REVIEW

100 YEARS OF VITAMIN D

Historical aspects of vitamin D

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Abstract

Vitamin D has many physiological functions including upregulation of intestinal calcium and phosphate absorption, mobilization of bone resorption, renal reabsorption of calcium as well as actions on a variety of pleiotropic functions. It is believed that many of the hormonal effects of vitamin D involve a 1,25-dihydroxyvitamin D_3-vitamin D receptor-mediated transcriptional mechanism involving binding to the cellular chromatin and regulating hundreds of genes in many tissues. This comprehensive historical review provides a unique perspective of the many steps of the discovery of vitamin D and its deficiency disease, rickets, stretching from 1650 until the present. The overview is divided into four distinct historical phases which cover the major developments in the field and in the process highlighting the: (a) first recognition of rickets or vitamin D deficiency; (b) discovery of the nutritional factor, vitamin D and its chemical structure; (c) elucidation of vitamin D metabolites including the hormonal form, 1,25-dihydroxyvitamin D_3; (d) delineation of the vitamin D cellular machinery, functions and vitamin D-related diseases which focused on understanding the mechanism of action of vitamin D in its many target cells.

Key Words

- vitamin D
- vitamin D metabolism
- rickets and osteomalacia
- calcium and phosphate homeostasis
- vitamin D analogs
- vitamin D function
- 7-dehydrocholesterol
- UV light

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Introduction

The history of vitamin D is a rich and storied subject and is now over 350 years old. It began in the early 1600s with the first descriptions of the human deficiency disease: rickets in children and osteomalacia in adults. Of course, there were no precise medical details that distinguished it from other bone diseases, but treatises describing the symptoms and lithographs from that time showing bone deformities resembling rickets leave little doubt that it was vitamin D deficiency. It took another 250 years to define the cause of vitamin D deficiency in the 1900–1920 period when physicians and biochemists elucidated the role of sunlight and identified the chemical structure of the two main forms of the vitamin D molecule, vitamin D_2 and vitamin D_3.

Another 50 years elapsed before the metabolism of vitamin D was first described in 1967 and the active form of vitamin D, namely 1,25-dihydroxyvitamin D ($1,25\text{-}(OH)_2D$), was discovered. The period of time since has witnessed the exciting realization that vitamin D has its own set of dedicated specialized machinery consisting of transport proteins, metabolic enzymes and vitamin D receptor (VDR) to mediate the actions of vitamin D, not only in bone but also in many other tissues around the body where it has a myriad of different physiological effects.

Before we get into the history of vitamin D, let us first remind the reader of the general aspects of its nomenclature, origins and principal functions. Vitamin D is a steroidal substance required by all vertebrates including humans to maintain blood calcium and phosphate within a narrow normal range and thereby support a healthy skeleton, muscle contraction, immune function and optimal cellular functions in many locations around the body (1). The name vitamin D is a term coined by nutritionists, and

is not a chemical term, which is defined as '*a substance with anti-rachitic properties that will cure rickets*'. In human biology, vitamin D usually refers to two substances: vitamin D_3 (usually known as cholecalciferol) of animal origin and vitamin D_2 (referred to as ergocalciferol) of plant or fungal origin. These two forms have roughly equal potencies, similar metabolic patterns and identical effects in the body.

Because of the four phases of vitamin D history, this review is divided into four sections each summarizing one particular time period:

1. 1650–1890: history of vitamin D deficiency (rickets)
2. 1890–1930: history of the discovery of vitamin D and its structural elucidation
3. 1930–1975: history of the discovery of vitamin D metabolites including 1,25-$(OH)_2D_3$
4. 1975–present: history of the discovery of the vitamin D cellular machinery, functions and vitamin D-related human diseases.

Since the different facets of the history of vitamin D represent interesting topics and span many centuries, they have been reviewed by many previous historians, including the current author, and interested readers are invited to further access these because they focus on different aspects of the overall story (2, 3, 4, 5, 6, 7, 8).

1650–1890: history of vitamin D deficiency (rickets)

There is no doubt that rickets was prevalent in Europe long before it was recognized as a specific disease in the 15th century, but the earliest documentation of the word 'rickets' was in a domestic receipt book of an English family in 1632 and the earliest printed record of rickets as a disease causing death in the London Bill of Mortality in 1634 (reviewed by (2, 3, 4)). The term *rickets* is thought to have its origins in the verb in the Dorset dialect to '*rucket*,' which means to breathe with difficulty. However, some claim the term rickets is derived from the Anglo-Saxon word '*wrikken*,' meaning to twist. Rickets and osteomalacia were first clearly described by Daniel Whistler in the Netherlands (1645) as a condition in which the skeleton was poorly mineralized and deformed (9). Francis Glisson (1650) provided the first documented records with his book entitled *De Rachitide* first published in Latin in 1650 and then translated into English in 1671 (10). It features a lithograph of children with bowing of the legs and skeletal deformities which are the hallmarks

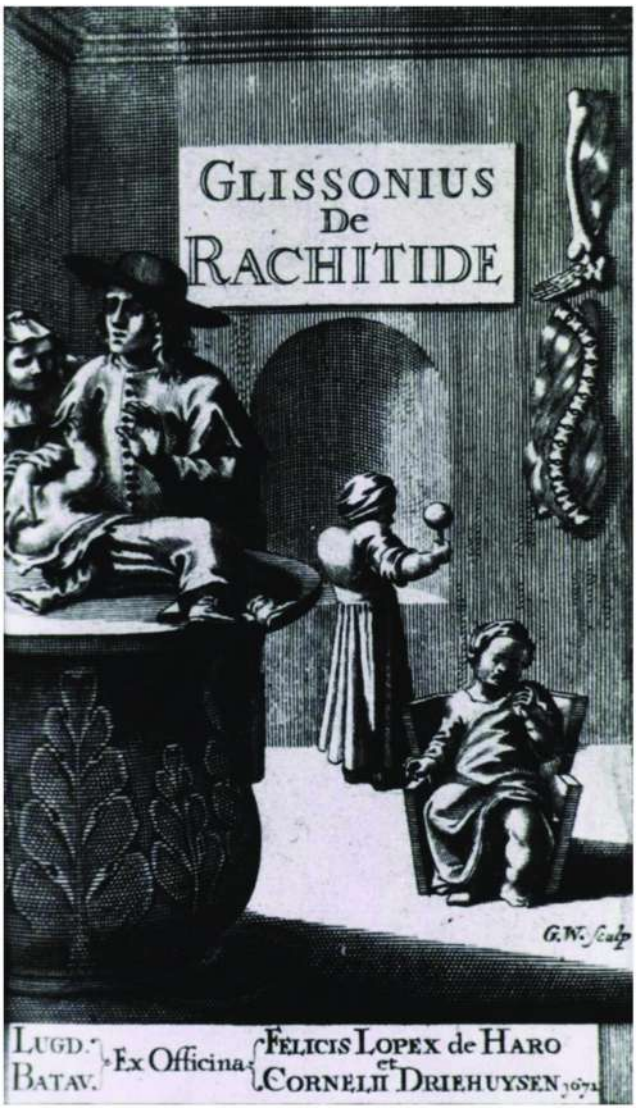

Figure 1
Lithograph from Glisson's *De Rachitide* (1671) (10) also depicted as the frontispiece of Hess AF's book (11) *Rickets Including Osteomalacia and Tetany*. Reproduced from the US National Library digital collection. Credit: Rickets, including osteomalacia and tetany / by Alfred F Hess.

of vitamin D deficiency. One of those Glisson lithographs was reproduced as a frontispiece in a landmark treatise on *Rickets Including Osteomalacia and Tetany* by AF Hess in 1929 (11). It is reproduced here as Fig. 1.

A more recent definition of vitamin D deficiency has grown to include defective chondrocyte differentiation and lack of mineralization of the growth plate, but the common feature of vitamin D deficiency is insufficiently mineralized or calcified bone matrix (1, 12, 13). Rickets is characterized by a deformed and misshaped skeleton, particularly bending and bowing of the long bones and enlargement of the epiphyses of the joints of the rib cage,

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arms, legs and neck. Victims have painful movements of the rib cage and difficulty breathing. In China, medical texts refer to deformities of the rib cage in severe rickets as 'chicken breast' (5). Severe rickets is often accompanied by pneumonia. The loss of the important role of vitamin D in strengthening the immune system compounds this problem. Though rarely is rickets life-threatening, it certainly lowers the quality of life for the afflicted individual and leads to secondary problems. One of these secondary effects of rickets occurs in young women who had vitamin D deficiency in childhood causing deformities of the pelvis which result in difficulties in childbirth (14). Shorter (14) speculates that rickets in early life must have resulted in numerous deaths of women during their first delivery.

Vitamin D deficiency is partly the result of inadequate skin synthesis of vitamin D_3 from 7-dehydrocholesterol compounded by a low dietary intake of vitamin D_2 from plant or fungal sources or vitamin D_3 from animal products. The advent of the Industrial Revolution in Western Europe heralded in massive air pollution in the form of smoke from mills and burning of fossil fuels. This dramatically reduced the amount of UV light reaching the ground. Since the workers needed for these new industrial jobs were required to move from their rural locations into dingy, poorly-lit cities, their exposure to UV light diminished and skin synthesis of vitamin D was reduced. Rickets resulted and was associated with lack of exposure to sufficient sunlight. Thus, the 18th and 19th centuries saw a higher increase in rickets in the industrialized cities of northern Europe. The Dickensian character Tiny Tim, of the novel *A Christmas Carol*, clearly represents a child with a deformed skeleton who must have been a common sight in the dark cities of the late 19th century (7). Rickets was particularly prevalent in the industrialized Britain of the 16th–20th centuries, and thus, it is no surprise that it was referred to in old texts as 'the English disease' (7, 15).

Despite the fact that rickets seemed to be associated with lack of exposure to sunlight, by the late 1700s, some, including Percival (16) in the UK, were advocating the use of cod liver oil for the treatment of rickets suggesting a nutritional aspect to vitamin D. In contrast, in the early 1800s, Sniadecki (17) in Poland was documenting the differential incidence in city-dwellers and rural-dwellers suggesting some environmental factor was involved. He speculated that sunlight or fresh air might be involved in the etiology of the disease. By the end of the 19th century, a rigorous debate roared on whether rickets was caused by the lack of some dietary substance or an environmental factor and how could these two points of view be reconciled.

1890–1930: history of the discovery of vitamin D and its structural elucidation

By the 1890s, some researchers such as Owen (18) and Palm (19), who clearly supported the environmental theory, produced evidence that there were big geographical differences in the incidence of rickets in different parts of the UK and northern and southern China. Palm, a medical missionary, went on to suggest that exposure of children to sunlight would cure rickets (19). Subsequently, researchers in Europe and the United States namely Buchholtz (1904), Raczynski (1913), Huldshinsky (1919), and later Chick (1922) and Hess & Weinstock (1924) performed experiments in which laboratory animals and children with rickets could be cured with sunlight or light from mercury arc lamps (7, 20, 21, 22, 23, 24). This clearly demonstrated that lack of exposure to UV light was one cause of rickets.

But the proponents of the theory that a dietary factor could also be involved continued with their experiments too. The early 20th century was a momentous period in nutritional research in which nutritionists showed that a diet of highly purified carbohydrates, protein, fat and salt is unable to fully support growth and life of experimental animals (25). By adding various 'trace factors', researchers were able to restore growth and a full range of physiological actions. The first of these trace factors was thiamin discovered by Funk (26) which cured neuritis in what Funk termed the '*vital amine or vitamin theory*.' Thiamin was later renamed vitamin B_1, but it was one of a number of vitamin substances that are defined as '*trace compounds which are derived from the diet and are required in small amounts per day and perform an essential role critical to life*.' Vitamin D was identified as one of these substances playing a critical role in skeletal growth and calcium and phosphate homeostasis. However, strictly speaking, vitamin D has been misnamed since it can also be derived from exposure to UV light and is not required to be in the diet. In practise and for a variety of social and religious reasons, many populations around the world do not receive adequate UV light, especially during the winter months, so that a dietary intake is essential.

The discovery of the nutritional factor, later termed vitamin D by McCollum (27), came largely as the result of the work of a number of researchers: Mellanby, McCollum, Steenbock and Hart working independently. Sir Edward Mellanby (28) in the UK reasoned that rickets might be due to a dietary deficiency and managed to produce beagle dogs with severe rickets by feeding them oatmeal and then cured their rickets with cod liver oil. Since cod liver oil is a mixture of lipids and a rich source of vitamin A, it was not clear what the active ingredient might be. McCollum (29),

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working first at the U Wisconsin and then Johns-Hopkins, heated and bubbled oxygen through the cod liver oil to destroy the vitamin A and found that the product still cured rickets. Building on the new vitamin nomenclature, he termed the new substance vitamin D. But how was the field to reconcile the apparently unconnected findings that UV light and a nutritional substance termed vitamin D could both cure rickets? Harry Steenbock also working at the U Wisconsin-Madison performed the definitive experiment. Steenbock & Black experimented with the diets of goats and found that sunlight or UV irradiation of the animals or their diets resulted in rickets being cured in the goats (30). Steenbock traced the bioactive substance in irradiated food to the non-saponifiable fraction of lipids in the diet and showed that it cured rickets (31). Dietary vitamin D was born.

Subsequently, Steenbock was able to show that irradiated yeast contained significant amounts of vitamin D, later shown to be vitamin D_2 and that the yeast could be irradiated and added to milk which formed the basis of the first food fortification with vitamin D (5). Though Steenbock and the University of Wisconsin filed a patent for milk fortification with vitamin D, the proceeds from this discovery were used to establish the Wisconsin Alumni Research Foundation (WARF) which was one of the prototypical organizations intended to allow universities to plough the benefits of their research into future research. WARF funded the research of a number of scientists inside and outside of the vitamin D field, included several Nobel laureates, with the proceeds of Steenbock's patent. Furthermore, vitamin D fortification of a variety of foodstuffs (including milk, margarine, bread and even beer) has become a major nutritional tool in the fight to prevent rickets and osteomalacia around the world (5).

In the late 1920s, Windaus and colleagues (32) isolated the key anti-rachitic substance from a mixture of irradiated plant sterols and named it vitamin D_1, although they did not identify its structure. Later, vitamin D_1 was shown to be a mixture of vitamin D_2 and tachysterol. A British group headed by Askew (33) successfully identified and determined the structure of the anti-rachitic, plant-derived sterol as vitamin D_2 or ergocalciferol. Windaus's group confirmed the structure of vitamin D_2 (34) and also isolated and identified the animal-derived, anti-rachitic vitamin D_3 or cholecalciferol and its skin precursor, 7-dehydrocholesterol (35). For his discovery of the structures of vitamin D_3, 7-dehydrocholesterol and several other sterols, Adolf Windaus was awarded the 1928 Nobel Prize for Chemistry (Fig. 2).

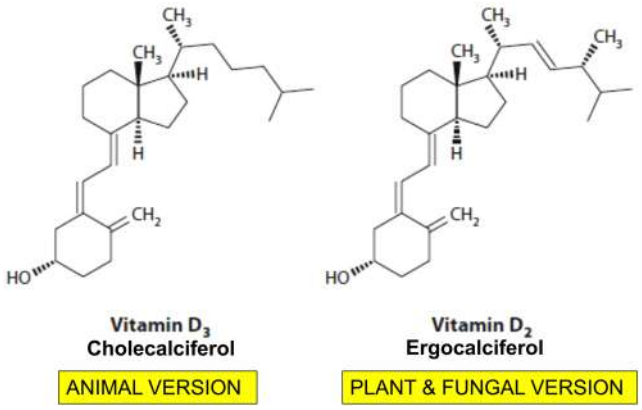

Figure 2
Structures of vitamin D_2 and D_3. The two versions of vitamin D differ only in their side chains vitamin D_2 possessing an additional C-22-23 double bond and a C-24 methyl group. The modifications make little significant difference in their metabolism or biological actions.

1930–1975: history of the discovery of vitamin D metabolites including 1,25-$(OH)_2D_3$

Chemically synthesized vitamin D_2 and vitamin D_3 have been available since the 1930s and paved the way for the study of their biological functions and metabolism. The physiological roles of vitamin D are primarily its roles in calcium and phosphate homeostasis (1) and include:

(1) stimulation of intestinal calcium and phosphate absorption;
(2) mobilization of calcium from bone;
(3) renal reabsorption of calcium.

All three of these functions serve to raise blood calcium and phosphate and ensure that these ions are available to ensure health and prevent rickets. Elucidating the details of these physiological functions became the main foci during the 1930–1960 time period, and research revealed that vitamin D was intimately connected to the roles of other calcium and phosphate-related hormones including parathyroid hormone (PTH) and calcitonin. Details of these connections are beyond the scope of this chapter and are described in reviews (1) and in other articles in this special series.

In the 1960s, there was considerable debate over whether the functions of vitamin D were carried out by vitamin D itself or its possible metabolites. Consequently, intense effort was put into studying the metabolism of vitamin D by using chemically synthesized radioactive versions of vitamin D_2 and vitamin D_3. The pioneer in this area was Egon Kodicek at the Dunn Nutritional Laboratories, U Cambridge UK. After 10 years of work, Kodicek (36) concluded that vitamin D was active without

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Table 1 History of the discovery of the major metabolites of vitamins D_2 and D_3.

Metabolite	Tissue source	Biosynthetic enzyme	Biological role	Discovery
Vitamin D_3 metabolites				
25-OH-D_3	Liver	25-Hydroxylase (CYP2R1)	Main circulating metabolite	Blunt *et al.* 1968 (38)
1,25-$(OH)_2D_3$	Kidney (major) Extra-renal sites	1α-Hydroxylase (CYP27B1)	Active hormonal form	Lawson *et al.* 1969 (39) Myrtle *et al.* 1970 (40) Holick *et al.* 1971 (41)
24,25-$(OH)_2D_3$	Kidney (major) Extra-renal sites	24-Hydroxylase (CYP24A1)	Principal catabolite	Suda *et al.* 1970a (48) Holick *et al.* 1972 (49)
25,26-$(OH)_2D_3$	Unknown	26-Hydroxylase (?)	Catabolite	Suda *et al.* 1970b (50)
25-OH-D_3-26,23-lactone	Kidney (major) Extra-renal sites	24-Hydroxylase (CYP24A1)	Presumed catabolite	Wichmann *et al.* 1979 (51)
1,24,25-$(OH)_3D_3$	Kidney (major) Extra-renal sites	24-Hydroxylase (CYP24A1)	Unknown possible catabolite	Holick *et al.* 1974 (52)
Calcitroic acid	Kidney (major) Extra-renal sites	24-Hydroxylase (CYP24A1)	Excretory form	Esvelt *et al.* 1981 (53)
Calcioic acid	Kidney (major)	24-Hydroxylase (CYP24A1)	Excretory form	Kaufmann *et al.* 2019 (76)
4α,25-$(OH)_2D_3$ 4β,25-$(OH)_2D_3$	Liver	General cytochrome P450 (CYP3A4)	Excretory form	Wang *et al.* 2013 (77)
Vitamin D_2 metabolites				
25-OH-D_2	Liver	25-Hydroxylase (CYP2R1)	Main circulating metabolite	Suda *et al.* 1969 (45)
1,25-$(OH)_2D_2$	Kidney (major)	1α-Hydroxylase (CYP27B1)	Active hormonal form	Jones *et al.* 1975 (46)
24,25-$(OH)_2D_2$	Kidney (major)	24-Hydroxylase (CYP24A1)	Principal catabolite	Jones *et al.* 1980 (47)
1,24,25-$(OH)_3D_2$	Kidney (major)	24-Hydroxylase (CYP24A1)	Presumed catabolite	Reddy *et al.* 1986 (78)

being metabolized. In retrospect, the radioactive vitamin D that his group were using was insufficiently labeled to detect its metabolites. However, Hector DeLuca, again at the U Wisconsin-Madison, and the final graduate student of Harry Steenbock, synthesized radioactive vitamin D_3 with much higher specific activity (37) and was able to demonstrate metabolism to more polar metabolites, the principal one being 25-hydroxyvitamin D_3 (25-OH-D_3) (38) made in the liver and the first identified natural vitamin D metabolite.

25-OH-D_3 proved to be more potent biologically than vitamin D_3 and was present in the bloodstream at a higher concentration (38). We now identify 25-OH-D_3 as the principal circulating form of vitamin D. But that is not the extent of vitamin D metabolism. Several other groups then entered or re-entered the picture, including Dr Kodicek's, as well as that of one of Dr DeLuca's former graduate students Dr Anthony Norman. Among the other polar products of vitamin D_3 was a metabolite even more potent than 25-OH-D_3, namely 1α,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$) which is now universally accepted as the hormonal form of vitamin D_3. Several groups including Dr Kodicek's, (39) Dr Norman's (40) and Dr DeLuca's (41) were credited with playing a role in the discovery and/or

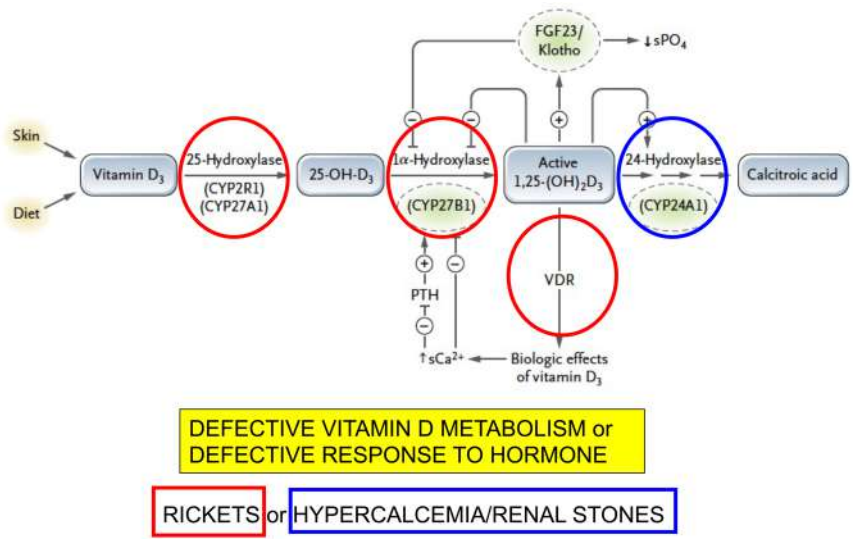

Figure 3
Metabolism and mechanism of action of vitamin D_3. Skin-synthesized or dietary vitamin D_3 is converted via a two-step hydroxylation process into the active hormonal form 1,25-$(OH)_2D_3$. The hormone binds to the vitamin D receptor (VDR) and regulates serum calcium (sCa^{2+}) and serum phosphate (sPO_4) levels ensuring sufficient minerals for normal cellular activity around the body including bone. Insufficient vitamin D results in insufficient 1,25-$(OH)_2D_3$ and vitamin deficiency rickets. Circled in red are the proteins in the vitamin D-specific machinery that when mutated also result in some type of rickets. Circled in blue is the enzyme CYP24A1 that when mutated results in elevated 1,25-$(OH)_2D_3$ and hypercalcemia and/or kidney stones.

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Table 2 History of the main protein components of the specific* vitamin D signal transduction machinery.

Protein	Abbreviation	Tissue location or source	Biological function	Discovery	Gene cloning
Vitamin D-binding globulin	DBP	Liver	Transport of vitamin D and its metabolites	Daiger *et al.* 1975 (64)	Cooke *et al.* 1991 (79)
Vitamin D receptor	VDR	Most tissues except liver	Regulation of vitamin D-dependent genes	Haussler 1969 (80) Brumbaugh *et al.* 1975 (55)	McDonnell *et al.* 1987 (56)
25-Hydroxylase	CYP2R1	Liver	25-hydroxylation of vitamins D_2 and D_3	Cheng *et al.* 2003 (81)	Cheng *et al.* 2004 (75)
1α-Hydroxylase	CYP27B1	Kidney (major) Extra-renal sites	1α-hydroxylation of 25-OH-D_2 & 25-OH-D_3	Fraser *et al.* 1970 (42)	St-Arnaud *et al.* 1997 (70) Takeyama *et al.* 1997 (71)
24-Hydroxylase	CYP24A1	Kidney (major) Extra-renal sites	24-hydroxylation of (& 23- & 26-hydroxylation) 25-OH-D_2 & 25-OH-D_3 Complete catabolism of vitamin D	Knutson *et al.* 1972 (66)	Ohyama & Okuda 1991 (72)

Other cellular proteins play a general role in vitamin D metabolism and action, for example, CYP3A4 but this degrades many other molecules and drugs.
*The specific vitamin D signal transduction machinery is specialized to transport, activate, mediate the biological effects of and catabolize vitamin D.

in the structural identification of 1,25-$(OH)_2D_3$. Kodicek's group administered a mixture of radioactive [4-^{14}C] and [1-^{3}H]vitamin D_3 preparations and showed that one polar metabolite lost its tritium atom during metabolism that aided in its identification as a 1-hydroxylated compound (39). Furthermore, the Cambridge group also showed that the hormone was biologically generated in the kidney (39, 42). Dr Norman's group showed that the new metabolite was associated with the chromatin of intestinal mucosal cells and had greater biological activity than even 25-OH-D_3 (40). Holick *et al.* (41) showed that the additional 1-hydroxyl group was in the 1α orientation and supported their identification of the metabolite as 1α,25-$(OH)_2D_3$ with mass spectrometry. Chemically synthesized 1,25-$(OH)_2D_3$ was first produced by Semmler *et al.* (43) and made commercially by a group headed by Dr Milan Uskokovic at Hoffmann-La Roche in the early 1970s and is known clinically by the name calcitriol (44).

The identification of the principal metabolites, 25-OH-D_3 and 1,25-$(OH)_2D_3$, spawned a frenzy of research activity in the vitamin D area and the discovery of a number of other vitamin D metabolites (1). Among these are the principal metabolites of vitamin D_2 including 25-OH-D_2 (45), 1,25-$(OH)_2D_2$ (46) and 24,25-$(OH)_2D_2$ (47). Also identified in that mixture of metabolites arising from radioactive vitamin D_3 were several compounds that are presumed to be inactive catabolites including, 24,25-$(OH)_2D_3$, 25,26-$(OH)_2D_3$, 25-OH-D_3-26,23-lactone, 1,24,25-$(OH)_3D_3$ and calcitroic acid (48, 49, 50, 51, 52, 53). A summary of the main metabolites of both vitamin D_3 and vitamin D_2 along with their tissue source, biosynthetic enzyme, details of first reporting and biological role is presented in Table 1 and depicted in a metabolic pathway diagram (Fig. 3).

1975–present: history of the discovery of the vitamin D cellular machinery, functions and vitamin D-related human diseases

The discovery of the active forms of vitamin D heralded in a search for

(a) the signal transduction mechanisms to explain how 1,25-$(OH)_2D_3$ was able to produce its various biological effects;
(b) identification of the enzymes responsible for the synthesis and catabolism of 1,25-$(OH)_2D_3$;
(c) a clear understanding of the regulation of the vitamin D endocrine system.

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Table 3 History of the main vitamin D-related genetic and acquired human diseases and animal models generated to study them.

Disease	Cause	Initial report	Animal model equivalent	Generated by
Vitamin D deficiency rickets	Lack of dietary vitamin D Lack of skin synthesis of D	F Glisson 1671 (10)	Beagle dog on oatmeal diet Lactating goat model	Mellanby 1919 (28) Steenbock & Black 1924 (30)
Vitamin D dependency rickets type 1A	Genetic defect in CYP27B1	Fraser *et al.* 1972 (82)	CYP27B1 null mouse	Kato 1999 (83) Panda *et al.* 2001 (84) St-Arnaud *et al.* 2003 (85)
Vitamin D dependency rickets type 1B	Genetic defect in CYP2R1	Cheng *et al.* 2004 (75)	CYP2R1 null mouse	Zhu *et al.* 2013 (86)
Vitamin D dependency rickets type 2	Genetic defect in VDR	Rosen *et al.* 1979 (87) Eil *et al.* 1981 (88)	VDR null mouse	Yoshizawa *et al.* 1997 (89) Li *et al.* 1998 (90)
Idiopathic infantile hypercalcemia	Genetic defect in CYP24A1	Lightwood 1953 (91) Schlingmann *et al.* 2011 (92)	CYP24A1 null mouse	St-Arnaud *et al.* 2000 (93)
Chronic kidney disease	Loss of Kidney CYP27B1 enzyme activity	DeLuca & Avioli 1970 (94) Brickman *et al.* 1974 (95)	Dog nephrectomy models	Rutherford *et al.* 1977 (96)

These studies began almost as soon as metabolism was recognized in the late 1960s when Mark Haussler, in AW Norman's laboratory, demonstrated that vitamin D metabolites were associated with the chromatin (54). Clear evidence of the protein that is now termed the vitamin D receptor (VDR) was produced by Haussler's lab (55). The VDR protein from various species was later purified and its gene was cloned by Haussler's group (56, 57). Study of the pure protein has led to a determination of its crystal structure (58). Parallel to these investigations of the VDR have come other studies on how it works both at the whole-body level in calcium and phosphate homeostasis and other pleiotropic functions (1, 8, 59) and at the cellular level in a classic steroid hormone super-family like process through a transcriptional mechanism (60). Over the past 30 years, Mark Haussler, Wes Pike and colleagues (61) have demonstrated that 1,25-$(OH)_2D_3$ works through a VDR-mediated mechanism that involves many coactivators and repressors to directly interact with and regulate hundreds of genes around the body. Other researchers, most notably Anthony Norman (62), have proposed that some of the actions of vitamin D occur through rapid non-genomic signaling pathways, possibly involving a plasma membrane VDR but this protein has never been fully characterized at the molecular level. Nevertheless, there remains some uncertainty that all vitamin D ligands and analogs produce their effects through a genomic VDR mechanism (63).

The history of two other components of the vitamin D machinery deserves some mention.

These are vitamin D-binding globulin (64, 65) and the cytochromes P450-containing enzymes that metabolize vitamin D into its many metabolites (66). Being a fat-soluble vitamin, vitamin D requires a protein to transport it around the body and the vitamin D-binding globulin (usually abbreviated as DBP) performs this function. DBP was first identified as Gc (group-specific component) in the 1970s, and its properties have been reviewed extensively by the father figure of the field Roger Bouillon, U Leuven, Belgium (65). DBP has a high affinity for most of the main metabolites of vitamin D, most notably 25-OH-D, and because of this, 25-OH-D is the main circulating form in the blood.

The cytochrome P450-containing enzymes (CYPs) responsible for vitamin D metabolism were first studied in the early 1970s in tissue extracts of liver and kidney (67, 68, 69) and then in tissue culture and given names based upon their hydroxylation activity: 25-hydroxylase, 1α-hydroxylase and 24-hydroxylase. In the early 1990–2005 period, all three enzymes were purified, cloned and expressed in cell culture systems, principally by Canadian group of St-Arnaud (70) as well as the Japanese groups of Kato S (71), Okuda (72) and Sakaki (73, 74) as well as Russell's group at the U Texas (75). The three enzymes are now known as CYP2R1, CYP27B1 and CYP24A1. A review of the CYP field and how these enzymes operate and how they are regulated is provided (66). A summary of the history of the signal transduction protein machinery for vitamin D including VDR, DBP and the various CYPs is provided in Table 2.

No review of the recent history of vitamin D would be complete without an overview of how defects in vitamin D metabolism result in human disease. It is now evident that vitamin D deficiency and rickets are caused by several genetic and acquired errors in vitamin D metabolism which involve any of the major protein components of the

vitamin D machinery described above. These are compiled into Table 3 where we document the disease name, the component of the vitamin D machinery affected, as well as the publication first describing it. Besides diseases involving too little 1,25-$(OH)_2D_3$ and resulting in rickets, diseases involving too much 1,25-$(OH)_2D_3$ which cause hypercalcemia are also included in Table 3. Most of these diseases involving a shortage of 1,25-$(OH)_2D_3$ are now treated with vitamin D analogs which were developed from knowledge of the metabolism and biological actions of vitamin D. Currently approved and marketed vitamin D analogs are listed in Table 4 along with their original publications.

Conclusions

The history of vitamin D is indeed a rich subject which has already stretched over 350 years and involved the four phases described in this review. While the chemical entity vitamin D remained unknown for all but 100 of those years, the significant medical consequences of vitamin D deficiency were evident for the whole of that time. Many physicians, nutritionists, biochemists, chemists and molecular biologists have worked to elucidate our current knowledge of the nature of vitamin D in addition to its metabolism, mechanism of action and biological activities. That knowledge has paid dividends by providing new therapies for the treatment of deficiency and excess vitamin D action. The field of vitamin D research is arguably one of the highlights of modern medicine.

Table 4 History of the commercially approved vitamin D drugs (vitamin D analogs) used to treat rickets and related diseases.

Vitamin D analog	Drug name	Marketed by	Field of use*	Initial report	Comments
25-OH-D_3	Calderol	Organon	Vitamin deficiency	Blunt & DeLuca 1969 (97)	First vitamin D metabolite
	Rayaldee	OPKO Renal	Chronic kidney disease		Licensed by Upjohn, Kalamazoo
1,25-$(OH)_2D_3$	Calcijex	Roche	Vitamin D dependency type 1A	Semmler *et al.* 1972 (43)	First vitamin D active analog
	Generic		Chronic kidney disease		
1α-OH-D_3	One-alpha	Leo Pharma	Vitamin D deficiency	Holick *et al.* 1973 (98)	1-hydroxylated prodrug not requiring activation by kidney
	Alfacalcidiol		Chronic kidney disease	Barton *et al.* 1973 (99)	
1α-OH-D_2	Hectorol	Genzyme/Sanofi	Chronic kidney disease	Lam *et al.* 1974 (100)	1-hydroxylated prodrug not requiring activation by kidney
	Doxercalciferol	Sandoz			
19-nor-1,25-$(OH)_2D_2$	Paricalcitol	Abbott	Chronic kidney disease	Takahashi F *et al.* 1997 (101)	Active 'low-calcemic' vitamin D analog
Calcipotriol	Daivonex	Leo Pharma	Psoriasis	Calverley 1987 (102)	Topical rapidly metabolized side-chain modified vitamin D analog

*Many of the vitamin D drugs used in chronic kdney disease stages 3–4 and beyond are used to suppress secondary hyperparathyroidism, as well as having a moderate serum calcium-raising activity.

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References

1 Jones G, Strugnell SA & DeLuca HF. Current understanding of the molecular actions of vitamin D. *Physiological Reviews* 1998 **78** 1193–1231. (https://doi.org/10.1152/physrev.1998.78.4.1193)

2 Swinburne LM. Rickets and the Fairfax family receipt books. *Journal of the Royal Society of Medicine* 2006 **99** 391–395. (https://doi.org/10.1258/jrsm.99.8.391)

3 O'Riordan JL & Bijvoet OL. Rickets before the discovery of vitamin D. *BoneKEy Reports* 2014 **3** 478. (https://doi.org/10.1038/bonekey.2013.212)

4 Mays S. The epidemiology of rickets in the 17th–19th centuries: some contributions from documentary sources and their value to palaeopathologists. *International Journal of Paleopathology* 2018 **23** 88–95. (https://doi.org/10.1016/j.ijpp.2017.10.011)

5 Jones G. Vitamin D. In *The Cambridge World History of Food. Part IVA4: The Nutrients- Deficiencies, Surfeits and Food-Related Disorders*, pp. 763–768. Eds KF Kiple & KC Ornelas. Cambridge: University of Cambridge Press, 2000.

6 Jones G. The discovery and the synthesis of the nutritional factor vitamin D. *International Journal of Paleopathology* 2018 **23** 96–99. (https://doi.org/10.1016/j.ijpp.2018.01.002)

7 Chesney RW. Theobald Palm and his remarkable observation: how the sunshine vitamin came to be recognized. *Nutrients* 2012 **4** 42–51. (https://doi.org/10.3390/nu4010042)

8 DeLuca HF. Historical overview of vitamin D. In *Vitamin D*, 3rd ed., chapter 1, pp. 2–12. Eds D Feldman, JW Pike & JS Adams. Academic Press, 2011.

9 Whistler D. De Morbo Puerili Anglorum, Quem Patrio Idiomate Indigenae Vocant. The Rickets. *MD Thesis*. Leiden, Netherlands: University of Leiden, 1645.

10 Glisson F. *De Rachitide Sive Morbo Puerili Quoi Vulgo. The Rickets Dicitur*. London: Sadler & Beaumont, 1650.

11 Hess AF. Frontispiece. In *Rickets Including Osteomalacia and Tetany*. Philadelphia: Lea & Febiger, 1929.

12 Pettifor JM. Nutritional rickets: deficiency of vitamin D, calcium, or both? *American Journal of Clinical Nutrition* 2004 **80** 1725S–1729S. (https://doi.org/10.1093/ajcn/80.6.1725S)

13 Munns CF, Shaw N, Kiely M, Specker BL, Thacher TD, Ozono K, Michigami T, Tiosano D, Mughal MZ, Makitie O, *et al.* Global consensus recommendations on prevention and management of nutritional rickets. *Journal of Clinical Endocrinology and Metabolism* 2016 **101** 394–415. (https://doi.org/10.1210/jc.2015-2175)

14 Shorter E. *A History of Women's Bodies New York*, pp. 1–398. New York: Perseus Books, 1982.

15 Belton N. Not only the English disease. *Acta Paediatrica Scandinavia* 1986 **323S** 68–75.

16 Percival T. *Essays Medical, Philosophical and Experimental on the Medical Use of Cod-Liver Oil*, vol. 2. London, 1789.

17 Sniadecki J. Jerdrzej Sniadecki (1768–1838) on the cure of rickets. (1840) Cited by W. Mozolowski. *Nature* 1939 **143** 121–124. (https://doi.org/10.1038/143121a0)

18 Owen I. Geographical distribution of rickets, acute and subacute rheumatism, chorea, cancer and urinary calculus in the British Islands. *BMJ* 1889 **1** 113–118.

19 Palm TA. The geographical distribution and etiology of rickets. *Practitioner* 1890 **45** 270–279.

20 Buchholz E. Ueber Lichtbehandlung der Rachitis und anderer Kinderkrankheiten. In *Verhandlungen der Gesellschaft fur der Abteilung fur Kinderheilkunde der 76*, vol. 21, p. 116. Breslau, Germany: Versammlung der Gesellschaft Deutscher Natturforcher und Aerzte in Breslau, 1904.

21 Raczynski J. Recherches experimentales sur la manque d'action du soleil comme cause de rachitisme. In *Comptes Rendues de l'Association de Pediatrics Paris*, pp. 308–309, 1913.

22 Huldschinsky K. Heilung von rachitis durch kunestliche hohensonne. *Deutsche Medizinische Wochenschrift* 1919 **45** 712–713. (https://doi.org/10.1055/s-0028-1137830)

23 Chick H, Palzell EJ & Hume EM. *Studies of Rickets in Vienna 1919–1922*. Medical Research Council, Special Report No 77, 1923.

24 Hess AF & Weinstock M. Antirachitic properties imparted to inert fluids and to green vegetables by ultra-violet irradiation. *Journal of Biological Chemistry* 1924 **62** 301–313. (https://doi.org/10.1016/S0021-9258(18)85064-5)

25 Hopkins FG. Feeding experiments illustrating the importance of accessory food factors in normal dietaries. *Journal of Physiology* 1912 **44** 425–460. (https://doi.org/10.1113/jphysiol.1912.sp001524)

26 Funk C. The preparation from yeast and certain foodstuffs of the substance the deficiency of which in diet occasions polyneuritis in birds. *Journal of Physiology* 1912 **45** 75–81. (https://doi.org/10.1113/jphysiol.1912.sp001537)

27 McCollum EV, Simmonds N, Becker JE & Shipley PG. Studies on experimental rickets XXI. An experimental demonstration of the existence of a vitamin which promotes calcium deposition. *Journal of Biological Chemistry* 1922 **53** 293–312. (https://doi.org/10.1016/S0021-9258(18)85783-0)

28 Mellanby E. An experimental investigation on rickets. *Lancet* 1919 **I** 407–412.

29 McCollum EV. The paths to the discovery of vitamins A and D. *Journal of Nutrition* 1967 **91** (Supplement 1) 11–16. (https://doi.org/10.1093/jn/91.2_Suppl.11)

30 Steenbock H & Black A. Fat-soluble vitamins. XVII. The induction of growth-promoting and calcifying properties in a ration by exposure to ultra-violet light. *Journal of Biological Chemistry* 1924 **61** 405–422. (https://doi.org/10.1016/S0021-9258(18)85139-0)

31 Steenbock H & Black A. The induction of growth-promoting and calcifying properties in fats and their unsaponifiable constituents by exposure to light. *Journal of Biological Chemistry* 1924 **64** 263–298.

32 Windaus A & Linsert O. Vitamin D1. *Justus Liebig's Annalen Der Chemie* 1928 **465** 148–166. (https://doi.org/10.1002/jlac.19284650108)

33 Askew FA, Bourdillon RB, Bruce HM, Jenkins RGC & Webster TA. The distillation of vitamin D. *Proceedings of the Royal Society* 1931 **B107** 76–90.

34 Windaus A, Linsert O, Luttringhaus A & Feidlich G. Crystalline vitamin D_2. *Justus Liebig's Annalen Der Chemie* 1932 **492** 226–241. (https://doi.org/10.1002/jlac.19324920111)

35 Windaus A, Schenk F & Van Werder FT. Uber das antirachitisch wirksame Bestrah-lungsprodukt aus 7-dehydrocholesterin. *Hoppe-Seyler's Zeitschrift für Physiologische Chemie* 1936 **241** 100–103. (https://doi.org/10.1515/bchm2.1936.241.1-3.100)

36 Kodicek E. The metabolism of vitamin D. In *Proceedings of the Fourth International Congress of Biochemistry*, vol. 11, pp. 198–208. Eds W Umbreit, H Molitor & L Pergammon. London: Pergammon, 1960.

37 Neville PF & DeLuca HF. The synthesis of. (1,2-^{3}H]vitamin D_3 and the tissue localization of a 0.25 µg (10 IU) dose per rat. *Biochemistry* 1966 **5** 2201–2207. (https://doi.org/10.1021/bi00871a007)

38 Blunt JW, DeLuca HF & Schnoes HK. 25-Hydroxycholecalciferol: a biologically active metabolite of vitamin D. *Biochemistry* 1968 **7** 3317–3322. (https://doi.org/10.1021/bi00850a001)

39 Lawson DEM, Wilson PW & Kodicek E. Metabolism of vitamin D. A new cholecalciferol involving loss of hydrogen at C-1 in chick intestinal nuclei. *Biochemical Journal* 1969 **115** 269–277. (https://doi.org/10.1042/bj1150269)

40 Myrtle JF, Haussler MR & Norman AW. Evidence for the biologically active form of cholecalciferol in the intestine. *Journal of Biological Chemistry* 1970 **245** 1190–1196. (https://doi.org/10.1016/S0021-9258(18)63306-X)

41 Holick MF, Schnoes HK, DeLuca HF, Suda T & Cousins RJ. Isolation and identification of 1,25- dihydroxycholecalciferol. A metabolite of vitamin D active in the intestine. *Biochemistry* 1971 **10** 2799–2804. (https://doi.org/10.1021/bi00790a023)

42 Fraser DR & Kodicek E. Unique biosynthesis by kidney of a biologically active vitamin D metabolite. *Nature* 1970 **228** 764–766. (https://doi.org/10.1038/228764a0)

43 Semmler EJ, Holick MF, Schnoes HK & DeLuca HF. The synthesis of 1,25-dihydroxycholecalciferol – a metabolically active form of vitamin D_3. *Tetrahedron Letters* 1972 **40** 4147–4150.

44 Baggiolini EG, Wovkulich PM, Iacobelli JA, Hennessy BM & Uskokovic MR. Preparation of 1α-hydroxylated vitamin D metabolites by total synthesis. In *Vitamin D: Chemical, Biochemical and Clinical Endocrinology of Calcium Metabolism*, pp. 1089–1100. Eds AW Norman, K Schaefer, D von Herrath & HG Grigoleit. Berlin: De Gruyter, 1982.

45 Suda T, DeLuca HF, Schnoes HK & Blunt JW. 25-Hydroxyergocalciferol: a biologically active metabolite of vitamin D_2. *Biochemical and Biophysical Research Communications* 1969 **35** 182–185. (https://doi.org/10.1016/0006-291x(69)90264-2)

46 Jones G, Schnoes HK & DeLuca HF. Isolation and identification of 1,25-dihydroxyvitamin D_2. *Biochemistry* 1975 **14** 1250–1256. (https://doi.org/10.1021/bi00677a025)

47 Jones G, Schnoes HK, Levan L & Deluca HF. Isolation and identification of 24-hydroxyvitamin D_2 and 24,25-dihydroxyvitamin D_2. *Archives of Biochemistry and Biophysics* 1980 **202** 450–457. (https://doi.org/10.1016/0003-9861(80)90449-x)

48 Suda T, DeLuca HF, Schnoes HK, Ponchon G, Tanaka Y & Holick MF. 21,25-Dihydroxycholecalciferol. A metabolite of vitamin D_3 preferentially active on bone. *Biochemistry* 1970 **9** 2917–2922. (https://doi.org/10.1021/bi00816a025)

49 Holick MF, Schnoes HK, DeLuca HF, Gray RW, Boyle IT & Suda T. Isolation and identification of 24,25-dihydroxycholecalciferol, a metabolite of vitamin D made in the kidney. *Biochemistry* 1972 **11** 4251–4255. (https://doi.org/10.1021/bi00773a009)

50 DeLuca HF, Suda T, Schnoes HK, Tanaka Y & Holick MF. 25,26-Dihydroxycholecalciferol, a metabolite of vitamin D_3 with intestinal calcium transport activity. *Biochemistry* 1970 **9** 4776–4780. (https://doi.org/10.1021/bi00826a022)

51 Wichmann JK, DeLuca HF, Schnoes HK, Horst RL, Shepard RM & Jorgensen NA. 25-Hydroxyvitamin D_3 26,23-lactone: a new in vivo metabolite of vitamin D. *Biochemistry* 1979 **18** 4775–4780. (https://doi.org/10.1021/bi00589a002)

52 Holick MF, Kleiner-Bossaller A, Schnoes HK, Kasten PM, Boyle IT & DeLuca HF. 1,24,25-Trihydroxyvitamin D_3. A metabolite of vitamin D_3 effective on intestine. *Journal of Biological Chemistry* 1973 **248** 6691–6696. (https://doi.org/10.1016/S0021-9258(19)43408-X)

53 Esvelt RP, Schnoes HK & DeLuca HF. Isolation and characterization of 1 alpha-hydroxy-23-carboxytetranorvitamin D: a major metabolite of 1,25-dihydroxyvitamin D_3. *Biochemistry* 1979 **18** 3977–3983. (https://doi.org/10.1021/bi00585a021)

54 Haussler MR, Myrtle JF & Norman AW. The association of a metabolite of vitamin D_3 with intestinal mucosa chromatin in vivo. *Journal of Biological Chemistry* 1968 **243** 4055–4064. (https://doi.org/10.1016/S0021-9258(18)93278-3)

55 Brumbaugh PF & Haussler MR. Specific binding of 1alpha,25-dihydroxycholecalciferol to nuclear components of chick intestine. *Journal of Biological Chemistry* 1975 **250** 1588–1594. (https://doi.org/10.1016/S0021-9258(19)41849-8)

56 McDonnell DP, Mangelsdorf DJ, Pike JW, Haussler MR & O'Malley BW. Molecular cloning of complementary DNA encoding the avian receptor for vitamin D. *Science* 1987 **235** 1214–1217. (https://doi.org/10.1126/science.3029866)

57 Baker AR, McDonnell DP, Hughes M, Crisp TM, Mangelsdorf DJ, Haussler MR, Pike JW, Shine J & O'Malley BW. Cloning and expression of full-length cDNA encoding human vitamin D receptor. *PNAS* 1988 **85** 3294–3298. (https://doi.org/10.1073/pnas.85.10.3294)

58 Rochel N, Wurtz JM, Mitschler A, Klaholz B & Moras D. The crystal structure of the nuclear receptor for vitamin D bound to its natural ligand. *Molecular Cell* 2000 **5** 173–179. (https://doi.org/10.1016/s1097-2765(00)80413-x)

59 DeLuca HF & Zierold C. Mechanisms and functions of vitamin D. *Nutritional Reviews* 1998 **56** 4–10.

60 Pike JW, Lee SM, Benkusky NA & Meyer MB. Genomic mechanisms governing mineral homeostasis and the regulation and maintenance of vitamin D metabolism. *Journal of Bone and Mineral Research Plus* 2021 **5** e10433. (https://doi.org/10.1002/jbm4.10433)

61 Haussler MR, Whitfield K, Haussler CA, Hsieh J-C & Jurutka PW. Nuclear vitamin D receptor: natural ligands, molecular structure–function and transcriptional control of vital genes. In *Vitamin D*, 3rd ed., chapter 8, pp. 137–170. Eds D Feldman, JW Pike & JS Adams. Academic Press, 2011.

62 Mizwicki MT & Norman AW. Vitamin D sterol/VDR conformational dynamics and non-genomic actions. In *Vitamin D*, 3rd ed., chapter 15, pp. 271–297. Eds D Feldman, JW Pike & JS Adams. Academic Press, 2011.

63 Bouillon R, Okamura WH & Norman AW. Structure-function relationships in the vitamin D endocrine system. *Endocrine Reviews* 1995 **16** 200–257. (https://doi.org/10.1210/edrv-16-2-200)

64 Daiger SP, Schanfield MS & Cavalli-Sforza LL. Group-specific component (Gc) proteins bind vitamin D and 25-hydroxyvitamin D. *PNAS* 1975 **72** 2076–2080. (https://doi.org/10.1073/pnas.72.6.2076)

65 Bouillon R, Schuit F, Antonio L & Rastinejad F. Vitamin D binding protein: a historic overview. *Frontiers in Endocrinology* 2020 **10** 1–21.

66 Jones G, Prosser DE & Kaufmann M. Cytochrome P450-mediated metabolism of vitamin D. *Journal of Lipid Research* 2014 **55** 13–31. (https://doi.org/10.1194/jlr.R031534)

67 Bhattacharyya MH & DeLuca HF. The regulation of rat liver calciferol-25-hydroxylase. *Journal of Biological Chemistry* 1973 **248** 2969–2973. (https://doi.org/10.1016/S0021-9258(19)43995-1)

68 Knutson JC & DeLuca HF. 25-Hydroxyvitamin D3-24-hydroxylase. Subcellular location and properties. *Biochemistry* 1974 **13** 1543–1548. (https://doi.org/10.1021/bi00704a034)

69 Gray RW, Omdahl JL, Ghazarian JG & DeLuca HF. 25-Hydroxycholecalciferol-1-hydroxylase subcellular location and properties. *Journal of Biological Chemistry* 1972 **247** 7528–7532. (https://doi.org/10.1016/S0021-9258(19)44557-2)

70 St-Arnaud R, Messerlian S, Moir JM, Omdahl JL & Glorieux FH. The 25-hydroxyvitamin D 1-alpha-hydroxylase gene maps to the pseudovitamin D-deficiency rickets (PDDR) disease locus. *Journal of Bone and Mineral Research* 1997 **12** 1552–1559. (https://doi.org/10.1359/jbmr.1997.12.10.1552)

71 Takeyama K, Kitanaka S, Sato T, Kobori M, Yanagisawa J & Kato S. 25-Hydroxy vitamin D_3 1alpha-hydroxylase and vitamin D synthesis. *Science* 1997 **277** 1827–1830. (https://doi.org/10.1126/science.277.5333.1827)

72 Ohyama Y & Okuda K. Isolation and characterization of a cytochrome P-450 from rat kidney mitochondria that catalyzes the 24-hydroxylation of 25-hydroxyvitamin D_3. *Journal of Biological Chemistry* 1991 **266** 8690–8695. (https://doi.org/10.1016/S0021-9258(18)31501-1)

73 Sakaki T, Sawada N, Nonaka Y, Ohyama Y & Inouye K. Metabolic studies using recombinant Escherichia coli cells producing rat mitochondrial CYP24: CYP24 can convert 1α,25-dihydroxyvitamin D3 to calcitroic acid. *European Journal of Biochemistry* 1999 **262** 43–48. (https://doi.org/10.1046/j.1432-1327.1999.00375.x)

74 Inouye K & Sakaki T. Enzymatic studies on the key enzymes of vitamin D metabolism; 1 alpha-hydroxylase (CYP27B1) and 24-hydroxylase (CYP24). *Biotechnology Annual Review* 2001 **7** 179–194. (https://doi.org/10.1016/s1387-2656(01)07037-5)

75 Cheng JB, Levine MA, Bell NH, Mangelsdorf DJ & Russell DW. Genetic evidence that the human CYP2R1 enzyme is a key vitamin D 25-hydroxylase. *PNAS* 2004 **101** 7711–7715. (https://doi.org/10.1073/pnas.0402490101)

76 Kaufmann M, Martineau C, Arabian A, Traynor M, St-Arnaud R & Jones G. Calcioic acid: in vivo detection and quantification of the terminal C24-oxidation product of 25-hydroxy vitamin D_3 and related intermediates in serum of mice treated with 24,25-dihydroxyvitamin D_3. *Journal of Steroid Biochemistry and Molecular Biology* 2019 **188** 23–28. (https://doi.org/10.1016/j.jsbmb.2018.12.001)

77 Wang Z, Schuetz EG, Xu Y & Thummel KE. Interplay between vitamin D and the drug metabolizing enzyme CYP3A4. *Journal of Steroid Biochemistry and Molecular Biology* 2013 **136** 54–58. (https://doi.org/10.1016/j.jsbmb.2012.09.012)

78 Reddy GS & Tserng KY. Isolation and identification of 1,24,25-trihydroxyvitamin D_2, 1,24,25,28-tetrahydroxyvitamin D_2 and 1,24,25,26-tetrahydroxyvitamin D_2: new metabolites of 1,25-dihydroxyvitamin D_2 produced in the kidney. *Biochemistry* 1986 **25** 5328–5336. (https://doi.org/10.1021/bi00366a051)

79 Cooke NE, McLeod JF, Wang XK & Ray K. Vitamin D binding protein: genomic structure, functional domains, and mRNA expression in tissues. *Journal of Steroid Biochemistry and Molecular Biology* 1991 **40** 787–793. (https://doi.org/10.1016/0960-0760(91)90304-n)

80 Haussler MR & Norman AW. Chromosomal receptor for a vitamin D metabolite. *PNAS* 1969 **62** 155–162. (https://doi.org/10.1073/pnas.62.1.155)

81 Cheng JB, Motola DL, Mangelsdorf DJ & Russell DW. Deorphanization of cytochrome P450 2R1: a microsomal vitamin D 25-hydroxylase. *Journal of Biological Chemistry* 2003 **278** 38084–38093. (https://doi.org/10.1074/jbc.M307028200)

82 Fraser D, Kooh SW, Kind HP, Holick MF, Tanaka Y & DeLuca HF. Pathogenesis of hereditary vitamin-D-dependent rickets. An inborn error of vitamin D metabolism involving defective conversion of 25-hydroxyvitamin D to 1 alpha,25-dihydroxyvitamin D. *New England Journal of Medicine* 1973 **289** 817–822. (https://doi.org/10.1056/NEJM197310182891601)

83 Kato S. Vitamin D 1alpha-hydroxylase knockout mice as a hereditary rickets animal model. *Endocrinology* 2001 **142** 2734–2735. (https://doi.org/10.1210/endo.142.7.8349)

84 Panda DK, Miao D, Tremblay ML, Sirois J, Farookhi R, Hendy GN & Goltzman D. Targeted ablation of the 25-hydroxyvitamin D 1alpha-hydroxylase enzyme: evidence for skeletal, reproductive, and immune dysfunction. *PNAS* 2001 **98** 7498–7503. (https://doi.org/10.1073/pnas.131029498)

85 St-Arnaud R, Dardenne O, Prud'homme J, Hacking SA & Glorieux FH. Conventional and tissue-specific inactivation of the 25-hydroxyvitamin D-1alpha-hydroxylase (CYP27B1). *Journal of Cellular Biochemistry* 2003 **88** 245–251. (https://doi.org/10.1002/jcb.10348)

86 Zhu JG, Ochalek JT, Kaufmann M, Jones G & Deluca HF. CYP2R1 is a major, but not exclusive, contributor to 25-hydroxyvitamin D production in vivo. *PNAS* 2013 **110** 15650–15655. (https://doi.org/10.1073/pnas.1315006110)

87 Rosen JF, Fleischman AR, Finberg L, Hamstra A & DeLuca HF. Rickets with alopecia: an inborn error of vitamin D metabolism. *Journal of Pediatrics* 1979 **94** 729–735. (https://doi.org/10.1016/s0022-3476(79)80139-0)

88 Eil C, Liberman UA, Rosen JF & Marx SJ. A cellular defect in hereditary vitamin-D-dependent rickets type II: defective nuclear uptake of 1,25-dihydroxyvitamin D in cultured skin fibroblasts. *New England Journal of Medicine* 1981 **304** 1588–1591. (https://doi.org/10.1056/NEJM198106253042608)

89 Yoshizawa T, Handa Y, Uematsu Y, Takeda S, Sekine K, Yoshihara Y, Kawakami T, Arioka K, Sato H, Uchiyama Y, *et al*. Mice lacking the vitamin D receptor exhibit impaired bone formation, uterine hypoplasia and growth retardation after weaning. *Nature Genetics* 1997 **16** 391–396. (https://doi.org/10.1038/ng0897-391)

90 Li YC, Pirro AE & Demay MB. Analysis of vitamin D-dependent calcium-binding protein messenger ribonucleic acid expression in mice lacking the vitamin D receptor. *Endocrinology* 1998 **139** 847–851. (https://doi.org/10.1210/endo.139.3.5803)

91 Lightwood R & Stapleton T. Idiopathic hypercalcaemia in infants. *Lancet* 1953 **265** 255–256. (https://doi.org/10.1016/s0140-6736(53)90187-1)

92 Schlingmann KP, Kaufmann M, Weber S, Irwin A, Goos C, John U, Misselwitz J, Klaus G, Kuwertz-Bröking E, Fehrenbach H, *et al*. Mutations in CYP24A1 and idiopathic infantile hypercalcemia. *New England Journal of Medicine* 2011 **365** 410–421. (https://doi.org/10.1056/NEJMoa1103864)

93 St-Arnaud R, Arabian A, Travers R, Barletta F, Raval-Pandya M, Chapin K, Depovere J, Mathieu C, Christakos S, Demay MB, *et al*. Deficient mineralization of intramembranous bone in vitamin D-24-hydroxylase-ablated mice is due to elevated 1,25-dihydroxyvitamin D and not to the absence of 24,25-dihydroxyvitamin D. *Endocrinology* 2000 **141** 2658–2666. (https://doi.org/10.1210/endo.141.7.7579)

94 DeLuca HF & Avioli LV. Treatment of renal osteodystrophy with 25-hydroxycholecalciferol. *Archives of Internal Medicine* 1970 **126** 896–899.

95 Brickman AS, Coburn JW, Massry SG & Norman AW. 1,25-Dihydroxyvitamin D_3 in normal man and patients with renal failure. *Annals of Internal Medicine* 1974 **80** 161–168. (https://doi.org/10.7326/0003-4819-80-2-161)

96 Rutherford WE, Bordier P, Marie P, Hruska K, Harter H, Greenwalt A, Blondin J, Haddad J, Bricker N & Slatopolsky E. Phosphate control and 25-hydroxycholecalciferol administration in preventing experimental renal osteodystrophy in the dog. *Journal of Clinical Investigation* 1977 **60** 332–341. (https://doi.org/10.1172/JCI108781)

97 Blunt JW & DeLuca HF. The synthesis of 25-hydroxycholecalciferol. A biologically active metabolite of vitamin D_3. *Biochemistry* 1969 **8** 671–675. (https://doi.org/10.1021/bi00830a031)

98 Holick MF, Semmler EJ, Schnoes HK & DeLuca HF. 1α-Hydroxy derivative of vitamin D_3: a highly potent analog of 1α,25-dihydroxyvitamin D_3. *Science* 1973 **180** 190–191. (https://doi.org/10.1126/science.180.4082.190)

99 Barton DH, Hesse RH, Pechet MM & Rizzardo E. A convenient synthesis of 1-hydroxy-vitamin D_3. *Journal of the American Chemical Society* 1973 **95** 2748–2749. (https://doi.org/10.1021/ja00789a090)

100 Lam HY, Schnoes HK & DeLuca HF. 1alpha-Hydroxyvitamin D_2: a potent synthetic analog of vitamin D_2. *Science* 1974 **186** 1038–1040. (https://doi.org/10.1126/science.186.4168.1038)

101 Takahashi F, Finch JL, Denda M, Dusso AS, Brown AJ & Slatopolsky E. A new analog of 1,25-$(OH)_2D_3$, 19-nor-1,25-$(OH)_2D_2$, suppresses serum PTH and parathyroid gland growth in uremic rats without elevation of intestinal vitamin D receptor content. *American Journal of Kidney Diseases* 1997 **30** 105–112. (https://doi.org/10.1016/s0272-6386(97)90571-0)

102 Calverley MJ. Synthesis of MC-903, a biologically active vitamin D metabolite analog. *Tetrahedron* 1987 **43** 4609–4619. (https://doi.org/10.1016/S0040-4020(01)86903-9)

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