An Update on Vitamin D Deficiency in the twenty-first century: nature and nurture

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Purpose of review
Here, we review the most up-to-date understanding of the pathogenesis, prevention and treatment of vitamin D deficient rickets in children. This will include recent advances in the genetic determinants of abnormal vitamin D metabolism, with the intention of aiding clinicians with establishing the diagnosis and implementing treatment plans for children presenting with vitamin D deficiency rickets.

Recent findings
Vitamin D deficiency rickets is a frequently encountered, but entirely preventable, disorder of bone mineral metabolism. Risk factors for developing vitamin D deficiency rickets include inadequate exposure to sunlight, exclusive breast feeding without vitamin D supplementation and inadequate intake of vitamin D, calcium or phosphorus. Other factors that may influence the development of vitamin D deficiency and/or rickets include genetic alterations or medications that alter vitamin D metabolism.

Summary
Vitamin D levels in individuals are influenced by environmental factors, as well as genetic factors. A thorough understanding of these factors is critical for the evaluation and treatment of a child presenting with rickets. There remains a great need for additional research to determine ideal vitamin D status across diverse populations, and to better understand how vitamin D status affects overall health.

Keywords
1,25-dihydroxyvitamin D, 25-hydroxyvitamin D, paediatrics, rickets, vitamin D, vitamin D receptor

INTRODUCTION

Rickets is a generalized skeletal disorder of children that is characterized by inadequate mineralization of newly formed bone osteoid and disruption of endochondral bone formation within the growth plate. In adults and older children with closed growth plates, defective mineralization of osteoid at the trabecular, endosteal and periosteal bone surfaces is termed osteomalacia. Impaired mineralization of cartilage and bone usually results from the lack of sufficient calcium and/or phosphorus in the extracellular fluid to enable formation of hydroxyapatite crystals, the mineralized component of bone tissue. Although calcium and/or phosphorus deficiency may result from inadequate dietary intake or increased renal loss, nutritional vitamin D deficiency is the most common cause of rickets in both developed and developing nations, highlighting the critical role that vitamin D metabolism plays in mineral homeostasis.

Rickets is an ancient disease [1] and the use of vitamin D-fortified foods and the availability of vitamin D for prevention and treatment of rickets represents one of the great medical successes of the twentieth century [2]. Nevertheless, in many parts of the world, vitamin D deficiency continues to be a public health problem. Reasons for the high prevalence of vitamin D deficiency are variable and influenced by culture and economics. The most notable causes of vitamin D deficiency include exclusive breastfeeding without vitamin D supplementation, inadequate diets, covering of the skin for religious or social purposes, use of UVB sunblock and obesity. In addition, the identification of patients who do not respond to vitamin D treatment has led to the recognition of children with dietary calcium

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KEY POINTS

- The Global Consensus recommended classifying vitamin D *sufficiency* as more than 50 nmol/l (20 ng/ml); insufficiency as 30–50 nmol/l (12–20 ng/ml); and *deficiency* of less than 12 ng/ml.
- Although CYP24A1 activity accounts for most of the catabolism of vitamin D metabolites, vitamin D metabolites can also be inactivated by CYP3A4 by a secondary pathway.
- Serum levels and genotypes for vitamin D binding protein can affect circulating concentrations of 25(OH)D.
- Vitamin D_2 seems to be metabolized more quickly than vitamin D_3, thus vitamin D_3 (cholecalciferol) is recommended for oral supplementation.

deficiency [3] as well as children with genetic and clinical disorders characterized by impaired activation and/or responsiveness of target tissues to vitamin D [4▪▪]. Equally important, in some children, rickets can result from calcium deficiency [5,6] or disorders that impair phosphate metabolism [7,8].

BIOCHEMICAL CRITERIA FOR VITAMIN D DEFICIENCY

The determination of vitamin D status is based on measurement of the circulating concentration of 25-hydroxy vitamin D (25(OH)D) rather than the fully active form of vitamin D, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) [9]. Although somewhat counterintuitive, 25(OH)D is measured because it is the most abundant circulating form of vitamin D and has a longer serum half-life than $1,25(OH)_2D$. Despite progress in developing sensitive and accurate assays for 25(OH)D, there remains a lack of consensus for the 25(OH)D concentration that is necessary for optimal health [10▪▪].

Global Consensus recommendations for the prevention and treatment of rickets in children were published in 2016 [11]. This expert panel recommended classifying vitamin D status according to serum 25(OH)D concentration as outlined in Table 1. This recommendation was made based upon strong evidence that the risk for nutritional rickets increases with 25(OH)D levels below 30 nmol/l. Other expert panels have offered other criteria for classifying vitamin D status (Table 1). It is important to note that all recommendations assume adequate intake and absorption of calcium and phosphorus, which may not always be the case.

In the past, 25(OH)D levels were measured via competitive-binding methods, high-performance liquid chromatography (HPLC) and radioimmunoassay. The most common methodology used today is an antibody-based automated assay that has high precision and accuracy, but results can be spuriously elevated in the presence of very high serum concentration of 1,25(OH)2D [12]. Newer methods for measuring vitamin D metabolites have emerged, such as liquid chromatography tandem mass spectrometry (LC-MS/MS), which in some cases can distinguish C3-epimers from nonepimerized vitamin D metabolites [13]. This distinction is important given vitamin D metabolites can be epimerized at the C3 position and form C3-epimers, which can lead to falsely elevated 25(OH)D levels, especially in infants who have higher C3-epimer levels [estimated to be 8.7–61.1% of total 25(OH)D in children less than 1 year of age] [14]. The physiologic significance of C3-epimers is unknown but these epimers are thought to have lower calcemic effects than vitamin D metabolites [15]. More recently, assays have been developed that enable the direct measurement of free 25(OH)D, which represents a tiny fraction of total 25(OH)D in serum [16▪], Nevertheless, there seems to be little clinical advantage to using these assays unless a patient has markedly reduced (e.g. severe liver disease) or markedly increased (e.g. pregnancy) serum levels of vitamin D binding protein (DBP) [17].

Table 1. Categorization of Vitamin D status by serum 25-OH-vitamin D concentration: Recommendations from various expert panels

Organization	Vitamin D sufficiency	Vitamin D insufficiency	Vitamin D deficiency
Global Consensus Recommendations on Prevention and Management of Nutritional Rickets	>20 ng/ml (>50 nmol/l)	12–20 ng/ml (30–50 nmol/l)	<12 ng/ml (<30 nmol/l)
American Academy of Pediatrics (AAP)	>20 ng/ml (>50 nmol/l)	15–20 ng/ml (40–50 nmol/l)	<15 ng/ml
Institute of Medicine (IOM)	>20 ng/ml (>50 nmol/l)	12–20 ng/ml (30–50 nmol/l)	<12 ng/ml (<30 nmol/l)
Pediatric Endocrine Society (PES)	>20 ng/ml (>50 nmol/l)	15–20 ng/ml (37.5–50 nmol/l)	<15 ng/ml (<37.5 nmol/l)
Endocrine Society	≥30 ng/ml (≥ 75 nmol/l)	20–29 ng/ml (50–72.5 nmol/l)	<20 ng/ml (<50 nmol/l)
National Kidney Foundation Kidney Disease Outcomes Quality Initiative (KDOQI)	>30 ng/ml (≥ 75 nmol/l)	16–30 ng/ml (40–75 nmol/l)	≤15 ng/ml (≤37.5 nmol/l)

FACTORS INFLUENCING VITAMIN D STATUS

The causes of vitamin D deficiency can be grouped according to extrinsic (environmental, secular or behavioural), and intrinsic (unique to the specific individual) risks. Overall, the most common causes of vitamin D deficiency are inadequate sun exposure or poor nutrition, but specific risk factors vary by age. In the newborn period, vitamin D status is heavily influenced by diet. Human breast milk contains nearly 22 IU/l (0.6 μg/l) of vitamin D, which is inadequate to meet the recommended 200 IU/day (5 μg/day). Breastfeeding mothers who choose not to provide vitamin D supplements to their baby can 'fortify' their breast milk by taking 4000–6400 IU of vitamin D per day, which does not lead to vitamin D intoxication in the mother and provides the nursing infant nearly 400 IU per day [18,19▪]. Standard infant formulas are fortified with nearly 400 IU/l (10 μg/l) vitamin D. Therefore, 500 ml of formula per day would meet the recommended vitamin D intake. Parents should be advised that plant-based infant formulas (e.g. almond milk-based, soy milk-based) may not contain adequate amounts of vitamin D and calcium unless fortified [20▪].

Premature infants are at even greater risk of vitamin D deficiency due to factors, including lack of sunlight exposure during postnatal hospitalization and greater risk of impaired gastrointestinal absorption. During childhood and adolescence, vitamin D deficiency is most commonly due to a combination of poor diet and lack of sunlight, which is the primary source of ultraviolet B (UVB) radiation. Vitamin D is fat soluble, so deficiency is common in children with fat malabsorption due to conditions such as inflammatory bowel disease, short gut syndrome, celiac disease, hepatobiliary disease or pancreatic insufficiency due to cystic fibrosis, and so on. Vitamin D deficiency is more common in individuals with obesity. This association is thought to be due to higher amounts of subcutaneous fat, which sequesters and therefore limits the bioavailability of vitamin D for systemic circulation [21▪], as well as decreased conversion of vitamin D to 25(OH)D [22]. Finally, certain drugs that induce expression of the hepatic P450 enzyme CYP3A4, such as rifampin, phenytoin and phenobarbital, can increase 25(OH)D catabolism and cause vitamin D deficiency [23].

VITAMIN D PHYSIOLOGY

Vitamin D exists as vitamin D_2 (ergocalciferol) and D_3 (cholecalciferol). Vitamin D_2 is synthesized in certain plants and fungi from ergosterol in response to ultraviolet-B (UV-B) irradiation. By contrast, vitamin D_3 is produced in the skin of animals from 7-dehydrocholesterol (7-DHC) exposed to UV-B (spectrum 280-320 UVB). In this two-step process, 7-DHC is first converted to pre-D3, which then isomerizes to D3 in a thermo-sensitive but nonenzymatic reaction. In humans, the degree of UV-B intensity and amount of melanin affects UV-B light reaching 7-DHC, therefore, darker skinned individuals are at a greater risk for developing vitamin D deficiency. Thinner skin, such as occurs in ageing, has reduced capacity to synthesize vitamin D in response to UV-B [24]. Both vitamin D_2 and D_3 are available for supplementation, but vitamin D_3 is preferred due to its superior pharmacokinetics (discussed below).

Vitamin D activation

Two steps of enzymatic hydroxylation are required to activate parent vitamin D to fully active, hormonal forms of vitamin D. In the liver, calciferol (vitamin D_2 or D_3) is first hydroxylated to 25(OH)D principally in microsomes via the CYP2R1 25-hydroxylase [25]. Additional enzymes can also function as 25-hydroxylases (e.g. CYP27A1 and CYP3A4), but these play minor roles in humans. In the kidneys, 25(OH)D is subsequently hydroxylated to $1,25(OH)_2D$ by 1-alpha hydroxylase (encoded by *CYP27B1*). Although CYP2R1 does not appear to be highly regulated, expression and/or activity of CYP27B1 in the proximal renal tubular cells are under tight control: increased by PTH and decreased by $1,25(OH)_2D$ and fibroblast growth factor 23 (FGF 23).

Vitamin D homeostasis

Serum concentrations of vitamin D metabolites are also regulated via degradative pathways. The principal mechanism for inactivation of vitamin D metabolites is by hydroxylation to $24,25(OH)_2D$ and $1,24,25(OH)_3D$, by 24-hydroxylase activity of CYP24A1. CYP24A1 is present in all cells that contain the vitamin D receptor (VDR), and therefore not only regulates circulating $1,25(OH)_2D$ but may also limit the levels of $1,25(OH)_2D$ in cells. Although CYP24A1 activity accounts for most of the catabolism of vitamin D metabolites, they can also be inactivated by CYP3A4 in the liver and small intestine via alternative hydroxylation (see Fig. 1).

In the circulation, nearly all vitamin D and its metabolites are bound to proteins, principally to vitamin D binding protein (DBP) but also in lesser amounts to albumin and lipoproteins. The affinity of DBP is greater for 25(OH)D3 than for 25(OH)D2, therefore serum concentrations of 25(OH)D3 have a greater circulating half-life than 25(OH)D2, which

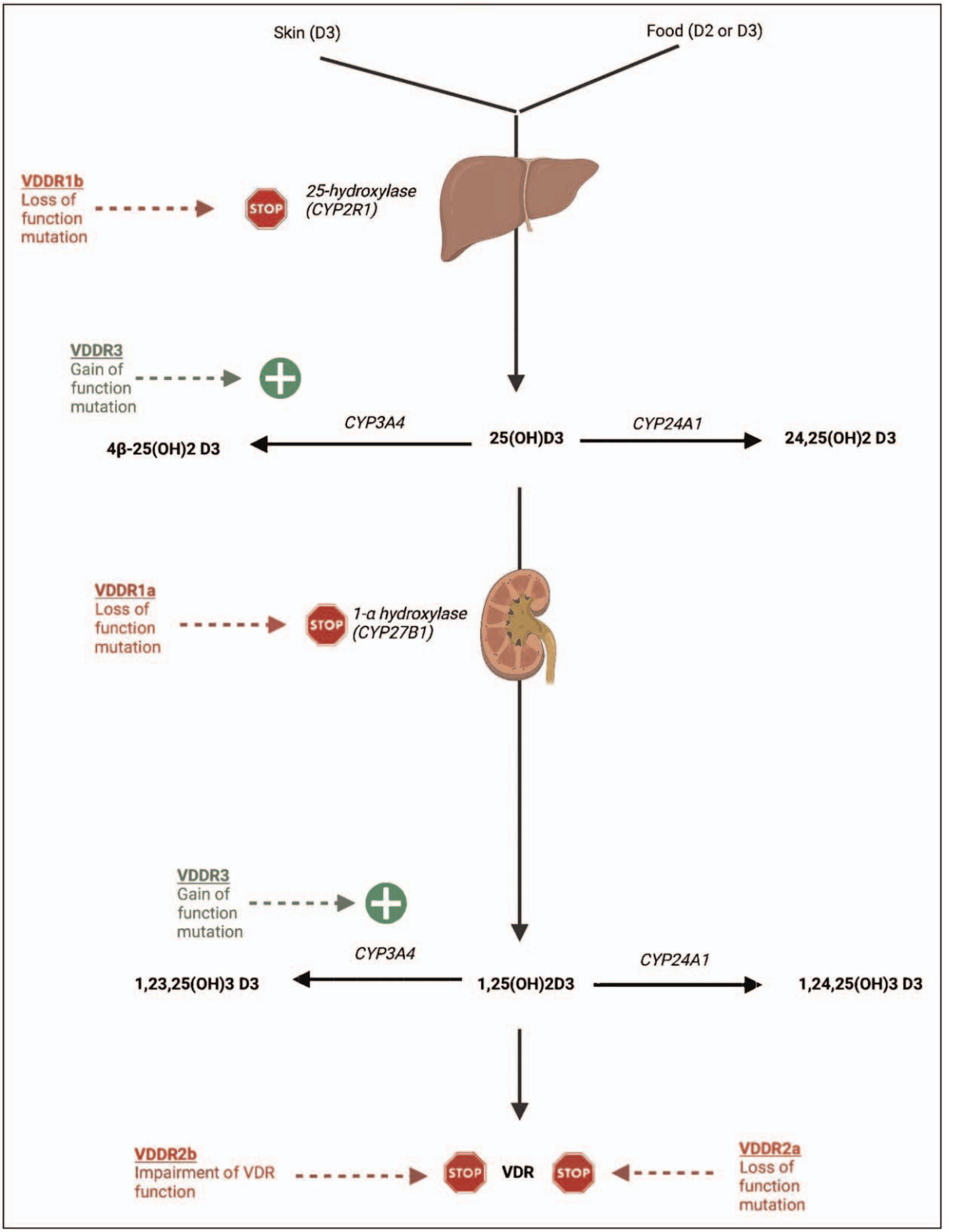

FIGURE 1. Vitamin D metabolism and nonnutritional causes of disordered Vitamin D metabolism: An overview of vitamin D metabolism, along with known genetic disorders affecting this pathway is outlined above. Red text and 'stop signs' illustrate loss of function mutations; green text and 'plus signs' illustrate gain of function mutations.

becomes clinically significant when vitamin D is not administered daily [26]. Serum concentrations of DBP do not appear to be influenced by race, but functional polymorphisms in the *GC* gene encoding DBP that affect binding affinity may influence circulating concentrations of vitamin D metabolites [27]. Surprisingly, complete absence of DBP due to a homozygous mutation in the *GC* gene is not associated with signs of vitamin D deficiency [28], which implicates free 25(OH)D rather than DBP-bound 25(OH)D as the more important form in most tissues [29].

Mechanism of action

Vitamin D supports calcium homeostasis through actions on the gut (intestinal absorption), kidneys (renal excretion) and bone (resorption). The primary mechanism by which vitamin D promotes bone mineralization is through the physiological actions of $1,25(OH)_2D$ on target cells, chiefly through induction of proteins in the intestine that promote calcium absorption. These actions are generally genomic and require interaction of $1,25(OH)_2D$ with nuclear VDR. The subsequent binding of the VDR to RXR generates a functional heterodimer that can interact with vitamin D response elements (VDREs) located on target genes and recruitment of coregulatory complexes that are required to stimulate or inhibit gene transcription. Importantly, these coregulatory complexes can be both gene and cell-specific, which provides selectivity of $1,25(OH)_2D$ action. It was recognized that some of the effects of vitamin D occurred too quickly to be due to the interactions of $1,25(OH)_2D$ with nuclear VDR, hence some actions of $1,25(OH)_2D$ were determined to be nongenomic in nature. The first example of nongenomic effects was seen via rapid calcium transport across intestinal lumen after 1,25 (OH)2D administration. Nongenomic effects of $1,25(OH)_2D$ occur through activation of intracellular signalling molecules (i.e. phospholipase C and A_2, phosphatidylinositol-3 kinase, p21ras) [30▪,31▪].

CLINICAL EVALUATION

The diagnosis of rickets is made based on history, physical examination, radiological appearance of the skeleton and biochemical findings. Examination findings include leg bowing (genu varum or valgum), poor growth, frontal bossing, craniosynostosis, prominence of costochondral junctions (rachitic rosary), indentation of the lower anterior thoracic wall (Harrison's groove), involution of the ribs and protrusion of the sternum (pigeon chest), softening of the occipital area (craniotabes), delayed closing of fontanelles and impaired development of teeth (delayed eruption, enamel hypoplasia and greater susceptibility to caries). Seizures are possible with severe hypocalcaemia, which is more common during periods of rapid growth and skeletal calcium accretion including infancy and adolescence. Sustained elevation of PTH levels, together with decreased calcium supply, results in reduced bone density (often misinterpreted as osteoporosis) and structural incompetence of the skeleton. In older children and adults this can cause pseudofractures (Looser's zones) that are manifested by a radiolucent line through the cortical plate, perpendicular to the long axis of bone, often with sclerosis seen at the margins.

Rickets is classically sub-divided into calcipenic and phosphopenic rickets. Studies now show that hypophosphatemia is responsible for the disturbance in the growth plate in both forms of rickets, but the mechanism for hypophosphatemia will vary by the underlying metabolic defect. Knowledge of this categorization and of respective clinical and laboratory features are essential for proper diagnosis and treatment.

In the early stage of vitamin D deficiency, serum levels of phosphate and calcium are typically normal, and serum levels of 1,25(OH)2D are elevated due to secondary hyperparathyroidism. ALP and PTH will be mildly elevated. Serum phosphorus may be normal or mildly depressed secondary to the phosphaturic effects of excess PTH. Osteopenic changes may be seen on x-ray. As vitamin D deficiency becomes more significant the biochemical abnormalities become more abnormal and serum levels of calcium are moderately depressed and hypophosphatemia is more pronounced. Serum concentrations of 1,25(OH)2D return to normal or are low as the serum concentration of 25(OH)D falls and becomes rate-limiting for CYP27B1. Rachitic changes including cupping and fraying of metaphyses can be visible by imaging and appreciated on physical examination. In severe vitamin D deficiency, biochemical findings are profoundly abnormal and bone changes are pronounced and can include fractures. Multiple fractures in nonmobile infants in specific locations such as the ribs are not common in children with typical nutritional rickets, and their presence should alert the clinician to possible nonaccidental trauma or another metabolic bone disease [32].

GENETIC CAUSES OF ABNORMAL VITAMIN D METABOLISM

In some children, vitamin D deficient rickets occurs as a consequence of genetic defects that affect activation, clearance, or responsiveness to vitamin D (Fig. 1) [4▪▪]. Because early investigators noted that treatment of these conditions required sustained

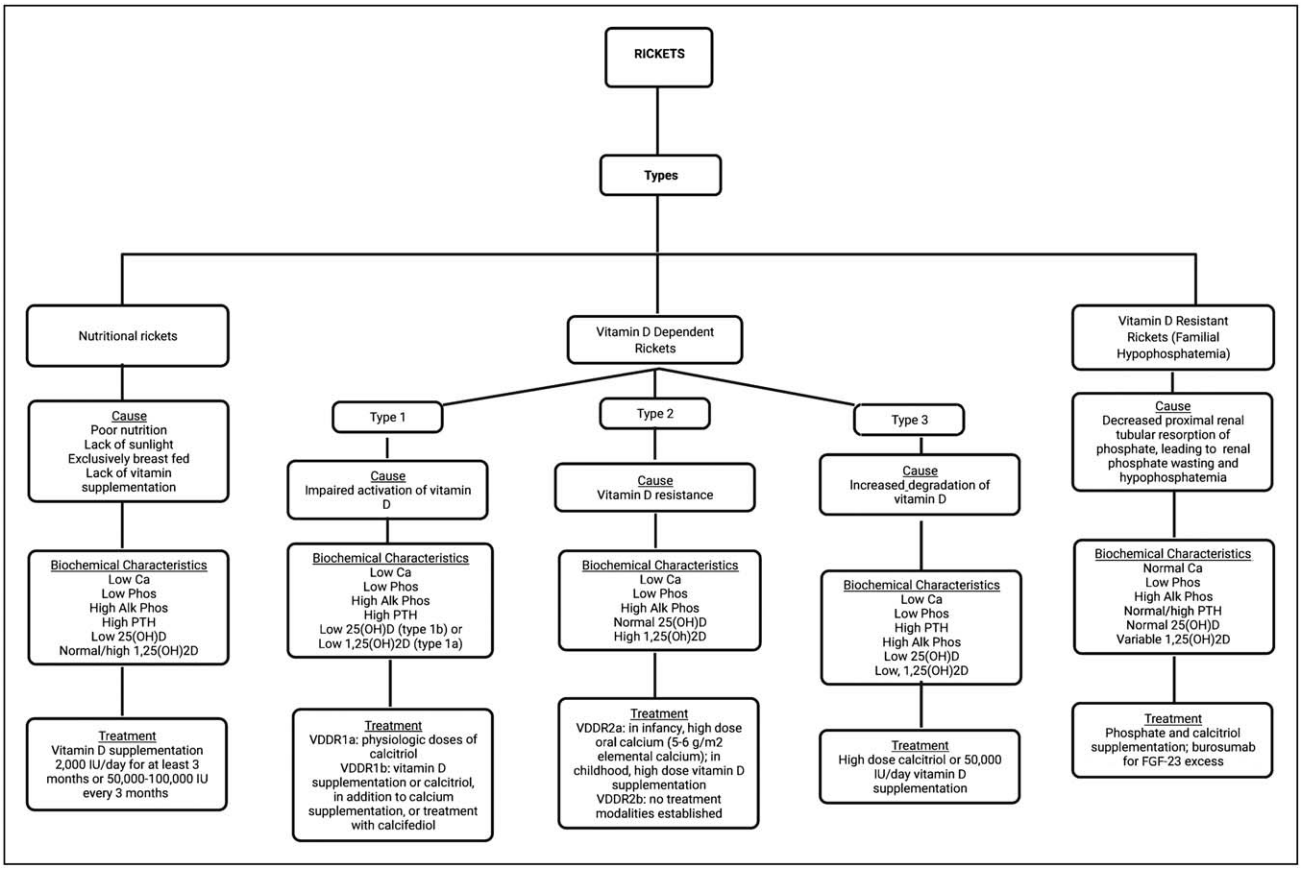

FIGURE 2. Types, causes, biochemical characteristics and treatment of childhood rickets. The mechanism, clinical characteristics and treatment of the different forms of childhood rickets is depicted above.

administration of very high doses of vitamin D, the children were termed 'vitamin D dependent' to distinguish them from those with typical vitamin D deficient rickets. Although the genetic disorders of vitamin D metabolism are uncommon, the practicing clinician needs to be aware of these diagnoses as the treatment approach differs from that of nutritional vitamin D deficient rickets (Fig. 2).

Vitamin D dependent rickets type 1A (VDDR1A) is due to a mutation in *CYP27B1* and leads to an inability to convert 25(OH)D to $1,25(OH)_2D$. Serum concentrations of 25(OH)D are normal to elevated, owing to decreased induction of CYP24A1 in the absence of $1,25(OH)_2D$. VDDR1A is treated with physiologic doses of calcitriol, which supplies the missing metabolite. VDDR1B is due to a mutation in *CYP2R1* and leads to the inability to convert calciferol to 25(OH)D. These patients require very high doses of vitamin D supplementation or physiologic doses of calcifediol or calcitriol.

Vitamin D dependent rickets type 2 (VDDR2) is due to a loss-of-function mutation in *VDR*, leading to vitamin D resistance. These patients have very high serum concentrations of $1,25(OH)_2D$ and normal or slightly elevated 25(OH)D levels due to impaired induction of CYP24A1 as a result of impaired VDR action. Treatment during infancy includes high dose oral calcium (5–6 g/m^2 elemental calcium) to counteract secondary hyperparathyroidism. Later in childhood, patients will require high-dose vitamin D. VDDR2b is a very uncommon disorder due to abnormal expression of proteins that interfere with VDR signalling. No treatment modalities have been established for VDDR2b.

Vitamin D dependent rickets type 3 (VDDR3) is a due to a recurrent gain-of-function mutation in *CYP3A4*, which leads to increased degradation of vitamin D and its metabolites. These patients require high-dose calcitriol or vitamin D2 or D3 supplementation [i.e. 50 000 IU/day (1250 μg/day)] [33].

PREVENTION AND TREAMENT OF NUTRITIONAL VITAMIN D DEFICIENCY

There is increasing evidence that vitamin D3 is more effective at maintaining serum levels of 25(OH)D than vitamin D2, even though both parent forms of vitamin D are comparable at increasing serum concentrations of 25(OH)D [34]. Current recommendations to prevent vitamin D deficiency are to ensure a

Table 2. Recommended Vitamin D doses for the treatment and prevention of Vitamin D deficiency, per the Global Consensus [11]

Age range	Daily treatment Vitamin D dose	Bolus treatment Vitamin D dose	Daily maintenance Vitamin D supplementation dose
0–3 months old	2000 IU (50 μg) daily for 3 months	Recommendation not available	400 IU (10 μg) daily
3–6 months old	2 000 IU (50 μg) daily for 3 months	Recommendation not available	400 IU (10 μg) daily
6–12 months old	2000 IU (50 μg) daily for 3 months	50 000 IU (1250 μg) every 3 months	400 IU (10 μg) daily
Greater than 12 months to 12 years old	3000–6000 IU (75–150 μg) daily for 3 months	150 000 IU (3750 μg) every 3 months	600 IU (15 mcg) daily
Greater than 12 years old	6000 IU (150 μg) daily for 3 months	300 000 IU (7500 μg) every 3 months	600 IU (15 μg) daily

daily vitamin D intake that maintains a 25(OH)D level more than 20 ng/ml for the majority of healthy individuals. It is also important to ensure an adequate calcium intake [11]. The recommended daily intake of vitamin D by age is summarized in Table 2. However, breast-fed neonates and children who are on vitamin D deficient diets may respond well to oral doses of 800–1500 IU/day all year up to age 2 years and during the winter months up to age 5 years without any signs of vitamin D intoxication [35]. In addition, patients who are treated with seizure medications, glucocorticoids, antifungals (e.g. ketoconazole), and medications for AIDS should receive two to three times more vitamin D for their age group [36]. Obese children also require higher doses of vitamin D [37]. Although the exact amount of daily vitamin D that is necessary to maintain vitamin D sufficiency in obese children is unknown, studies in obese adults have proposed that about 2.5 IU/kg of vitamin D is required for every unit increment in 25(OH)D (nanograms per millilitre) [38]. Children should maintain the RDA for calcium as well.

Children with vitamin D deficiency rickets will require treatment with a cumulative dose of 80 000–600 000 IU (2.5–15 mg) of vitamin D, dependent upon the age of the patient, with adequate calcium intake to effectively cure rickets [36]. Consensus guidelines for treatment of vitamin D deficiency are presented in Table 2. Transdermal forms of vitamin D3 are available [39▪] as is calcifediol, which due to its greater water solubility can provide a more rapid correction of vitamin D deficiency in patients with fat malabsorption or defects in 25-hydroxylation [40].

In addition to vitamin D, calcitriol is indicated in the presence of hypocalcaemia at doses of 10–100 ng/kg/day divided twice daily. Calcitriol is stopped when the serum calcium level normalizes. Supplemental calcium should be given at a dose of 50–75 mg elemental calcium/kg/day in three divided doses in the initial phase of treatment to avoid hypocalcaemia secondary to 'hungry bone' syndrome, especially with high-dose 'stoss' therapy. Supplemental oral calcium is stopped when PTH and 25(OH)D levels have normalized and vitamin D supplementation has been decreased to maintenance dosing.

CONCLUSION

Vitamin D deficiency/nutritional rickets is a common but preventable paediatric health problem that can have permanent effects into adulthood. There are limited randomized, controlled studies on the level of 25(OH)D for optimal bone health and varying practices regarding treatment of nutritional rickets. Recent discoveries in vitamin D binding protein polymorphisms, alternate vitamin D catabolism pathways and genomic versus nongenomic actions are important factors to consider when evaluating and treating paediatric patients but have made the job of establishing general criteria more complicated.

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Conflicts of interest

There are no conflicts of interest.

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