chemical properties and kinetics for a same biological effect. Therefore, regarding vitamin D supplementation, there is not sufficient evidence to recommend one compound over the other. Further studies are needed in this field, comparing the long-term efficacy and safety of calcidiol versus cholecalciferol in the different pathologic conditions listed above. These studies have to take into account the different kinetics of the two drugs, so that the real comparison would begin not in a transient situation, but once the steady state has been attained. One approach could be to design studies employing daily or weekly doses of both compounds which are recommended in daily practice according to the published guidelines, to correct vitamin D deficiency, assessing calcium absorption, calcemia, PTH, and calciuria.

Regarding active vitamin D analogs ("D-hormones"), discrepancies exist between in vitro and in vivo settings, meaning that further evidence is needed in humans to make recommendations for osteoporosis, and also to clarify the U-shaped curves for the observed effects. Further studies are needed to assess the effect of 1 alpha-hydroxylated compounds on fracture risk when used in combination with the most effective bone antiresorptive or proformative agents.

Moreover, the effects of intermittent therapy with calcitriol and similar compounds on BMD, falls and fracture risk, and on more sensitive biomarkers, their effectiveness

in specific forms of secondary osteoporosis, and the long-term complications of such therapies, have yet to be determined, in order to define the optimal intermittent regimen for a given outcome.

Finally, in all these studies, the effect of calcium supplementation or optimization needs to be assessed.

Acknowledgments This paper was derived from a Working Group meeting supported by the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO).

References

1. G. Jones, D.E. Prosser, M. Kaufmann, Cytochrome P450-mediated metabolism of vitamin D. J. Lipid Res. **55**, 13 (2014)
2. M. Imawari, Y. Akanuma, H. Itakura, Y. Muto, K. Kosaka, D.S. Goodman, The effects of diseases of the liver on serum 25-hydroxyvitamin D and on the serum binding protein for vitamin D and its metabolites. J. Lab. Clin. Med. **93**, 171 (1979)
3. J.E. Zerwekh, Blood biomarkers of vitamin D status. Am. J. Clin. Nutr. **87**, 1087S–1091S (2008)
4. L. Cianferotti, C. Marcocci, Subclinical vitamin D deficiency. Best Pract. Res. Clin. Endocrinol. Metab. **26**, 523 (2012)
5. N. Binkley, D. Wiebe, Clinical controversies in vitamin D: 25(OH)D measurement, target concentration, and supplementation. J. Clin. Densitom. **16**, 402 (2013)
6. M. Abboud, D.A. Puglisi, B.N. Davies, M. Rybchyn, N.P. Whitehead, K.E. Brock, L. Cole, C. Gordon-Thomson, D.R. Fraser, R.S. Mason, Evidence for a specific uptake and retention mechanism for 25-hydroxyvitamin D (25OHD) in skeletal muscle cells. Endocrinology **154**, 3022 (2013)
7. F. Molnár, R. Sigüeiro, Y. Sato, C. Araujo, I. Schuster, P. Antony, J. Peluso, C. Muller, A. Mouriño, D. Moras, N. Rochel, 1α,25(OH)2-3-epi-vitamin D3, a natural physiological metabolite of vitamin D3: its synthesis, biological activity and crystal structure with its receptor. Plos One **6**, e18124 (2011)
8. M.F. Holick, Vitamin D deficiency. N. Engl. J. Med. **357**, 266 (2007)
9. C.M. Girgis, R.J. Clifton-Bligh, M.W. Hamrick, M.F. Holick, J.E. Gunton, The roles of vitamin D in skeletal muscle: form, function, and metabolism. Endocr. Rev. **34**, 33 (2013)
10. P. Gerdhem, K.A. Ringsberg, K.J. Obrant, K. Akesson, Association between 25-hydroxy vitamin D levels, physical activity, muscle strength and fractures in the prospective population-based OPRA Study of Elderly Women. Osteoporos. Int. **16**, 1425 (2005)
11. J.K. Lai, R.M. Lucas, M.S. Clements, A.W. Roddam, E. Banks, Hip fracture risk in relation to vitamin D supplementation and serum 25-hydroxyvitamin D levels: a systematic review and meta-analysis of randomised controlled trials and observational studies. BMC Public Health. **10**, 331 (2010)
12. P. Pludowski, M.F. Holick, S. Pilz, C.L. Wagner, B.W. Hollis, W.B. Grant, Y. Shoenfeld, E. Lerchbaum, D.J. Llewellyn, K. Kienreich, M. Soni, Vitamin D effects on musculoskeletal health, immunity, autoimmunity, cardiovascular disease, cancer, fertility, pregnancy, dementia and mortality-a review of recent evidence. Autoimmun. Rev. **12**, 976 (2013)
13. H.A. Bischoff-Ferrari, E. Giovannucci, W.C. Willett, T. Dietrich, B. Dawson-Hughes, Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. Am. J. Clin. Nutr. **84**, 18 (2006)
14. M. Wacker, M.F. Holick, Vitamin D—effects on skeletal and extraskeletal health and the need for supplementation. Nutrients. **5**, 111 (2013)
15. B.W. Hollis, C.L. Wagner, Clinical review: the role of the parent compound vitamin D with respect to metabolism and function: Why clinical dose intervals can affect clinical outcomes. J. Clin. Endocrinol. Metab. **98**, 4619 (2013)
16. R.F. Chun, B.E. Peercy, E.S. Orwoll, C.M. Nielson, J.S. Adams, M. Hewison, Vitamin D and DBP: the free hormone hypothesis revisited. J Steroid Biochem Mol Biol. **144**, 132 (2014)
17. J.C. Gallagher, A. Sai, T. Templin 2nd, L. Smith, Dose response to vitamin D supplementation in postmenopausal women: a randomized trial. Ann. Intern. Med. **156**, 425 (2012)
18. B. Dawson-Hughes, A. Mithal, J.P. Bonjour, S. Boonen, P. Burckhardt, G.E. Fuleihan, R.G. Josse, P. Lips, J. Morales-Torres, N. Yoshimura, IOF position statement: vitamin D recommendations for older adults. Osteoporos. Int. **21**, 1151 (2010)
19. M.F. Holick, N.C. Binkley, H.A. Bischoff-Ferrari, C.M. Gordon, D.A. Hanley, R.P. Heaney, M.H. Murad, C.M. Weaver, Guidelines for preventing and treating vitamin D deficiency and insufficiency revisited. J. Clin. Endocrinol. Metab. **97**, 1153 (2012)
20. R. Rizzoli, S. Boonen, M.L. Brandi, O. Bruyère, C. Cooper, J.A. Kanis, J.M. Kaufman, J.D. Ringe, G. Weryha, J.Y. Reginster, Vitamin D supplementation in elderly or postmenopausal women: a 2013 update of the 2008 recommendations from the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO). Curr. Med. Res. Opin. **29**, 305 (2013)
21. H.A. Bischoff-Ferrari, W.C. Willett, E.J. Orav, P. Lips, P.J. Meunier, R.A. Lyons, L. Flicker, J. Wark, R.D. Jackson, J.A. Cauley, H.E. Meyer, M. Pfeifer, K.M. Sanders, H.B. Stähelin, R. Theiler, B. Dawson-Hughes, A pooled analysis of vitamin D dose requirements for fracture prevention. N. Engl. J. Med. **367**, 40 (2012)
22. K.M. Sanders, A.L. Stuart, E.J. Williamson, J.A. Simpson, M.A. Kotowicz, D. Young, G.C. Nicholson, Annual high-dose oral vitamin D and falls and fractures in older women: a randomized controlled trial. JAMA **303**, 1815 (2010)
23. M. Rossini, S. Adami, O. Viapiana, E. Fracassi, L. Idolazzi, M.R. Povino, D. Gatti, Dose-dependent short-term effects of single high doses of oral vitamin D(3) on bone turnover markers. Calcif. Tissue Int. **91**, 365 (2012)
24. M.R. Haussler, P.W. Jurutka, M. Mizwicki, A.W. Norman, Vitamin D receptor (VDR)-mediated actions of $1\alpha,25(OH)_2$vitamin D_3: genomic and non-genomic mechanisms. Best Pract. Res. Clin. Endocrinol. Metab. **25**, 543 (2011)
25. H.F. Deluca, History of the discovery of vitamin D and its active metabolites. Bonekey Rep. **3**, 479 (2014)
26. J. Lund, H.F. DeLuca, Biologically active metabolite of vitamin D3 from bone, liver, and blood serum. J. Lipid Res. **7**, 739 (1966)
27. L.V. Avioli, S.W. Lee, J.E. McDonald, J. Lund, H.F. DeLuca, Metabolism of vitamin D3-3H in human subjects: distribution in blood, bile, feces, and urine. J. Clin. Invest. **46**, 983 (1967)
28. J.W. Blunt, H.F. DeLuca, H.K. Schnoes, 25-hydroxycholecalciferol. A biologically active metabolite of vitamin D3. Biochemistry **7**, 3317 (1968)
29. J.W. Blunt, Y. Tanaka, H.F. DeLuca, Biological activity of 25-hydroxycholecalciferol, a metabolite of vitamin D3. Proc. Natl. Acad. Sci. USA **61**, 1503 (1968)
30. J.W. Blunt, H.F. DeLuca, The synthesis of 25-hydroxycholecalciferol. A biologically active metabolite of vitamin D3. Biochemistry **8**, 671 (1969)
31. G. Ponchon, A.L. Kennan, H.F. DeLuca, "Activation" of vitamin D by the liver. J. Clin. Invest. **48**, 2032 (1969)

32. D.R. Fraser, E. Kodicek, Unique biosynthesis by kidney of a biological active vitamin D metabolite. Nature **228**, 764 (1970)
33. D.E. Lawson, D.R. Fraser, E. Kodicek, H.R. Morris, D.H. Williams, Identification of 1,25-dihydroxycholecalciferol, a new kidney hormone controlling calcium metabolism. Nature **230**, 228 (1971)
34. I.T. Boyle, L. Miravet, R.W. Gray, M.F. Holick, H.F. Deluca, The response of intestinal calcium transport to 25-hydroxy and 1,25-dihydroxy vitamin D in nephrectomized rats. Endocrinology **90**, 605 (1972)
35. M.F. Holick, M. Garabedian, H.F. DeLuca, 1,25-dihydroxycholecalciferol: metabolite of vitamin D3 active on bone in anephric rats. Science **176**, 1146 (1972)
36. J.G. Haddad, K.J. Chyu, Competitive protein-binding radioassay for 25-hydroxycholecalciferol. J. Clin. Endocrinol. Metab. **33**, 992 (1971)
37. R. Belsey, H.F. Deluca, J.T. Potts Jr, Competitive binding assay for vitamin D and 25-OH vitamin D. J. Clin. Endocrinol. Metab. **33**, 554 (1971)
38. M.F. Holick, H.K. Schnoes, H.F. DeLuca, Identification of 1,25-dihydroxycholecalciferol, a form of vitamin D3 metabolically active in the intestine. Proc. Natl. Acad. Sci. USA **68**, 803 (1971)
39. A.W. Norman, J.F. Myrtle, R.J. Midgett, H.G. Nowicki, V. Williams, G. Popják, 1,25-dihydroxycholecalciferol: identification of the proposed active form of vitamin D3 in the intestine. Science **173**, 51 (1971)
40. Y. Tanaka, H.F. DeLuca, J. Omdahl, M.F. Holick, Mechanism of action of 1,25-dihydroxycholecalciferol on intestinal calcium transport. Proc. Natl. Acad. Sci USA **68**, 1286 (1971)
41. C.A. Frolik, H.F. Deluca, 1,25-dihydroxycholecalciferol: the metabolite of vitamin D responsible for increased intestinal calcium transport. Arch. Biochem. Biophys. **147**, 143 (1971)
42. M. Garabedian, M.F. Holick, H.F. Deluca, I.T. Boyle, Control of 25-hydroxycholecalciferol metabolism by parathyroid glands. Proc. Natl. Acad. Sci. USA **69**, 1673 (1972)
43. E.B. Mawer, C.M. Taylor, J. Backhouse, G.A. Lumb, S.W. Stanbury, Failure of formation of 1,25-dihydroxycholecalciferol in chronic renal insufficiency. Lancet **1**, 626 (1973)
44. B.S. Chertow, D.J. Baylink, J.E. Wergedal, M.H. Su, A.W. Norman, Decrease in serum immunoreactive parathyroid hormone in rats and in parathyroid hormone secretion in vitro by 1,25-dihydroxycholecalciferol. J. Clin. Invest. **56**, 668 (1975)
45. C.A. Frolik, H.F. DeLuca, The stimulation of 1,25-dihydroxycholecalciferol metabolism in vitamin D-deficient rats by 1,25-dihydroxycholecalciferol treatment. J. Clin. Invest. **52**, 543 (1973)
46. Y. Tanaka, H.F. Deluca, Bone mineral mobilization activity of 1,25-dihydroxycholecalciferol, a metabolite of vitamin D. Arch. Biochem. Biophys. **146**, 574 (1971)
47. L.G. Raisz, C.L. Trummel, M.F. Holick, H.F. DeLuca, 1,25-dihydroxycholecalciferol: a potent stimulator of bone resorption in tissue culture. Science **175**, 768 (1972)
48. M. Garabedian, Y. Tanaka, M.F. Holick, H.F. Deluca, Response of intestinal calcium transport and bone calcium mobilization to 1,25-dihydroxyvitamin D3 in thyroparathyroidectomized rats. Endocrinology **94**, 1022 (1974)
49. R.W. Gray, A.E. Caldas, D.R. Wilz, J. Lemann Jr, G.A. Smith, H.F. DeLuca, Metabolism and excretion of 3H-1,25-(OH)2-vitamin D3 in healthy adults. J. Clin. Endocrinol. Metab. **46**, 756 (1978)
50. T.L. Clemens, G.N. Hendy, R.F. Graham, E.G. Baggiolini, M.R. Uskokovic, J.L. O'Riordan, A radioimmunoassay for 1,25-dihydroxycholecalciferol. Clin. Sci. Mol. Med. **54**, 329 (1978)
51. Consensus Development Conference, Diagnosis, prophylaxis and treatment of osteoporosis. Am. J. Med. **94**, 646 (1993)
52. M.F. Holick, E.J. Semmler, H.K. Schnoes, H.F. DeLuca, 1 - Hydroxy derivative of vitamin D 3: a highly potent analog of 1,25-dihydroxyvitamin D 3. Science **180**, 190 (1973)
53. K. Ueno, Y. Okamiya, T. Makita, S. Kurozumi, H. Kawashima, Y. Hashimoto, Studies on biopharmacological activity of active vitamin D3 analogues (VII) Effect of 1 alpha-hydroxycholecalciferol on renal function in rats and Beagle dogs (Japanese). Nihon. Yakurigaku Zasshi. **75**, 617 (1979)
54. S. Morimoto, S. Imanaka, E. Koh, T. Shiraishi, T. Nabata, S. Kitano, Y. Miyashita, Y. Nishii, T. Ogihara, Comparison of the inhibitions of proliferation of normal and psoriatic fibroblasts by 1 alpha,25-dihydroxyvitamin D3 and synthetic analogues of vitamin D3 with an oxygen atom in their side chain. Biochem. Int. **19**, 1143 (1989)
55. H. Tsurukami, T. Nakamura, K. Suzuki, K. Sato, Y. Higuchi, Y. Nishii, A novel synthetic vitamin D analogue, 2 beta-(3-hydroxypropoxy)1 alpha, 25-dihydroxyvitamin D3 (ED-71), increases bone mass by stimulating the bone formation in normal and ovariectomized rats. Calcif. Tissue Int. **54**, 142 (1994)
56. Y. Tanaka, T. Nakamura, S. Nishida, K. Suzuki, S. Takeda, K. Sato, Y. Nishii, Effects of a synthetic vitamin D analog, ED-71, on bone dynamics and strength in cancellous and cortical bone in prednisolone-treated rats. J. Bone Miner. Res. **11**, 325 (1996)
57. Y. Uchiyama, Y. Higuchi, S. Takeda, T. Masaki, A. Shira-Ishi, K. Sato, N. Kubodera, K. Ikeda, E. Ogata, ED-71, a vitamin D analog, is a more potent inhibitor of bone resorption than alfacalcidol in an estrogen-deficient rat model of osteoporosis. Bone **30**, 582 (2002)
58. P.H. de Freitas, T. Hasegawa, S. Takeda, M. Sasaki, C. Tabata, K. Oda, M. Li, H. Saito, N. Amizuka, Eldecalcitol, a second-generation vitamin D analog, drives bone minimodeling and reduces osteoclastic number in trabecular bone of ovariectomized rats. Bone **49**, 335 (2011)
59. S. Harada, T. Mizoguchi, Y. Kobayashi, Y. Nakamichi, S. Takeda, S. Sakai, F. Takahashi, H. Saito, H. Yasuda, N. Udagawa, T. Suda, N. Takahashi, Daily administration of eldecalcitol (ED-71), an active vitamin D analog, increases bone mineral density by suppressing RANKL expression in mouse trabecular bone. J. Bone Miner. Res. **27**, 461 (2012)
60. K.H. Lau, D.J. Baylink, Vitamin D therapy of osteoporosis: plain vitamin D therapy versus active vitamin D analog (D-hormone) therapy. Calcif. Tissue Int. **65**, 295 (1999)
61. S. O'Donnell, D. Moher, K. Thomas, D.A. Hanley, A. Cranney, Systematic review of the benefits and harms of calcitriol and alfacalcidol for fractures and falls. J. Bone Miner. Metab. **26**, 531 (2008)
62. M.L. Brandi, S. Minisola, Calcidiol [25(OH)D3]: from diagnostic marker to therapeutical agent. Curr. Med. Res. Opin. **29**, 1565 (2013)
63. L.J. Peppone, S. Hebl, J.Q. Purnell, M.E. Reid, R.N. Rosier, K.M. Mustian, O.G. Palesh, A.J. Huston, M.N. Ling, G.R. Morrow, The efficacy of calcitriol therapy in the management of bone loss and fractures: a qualitative review. Osteoporos. Int. **21**, 1133 (2010)
64. J.A. Eisman, R. Bouillon, Vitamin D: direct effects of vitamin D metabolites on bone: lessons from genetically modified mice. Bonekey Rep. **3**, 499 (2014)
65. L. Ovesen, C. Brot, J. Jakobsen, Food contents and biological activity of 25-hydroxyvitamin D: a vitamin D metabolite to be reckoned with? Ann. Nutr. Metab. **47**, 107 (2003)
66. J.E. Smith, D.S. Goodman, The turnover and transport of vitamin D and of a polar metabolite with the properties of 25-hydroxycholecalciferol in human plasma. J. Clin. Invest. **50**, 2159 (1971)
67. T.C. Stamp, J.M. Round, J.G. Haddad, Effect of oral vitamin D, 25-hydroxycholecalciferol (25-HCC) and whole-body ultra-

violet irradiation on plasma 25-HCC levels in man. Clin. Sci. **44**, 3P (1973)
68. T.C. Stamp, Intestinal absorption of 25-hydroxycholecalciferol. Lancet **2**, 121 (1974)
69. J.G. Haddad, T.C. Stamp, Circulating 25-hydroxyvitamin D in man. Am. J. Med. **57**, 57 (1974)
70. J.G. Haddad Jr, S. Rojanasathit, Acute administration of 25-hydroxycholecalciferol in man. J. Clin. Endocrinol. Metab. **42**, 284 (1976)
71. K.S. Jones, I. Schoenmakers, L.J. Bluck, S. Ding, A. Prentice, Plasma appearance and disappearance of an oral dose of 25-hydroxyvitamin D2 in healthy adults. Br. J. Nutr. **107**, 1128 (2012)
72. T.C. Stamp, J.G. Haddad, C.A. Twigg, Comparison of oral 25-hydroxycholecalciferol, vitamin D, and ultraviolet light as determinants of circulating 25-hydroxyvitamin D. Lancet **1**, 1341 (1977)
73. B.W. Hollis, H.R. Conrad, J.W. Hibbs, Changes in plasma 25-hydroxycholecalciferol and selected blood parameters after injection of massive doses of cholecalciferol or 25-hydroxycholecalciferol in non-lactating dairy cows. J. Nutr. **107**, 606 (1977)
74. M.J. Barger-Lux, R.P. Heaney, S. Dowell, T.C. Chen, M.F. Holick, Vitamin D and its major metabolites: serum levels after graded oral dosing in healthy men. Osteoporos. Int. **8**, 222 (1998)
75. A. Jetter, A. Egli, B. Dawson-Hughes, H.B. Staehelin, E. Stoecklin, R. Goessl, J. Henschkowski, H.A. Bischoff-Ferrari, Pharmacokinetics of oral vitamin D(3) and calcifediol. Bone **59**, 14 (2014)
76. K.D. Cashman, K.M. Seamans, A.J. Lucey, E. Stöcklin, P. Weber, M. Kiely, T.R. Hill, Relative effectiveness of oral 25-hydroxyvitamin D3 and vitamin D3 in raising wintertime serum 25-hydroxyvitamin D in older adults. Am. J. Clin. Nutr. **95**, 1350 (2012)
77. S.H. Chou, T.K. Chung, B. Yu, Effects of supplemental 25-hydroxycholecalciferol on growth performance, small intestinal morphology, and immune response of broiler chickens. Poult. Sci. **88**, 2333 (2009)
78. C. Lauridsen, U. Halekoh, T. Larsen, S.K. Jensen, Reproductive performance and bone status markers of gilts and lactating sows supplemented with two different forms of vitamin D. J. Anim. Sci. **88**, 202 (2010)
79. N. Sahin, T.A. Balci, O. Kucuk, M.O. Smith, K. Sahin, Effects of 25-hydroxycholecalciferol and soy isoflavones supplementation on bone mineralisation of quail. Br. Poult. Sci. **50**, 709 (2009)
80. E.A. Hines, J.D. Coffey, C.W. Starkey, T.K. Chung, J.D. Starkey, Improvement of maternal vitamin D status with 25-hydroxycholecalciferol positively impacts porcine fetal skeletal muscle development and myoblast activity. J. Anim. Sci. **91**, 4116 (2013)
81. T.J. Hahn, L.R. Halstead, S.L. Teitelbaum, B.H. Hahn, Altered mineral metabolism in glucocorticoid-induced osteopenia. Effect of 25-hydroxyvitamin D administration. J. Clin. Invest. **64**, 655 (1979)
82. R.M. Francis, M. Peacock, J.H. Storer, A.E. Davies, W.B. Brown, B.E. Nordin, Calcium malabsorption in the elderly: the effect of treatment with oral 25-hydroxyvitamin D3. Eur. J. Clin. Invest. **13**, 391 (1983)
83. S. Russo, L. Carlucci, C. Cipriani, A. Ragno, S. Piemonte, R.D. Fiacco, J. Pepe, V. Fassino, S. Arima, E. Romagnoli, S. Minisola, Metabolic changes following 500 μg monthly administration of calcidiol: a study in normal females. Calcif. Tissue Int. **89**, 252 (2011)
84. M. Peacock, G. Liu, M. Carey, R. McClintock, W. Ambrosius, S. Hui, C.C. Johnston, Effect of calcium or 25OH vitamin D3 dietary supplementation on bone loss at the hip in men and women over the age of 60. J. Clin. Endocrinol. Metab. **85**, 3011 (2000)
85. M. Sosa, P. Láinez, A. Arbelo, M.C. Navarro, The effect of 25-dihydroxyvitamin D on the bone mineral metabolism of elderly women with hip fracture. Rheumatology (Oxford) **39**, 1263 (2000)
86. H.A. Bischoff-Ferrari, B. Dawson-Hughes, E. Stöcklin, E. Sidelnikov, W.C. Willett, J.O. Edel, H.B. Stähelin, S. Wolfram, A. Jetter, J. Schwager, J. Henschkowski, A. von Eckardstein, A. Egli, Oral supplementation with 25(OH)D3 versus vitamin D3: effects on 25(OH)D levels, lower extremity function, blood pressure, and markers of innate immunity. J. Bone Miner. Res. **27**, 160 (2012)
87. M.L. Bianchi, C. Colombo, B.M. Assael, A. Dubini, M. Lombardo, S. Quattrucci, S. Bella, M. Collura, B. Messore, V. Raia, F. Poli, R. Bini, C.V. Albanese, V. De Rose, D. Costantini, G. Romano, E. Pustorino, G. Magazzù, S. Bertasi, V. Lucidi, G. Traverso, A. Coruzzo, A.D. Grzejdziak, Treatment of low bone density in young people with cystic fibrosis: a multicentre, prospective, open-label observational study of calcium and calcifediol followed by a randomised placebo-controlled trial of alendronate. Lancet Respir. Med. **1**, 377 (2013)
88. A. Drincic, E. Fuller, R.P. Heaney, L.A. Armas, 25-Hydroxyvitamin D response to graded vitamin D_3 supplementation among obese adults. J. Clin. Endocrinol. Metab. **98**, 4845 (2013)
89. S. Petta, C. Cammà, C. Scazzone, C. Tripodo, V. Di Marco, A. Bono, D. Cabibi, G. Licata, R. Porcasi, G. Marchesini, A. Craxí, Low vitamin D serum level is related to severe fibrosis and low responsiveness to interferon-based therapy in genotype 1 chronic hepatitis C. Hepatology **51**, 1158 (2010)
90. L.V. Avioli, S.J. Birge, S.W. Lee, Effects of prednisone on vitamin D metabolism in man. J. Clin. Endocrinol. Metab. **28**, 1341 (1968)
91. M. Carré, O. Ayigbedé, L. Miravet, H. Rasmussen, The effect of Prednisolone upon the metabolism and action of 25-hydroxy- and 1,25-dihydroxyvitamin D3. Proc. Natl. Acad. Sci. USA **71**, 2996 (1974)
92. S.A. Hamed, Influences of bone and mineral metabolism in epilepsy. Expert Opin. Drug Saf. **10**, 265 (2011)
93. Al Mutair, A.N., Nasrat, G.H., Russell, D.W.: mutation of the CYP2R1 vitamin D 25-hydroxylase in a Saudi Arabian family with severe vitamin D deficiency. J. Clin. Endocrinol. Metab. **97**, E2022 (2012)
94. I. Garcia-Delgado, S. Prieto, L. Gil-Fraguas, E. Robles, J.J. Rufilanchas, F. Hawkins, Calcitonin, etidronate, and calcidiol treatment in bone loss after cardiac transplantation. Calcif. Tissue Int. **60**, 155 (1997)
95. M. Talalaj, L. Gradowska, E. Marcinowska-Suchowierska, M. Durlik, Z. Gaciong, M. Lao, Efficiency of preventive treatment of glucocorticoid-induced osteoporosis with 25-hydroxyvitamin D3 and calcium in kidney transplant patients. Transplant. Proc. **28**, 3485 (1996)
96. J.E. Compston, B. Creamer, Plasma levels and intestinal absorption of 25-hydroxyvitamin D in patients with small bowel resection. Gut **18**, 171 (1977)
97. A.J. Batchelor, G. Watson, J.E. Compston, Changes in plasma half-life and clearance of 3H-25-hydroxyvitamin D3 in patients with intestinal malabsorption. Gut **23**, 1068 (1982)
98. M. Davies, E.B. Mawer, E.L. Krawitt, Comparative absorption of vitamin D3 and 25-hydroxyvitamin D3 in intestinal disease. Gut **21**, 287 (1980)
99. J. Michaud, J. Naud, D. Ouimet, C. Demers, J.L. Petit, F.A. Leblond, A. Bonnardeaux, M. Gascon-Barré, V. Pichette, Reduced hepatic synthesis of calcidiol in uremia. J. Am. Soc. Nephrol. **21**, 1488 (2010)

100. G. Jean, J.C. Terrat, T. Vanel, J.M. Hurot, C. Lorriaux, B. Mayor, C. Chazot, Daily oral 25-hydroxycholecalciferol supplementation for vitamin D deficiency in haemodialysis patients: effects on mineral metabolism and bone markers. Nephrol. Dial. Transplant. **23**, 3670 (2008)
101. H. Schmidt-Gayk, C. Grawunder, W. Tschöpe, W. Schmitt, E. Ritz, V. Pietsch, K. Andrassay, R. Bouillon, 25-hydroxy-vitamin-D in nephrotic syndrome. Lancet **2**, 105 (1977)
102. I.H. de Boer, M.C. Sachs, P.A. Cleary, A.N. Hoofnagle, J.M. Lachin, M.E. Molitch, M.W. Steffes, W. Sun, B. Zinman, J.D. Brunzell, Diabetes Control and Complication Trial/Epidemiology of Diabetes Interventions and Complications Study Research Group. Circulating vitamin D metabolites and kidney disease in type 1 diabetes. J. Clin. Endocrinol. Metab. **97**, 4780 (2012)
103. C. Foresta, G. Strapazzon, L. De Toni, L. Perilli, A. Di Mambro, B. Muciaccia, L. Sartori, R. Selice, Bone mineral density and testicular failure: evidence for a role of vitamin D 25-hydroxylase in human testis. J. Clin. Endocrinol. Metab. **96**, E646 (2011)
104. A. Caniggia, R. Nuti, F. Lore, G. Martini, V. Turchetti, G. Righi, Long-term treatment with calcitriol in postmenopausal osteoporosis. Metabolism. **39**, 43 (1990)
105. A. Caniggia, R. Nuti, F. Loré, G. Martini, B. Frediani, S. Giovani, Total body absorptiometry in postmenopausal osteoporosis patients treated with 1 alpha-hydroxylated vitamin D metabolites. Osteoporos. Int. **3**, 181 (1993)
106. M.W. Tilyard, G.F. Spears, J. Thomson, S. Dovey, Treatment of postmenopausal osteoporosis with calcitriol or calcium. N. Engl. J. Med. **326**, 357 (1992)
107. P. Sambrook, N.K. Henderson, A. Keogh, P. MacDonald, A. Glanville, P. Spratt, P. Bergin, P. Ebeling, J. Eisman, Effect of calcitriol on bone loss after cardiac or lung transplantation. J. Bone Miner. Res. **15**, 1818 (2000)
108. J.C. Gallagher, S.E. Fowler, J.R. Detter, S.S. Sherman, Combination treatment with estrogen and calcitriol in the prevention of age-related bone loss. J. Clin. Endocrinol. Metab. **86**, 3618 (2001)
109. J.C. Gallagher, P.B. Rapuri, L.M. Smith, An age-related decrease in creatinine clearance is associated with an increase in number of falls in untreated women but not in women receiving calcitriol treatment. J. Clin. Endocrinol. Metab. **92**, 51 (2007)
110. Y. Rhee, M. Kang, Y. Min, D. Byun, Y. Chung, C. Ahn, K. Baek, J. Mok, D. Kim, D. Kim, H. Kim, Y. Kim, S. Myoung, D. Kim, S.K. Lim, Effects of a combined alendronate and calcitriol agent (Maxmarvil) on bone metabolism in Korean postmenopausal women: a multicenter, double-blind, randomized, placebo-controlled study. Osteoporos. Int. **17**, 1801 (2006)
111. H.W. Suh, H.O. Kim, Y.S. Kim, S. Sunwoo, J.A. Lee, H.R. Lee, B. Kim, D.H. Kim, Y.S. Choi, Y.S. Cheong, K. Yum, Y.J. Yang, B.Y. Yu, C.H. Cho, S.B. Park, D.H. Shin, Korea Post-Marketing Surveillance Research Group. The efficacy and safety of a combined alendronate and calcitriol agent (maxmarvil): a post-marketing surveillance study in korean postmenopausal women with osteoporosis. Korean. J Fam. Med. **33**, 346 (2012)
112. D.L. Trump, D.M. Potter, J. Muindi, A. Brufsky, C.S. Johnson, Phase II trial of high-dose, intermittent calcitriol (1,25 dihydroxyvitamin D3) and dexamethasone in androgen-independent prostate cancer. Cancer **106**, 2136 (2006)
113. H. Orimo, E. Schacht, The D-hormone analog alfacalcidol: the pioneer beyond the horizon of osteoporosis treatment. J. Rheumatol. Suppl. **76**, 4 (2005)
114. R.M. Francis, I.T. Boyle, C. Moniz, A.M. Sutcliffe, B.S. Davis, G.H. Beastall, R.A. Cowan, N. Downes, A comparison of the effects of alfacalcidol treatment and vitamin D2 supplementation on calcium absorption in elderly women with vertebral fractures. Osteoporos. Int. **6**, 284 (1996)
115. R. Nuti, G. Bianchi, M.L. Brandi, R. Caudarella, E. D'Erasmo, C. Fiore, G.C. Isaia, G. Luisetto, M. Muratore, P. Oriente, S. Ortolani, Superiority of alfacalcidol compared to vitamin D plus calcium in lumbar bone mineral density in postmenopausal osteoporosis. Rheumatol. Int. **26**, 445 (2006)
116. J.Y. Reginster, D. Kuntz, W. Verdickt, M. Wouters, L. Guillevin, C.J. Menkès, K. Nielsen, Prophylactic use of alfacalcidol in corticosteroid-induced osteoporosis. Osteoporos. Int. **9**, 75 (1999)
117. J.D. Ringe, A. Dorst, H. Faber, E. Schacht, V.W. Rahlfs, Superiority of alfacalcidol over plain vitamin D in the treatment of glucocorticoid-induced osteoporosis. Rheumatol. Int. **24**, 63 (2004)
118. L. Dukas, H.A. Bischoff, L.S. Lindpaintner, E. Schacht, D. Birkner-Binder, T.N. Damm, B. Thalmann, H.B. Stähelin, Alfacalcidol reduces the number of fallers in a community-dwelling elderly population with a minimum calcium intake of more than 500 mg daily. J. Am. Geriatr. Soc. **52**, 230 (2004)
119. L. Dukas, E. Schacht, Z. Mazor, H.B. Stähelin, Treatment with alfacalcidol in elderly people significantly decreases the high risk of falls associated with a low creatinine clearance of <65 ml/min. Osteoporos. Int. **16**, 198 (2005)
120. J.D. Ringe, P. Farahmand, E. Schacht, Alfacalcidol in men with osteoporosis: a prospective, observational, 2-year trial on 214 patients. Rheumatol. Int. **33**, 637 (2013)
121. J.C. Gallagher, The effects of calcitriol on falls and fractures and physical performance tests. J. Steroid Biochem. Mol. Biol. **89–90**, 497 (2004)
122. J.D. Ringe, P. Farahmand, E. Schacht, A. Rozehnal, Superiority of a combined treatment of Alendronate and Alfacalcidol compared to the combination of Alendronate and plain vitamin D or Alfacalcidol alone in established postmenopausal or male osteoporosis (AAC-Trial). Rheumatol. Int. **27**, 425 (2007)
123. T. Matsumoto, T. Miki, H. Hagino, T. Sugimoto, S. Okamoto, T. Hirota, Y. Tanigawara, Y. Hayashi, M. Fukunaga, M. Shiraki, T. Nakamura, A new active vitamin D, ED-71, increases bone mass in osteoporotic patients under vitamin D supplementation: a randomized, double-blind, placebo-controlled clinical trial. J. Clin. Endocrinol. Metab. **90**, 5031 (2005)
124. T. Matsumoto, N. Kubodera, ED-71, a new active vitamin D3, increases bone mineral density regardless of serum 25(OH)D levels in osteoporotic subjects. J. Steroid Biochem. Mol. Biol. **103**, 584 (2007)
125. T. Matsumoto, M. Ito, Y. Hayashi, T. Hirota, Y. Tanigawara, T. Sone, M. Fukunaga, M. Shiraki, T. Nakamura, A new active vitamin D3 analog, eldecalcitol, prevents the risk of osteoporotic fractures–a randomized, active comparator, double-blind study. Bone **49**, 605 (2011)
126. H. Hagino, T. Takano, M. Fukunaga, M. Shiraki, T. Nakamura, T. Matsumoto, Eldecalcitol reduces the risk of severe vertebral fractures and improves the health-related quality of life in patients with osteoporosis. J. Bone Miner. Metab. **31**, 183 (2013)
127. M.P. Caraceni, G.C. Gandolini, F. Campanini, M.A. Iannetta, S. Ortolani, Vitamin D metabolites in spasmophilia. Biomed. Pharmacother. **44**, 479 (1990)
128. A. Avenell, J.C. Mak, D. O'Connell, Vitamin D and vitamin D analogues for preventing fractures in post-menopausal women and older men. Cochrane Database Syst. Rev. **4**, CD000227 (2014)