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Marie B. Demay

*J Clin Invest.* 2018;128(9):3736-3738. <https://doi.org/10.1172/JCI122046>.

## Commentary

While disorders of impaired vitamin D activation and action have long been appreciated, the consequences of abnormalities in pathways leading to the inactivation of vitamin D metabolites have only recently been identified. Two recent articles have shed new light on this area of vitamin D biology. The report by Martineau et al., published in the *JCI*, describes a pathway in which binding of the vitamin D metabolite 24R,25(OH)<sub>2</sub>D<sub>3</sub> to its effector molecule FAM57B2 plays an important role in endochondral ossification during bone repair. This work follows, and adds to, another recent *JCI* publication by Roizen et al., showing that rapid inactivation of vitamin D metabolites causes vitamin D deficiency, leading to vitamin D–dependent rickets.

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# The good and the bad of vitamin D inactivation

Marie B. Demay

Endocrine Unit, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts, USA.

**While disorders of impaired vitamin D activation and action have long been appreciated, the consequences of abnormalities in pathways leading to the inactivation of vitamin D metabolites have only recently been identified. Two recent articles have shed new light on this area of vitamin D biology. The report by Martineau et al., published in the *JCI*, describes a pathway in which binding of the vitamin D metabolite 24R,25(OH)<sub>2</sub>D<sub>3</sub> to its effector molecule FAM57B2 plays an important role in endochondral ossification during bone repair. This work follows, and adds to, another recent *JCI* publication by Roizen et al., showing that rapid inactivation of vitamin D metabolites causes vitamin D deficiency, leading to vitamin D-dependent rickets.**

## Metabolites of vitamin D

Vitamin D deficiency is associated with impaired intestinal calcium absorption that leads to osteomalacia and rickets. The antirachitic properties of vitamin D were first established in the early part of the 20th century (1, 2). Since then, many key steps in the synthesis, metabolism, and action of vitamin D have been elucidated, greatly improving the diagnosis and treatment of disorders of vitamin D action. Genetic studies have identified mutations in pathways required for activation of the vitamin D prohormone to the active 1,25-dihydroxyvitamin D hormone (Figure 1A), which plays an important role in the absorption of calcium from the intestine, thereby promoting skeletal growth and mineralization.

While treatment with vitamin D or vitamin D-rich nutrients prevents the development of nutritional rickets, rickets associated with disorders of vitamin D activation is resistant to vitamin D therapy. Identification of the enzyme responsible for 25-hydroxylation of vitamin D in the liver remained elusive until a rare form of rickets was shown to result from inactivating mutations of *CYP2R1* (3, 4). The rare occurrence of this disorder and the relatively mild phenotype of affected

individuals suggest compensatory effects of a second enzyme contributing to the 25-hydroxyvitamin D pool (5).

Homozygous inactivating mutations of *CYP27B1*, the vitamin D 1- $\alpha$  hydroxylase, lead to pseudovitamin D deficiency rickets (6). Affected individuals are unable to synthesize 1,25-dihydroxyvitamin D; thus, impaired intestinal calcium absorption leads to hypocalcemia, secondary hyperparathyroidism, rickets, and osteomalacia. This disorder is effectively treated with physiological doses of 1- $\alpha$  hydroxylated vitamin D metabolites. Although inactivating vitamin D receptor mutations also lead to impaired intestinal calcium absorption, affected individuals have very high concentrations of 1,25-dihydroxyvitamin D due to induction of *CYP27B1* by the high levels of parathyroid hormone (PTH) and impaired catabolism of 1,25-dihydroxyvitamin D (7). Consistent with the high levels of circulating 1,25-dihydroxyvitamin D, individuals are resistant to treatment with vitamin D metabolites and may require parenteral calcium to circumvent the defect in intestinal calcium absorption.

Although the molecular basis for these disorders of impaired vitamin D activation and action has been appreciated for over two decades, abnormalities

in pathways of vitamin D inactivation have only recently been identified (Figure 1B). Homozygous mutations in the vitamin D 24-hydroxylase *CYP24A1*, the major enzyme responsible for inactivation of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D, lead to idiopathic infantile hypercalcemia (IIH). This disorder is characterized by increased serum concentrations of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D, hypercalcemia, and hypercalciuria, which leads to nephrocalcinosis. This phenotype emphasizes the nonredundant effects of *CYP24A1* in regulating the endocrine actions of active vitamin D metabolites (8, 9).

## Novel actions of 24R,25-dihydroxyvitamin D<sub>3</sub>

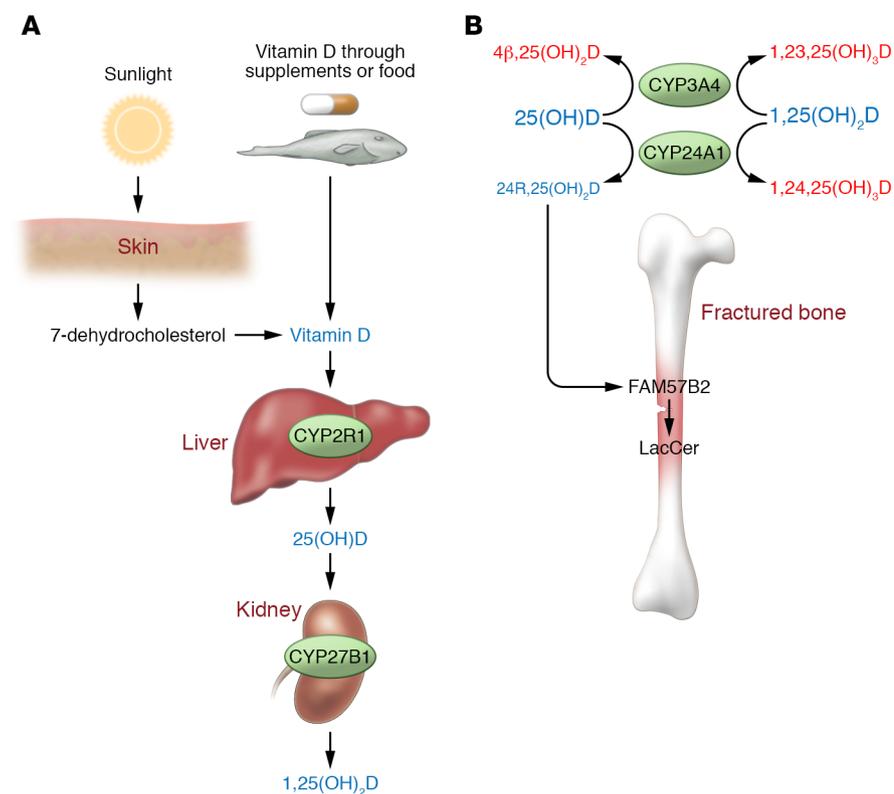
While studies in avian models have implicated a role for 24R,25-dihydroxyvitamin D<sub>3</sub> [24R,25(OH)<sub>2</sub>D<sub>3</sub>] in avian fracture repair (10), it is not known whether individuals with IIH have abnormalities in fracture healing. However, a recent study by Martineau et al. identified a nonredundant role for 24R,25(OH)<sub>2</sub>D<sub>3</sub> in murine fracture repair (11). In this study, they demonstrate that mice lacking *CYP24A1* exhibit impaired fracture healing that is corrected by administration of 24R,25(OH)<sub>2</sub>D<sub>3</sub>, but not by 1,25-dihydroxyvitamin D, traditionally thought to be the active metabolite of vitamin D. They identified the transmembrane protein FAM57B2, which interacts specifically with 24R,25(OH)<sub>2</sub>D<sub>3</sub>. The binding of 24R,25(OH)<sub>2</sub>D<sub>3</sub> to FAM57B2 in the fracture callus leads to the production of lactosylceramide, which is necessary for the effects of 24R,25(OH)<sub>2</sub>D<sub>3</sub> on callus formation and fracture healing.

It is unclear whether 24R,25(OH)<sub>2</sub>D<sub>3</sub> needs to be synthesized at the fracture site, or whether circulating levels are sufficient to exert its beneficial effects. The observation that an absence of 24R,25(OH)<sub>2</sub>D<sub>3</sub> impairs the maturation of chondrocytes, which are in an avascular part of the repair callus, sug-

► **Related Article:** <https://doi.org/10.1172/JCI98093>

**Conflict of interest:** The author has declared that no conflict of interest exists.

**Reference information:** *J Clin Invest.* 2018;128(9):3736–3738. <https://doi.org/10.1172/JCI122046>.



**Figure 1. Vitamin D synthesis and metabolism.** (A) Ultraviolet radiation results in the conversion of 7-dehydrocholesterol to pre-vitamin D, which isomerizes to vitamin D in the skin. Vitamin D can also be obtained from nutrition. Vitamin D-binding protein transports vitamin D to the liver, where it undergoes 25-hydroxylation by CYP2R1. CYP27B1 further hydroxylates 25-hydroxyvitamin D at the 1- $\alpha$  position, resulting in the formation of the active hormone 1,25-dihydroxyvitamin D. Although this latter enzyme is widely expressed, the kidney is thought to be the major source of circulating 1,25-dihydroxyvitamin D. (B) Vitamin D metabolites are 24-hydroxylated by CYP24A1. While these metabolites were previously thought to be inactive, binding of 24R,25(OH)<sub>2</sub>D<sub>3</sub> to FAM57B2 in the fracture callus leads to the production of lactosylceramide (LacCer), which is essential for the effects of 24,25(OH)<sub>2</sub>D on callus formation and fracture healing. While CYP3A4 plays a minor role in normal vitamin D physiology, a dominant gain-of-function mutation in CYP3A4 leading to accelerated vitamin D inactivation has recently been identified as the molecular basis for two cases of early-onset rickets. Vitamin D metabolites thought to be inactive are shown in red.

gests the importance of local synthesis of 24R,25(OH)<sub>2</sub>D<sub>3</sub>. Why the absence of 24R,25(OH)<sub>2</sub>D<sub>3</sub> does not result in similar impairment of maturation of the endochondral skeleton remains unknown (12). However, potential paracrine signaling between the maturing growth plate, perichondrium, and marrow may lead to a functional redundancy that is absent in the fracture callus.

### Mutation in an alternative pathway of vitamin D inactivation leads to rickets

Activating mutations of CYP24A1 would be expected to lead to rickets as a result of accelerated inactivation of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D, but no mutations have been reported.

However, another recent publication in the *JCI* by Roizen et al. identified a dominant gain-of-function mutation in CYP3A4 leading to accelerated vitamin D inactivation in two cases of early-onset rickets (13). While this enzyme is thought to play a minor role in normal vitamin D physiology, induction of CYP3A4 underlies the accelerated metabolism of vitamin D observed with several pharmacological agents. The mutation in the substrate recognition site of CYP3A4 seen in affected individuals increases the rate of 1,25-dihydroxyvitamin D inactivation by 10-fold versus WT CYP3A4, and by 2-fold versus CYP24A1, the principal pathway for inactivation of active vitamin D metabolites (13). Interestingly, a dramatic variability in CYP3A4

expression and catalytic activity has been reported in the general population (14). While this variability could be due to genetic polymorphisms, induction of this enzyme by pharmacological agents and other exogenous compounds may also play a role. Notably, induction of the alternative CYP3A4 pathway of vitamin D metabolism by rifampin is being used to treat IIH patients with CYP24A1 mutations (15).

### Unanswered questions

While the effect of mutations that impair activation of the vitamin D prohormone to 1,25-dihydroxyvitamin D have long been appreciated, recently identified abnormalities in vitamin D catabolism raise the question as to whether altered activity or polymorphisms of these enzymes contribute to the unexplained variability in vitamin D intake required to maintain vitamin D sufficiency in the general population. Whether individuals with IIH, who cannot synthesize 24R,25(OH)<sub>2</sub>D<sub>3</sub>, have impaired fracture healing is not yet known. However, investigations of FAM57B2 structure and function have the potential to identify novel therapeutic targets for skeletal repair.

Address correspondence to: Marie B. Demay, Endocrine Unit, Thier 11, Massachusetts General Hospital, 50 Blossom St., Boston, Massachusetts 02114, USA. Phone: 617.726.3966; Email: demay@helix.mgh.harvard.edu.

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