PERSPECTIVE

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# Vitamin D Metabolism Revised: Fall of Dogmas

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Vitamin D is one of the most frequently used medicinal products around the world. The dietary intake is irregular because few food items contain vitamin D and is usually well below human requirements, so that its synthesis in the skin is the most important source of vitamin D. The global supply of vitamin D is usually considered as a passive series of events, not controlled by enzymes or hormones. In the nearly 100 years after its discovery, we learned that vitamin D has a complex metabolism and steroidlike hormonal action. Vitamin D is totally inactive and requires a complex metabolism, first in the liver (mostly but not exclusively by CYP2R1) into 25-hydroxyvitamin D (25OHD), followed by a second hydroxylation by CYP27B1 into 1,25-dihydroxyvitamin D (1,25$(OH)_2$D). CYP2R1 is usually considered to be constitutively expressed. Therefore, the production of 25OHD is considered to be mostly substrate dependent so that serum 25OHD reflects the global supply of vitamin D. CYP27B1 in the kidney is the unique source of circulating 1,25D and is tightly regulated by different ions and hormones so that it behaves as a classical feedback regulated hormonal system. CYP27B1 is also widely expressed in many extra renal tissues so that 1,25$(OH)_2$D also behaves in a paracrine/autocrine fashion. In these tissues its activity is regulated by a variety of mechanisms different from what happens in the kidney. Although there are probably around 50 known metabolites of vitamin D, measurement of serum 25OHD is clinically used to define the vitamin D status, whereas serum 1,25$(OH)_2$D is used to assess the biological activity of the vitamin D endocrine system. All metabolites of vitamin D in serum are bound with relatively high affinity to a specific binding protein, vitamin D binding protein (DBP). This protein is highly polymorphic and circulates in serum in high concentrations so that the free concentrations of all vitamin D metabolites are very low. 1,25$(OH)_2$D binds to the vitamin D receptor (VDR), present in most cells. This hormonal system functions as most steroid and thyroid hormones and regulates a very large number of genes involved in calcium and phosphate transport but also regulates a very large number of genes (up to 10% of all genes of the some organisms such as the zebrafish) not involved in ion transport or bone metabolism (reviewed in Bouillon and colleagues(1)).

However, a few recent publications have challenged some aspects of our understanding of vitamin D metabolism including the concept of stable expression of the hepatic 25-hydroxylases.[2,3] We review these new data regarding CYP2R1, discuss their potential implications, and extend this review to examine the overall metabolism of vitamin D to explore whether old dogmas still hold today.

Obesity and the metabolic syndrome are associated with low vitamin D status.[1] Prospective studies suggest that low nonepimeric 25OHD or increased 3-epi-25OHD concentrations are associated with higher risk for type 2 diabetes.[4] The causality (in whatever direction) between obesity/diabetes and low vitamin D status is, however, not proven. In a recent *JBMR* article, Roizen and colleagues clearly demonstrated that the serum concentration of 25OHD is substantially lower (~−20%) in serum of obese mice (fed a high-fat diet) compared with normal-weight mice, whereas serum concentrations of vitamin $D_3$ itself were similar in both groups.[2] This is not a surprise because serum 25OHD concentrations in overweight or obese humans are virtually systematically lower than in normal subjects in many different areas of the world having different sun exposure or dietary habits.[5] Their novel finding, however, was that the mRNA of major vitamin D-25-hydroxylase (CYP2R1) is markedly (~40%) lower in livers of obese mice (fed a high-fat diet) compared with livers from normal mice. They confirm that by finding lower protein expression (~50% decrease) of CYP2R1. The gene expression of some other potential 25-hydroxylases (CYP27A1 and CYP3A4) as well as the major catabolizing enzyme (CYP24A1) were not changed by diet-induced obesity. Finally, the authors measured the 25-hydroxylase activity by incubating mouse liver homogenates with vitamin $D_2$ and found a ~70% reduction in the overall enzymatic activity. As the substrate concentration was in the millimolar range, such an assay is not specific for the high-affinity, low-capacity CYP2R1 but represents a combined activity of all 25-hydroxylases including those that hydrolyze vitamin $D_2$ less well than $D_3$. They also used the ratio of serum 25OHD to serum vitamin D concentrations as a marker of 25-hydroxylase activity and found a strong positive correlation between this ratio and liver mRNA expression of CYP2R1.

Aatsinki and colleagues addressed a similar question about the origin of fairly systematic low serum 25OHD concentrations in diabetic subjects compared with their euglycemic controls, by studying high-fat-diet-induced obesity and type 2 diabetes in mice.[3] In addition, they also studied the effect of 24-hour fasting and of streptozotocin-induced type 1 diabetes. All these

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metabolic situations decreased the hepatic mRNA and protein concentration of CYP2R1. Fasting for 24 hours, type 1 diabetes, or type 2 diabetes decreased the mRNA of CYP2R1 in liver by 80%, 43%, and 45%, respectively, and generated a decrease of about 30% in protein concentration (estimated by Western blot). In vitro measurement of total 25-hydroxylase activity indicated a more than 50% decrease during 12- to 24-hour fasting. In addition, these authors demonstrated that the *decrease* in CYP2R1 was mediated by PPARγ-coactivator-1α (PGC1α), the key control enzyme induced by metabolic diseases such as fasting or type 1 or type 2 diabetes. By using several in vitro and in vivo gene KO and overexpression experiments, they showed that the control of CYP2R1 gene expression by PGC1α required the presence of another nuclear receptor, estrogen-related receptor α (ERRα), known to bind tightly to this and other nuclear receptors (such as VDR and glucocorticoid receptor [GR]). Activation of the GR receptor by dexamethasone also decreased hepatic CYP2R1 mRNA and protein concentrations (by 50% and 26%, respectively), again mediated by induction of PGC1α. PGC1α also *induced* hepatic and renal expression of CYP24A1 several-fold, again mediated by the GR-PGC1α-ERRα pathway (but much less than the 100-fold induction by $1,25(OH)_2D$). Other major fat-regulating nuclear receptors are less likely involved, as the ENCODE project did not find consensus sequences for nuclear receptor (VDR) binding sites in promoters of genes involved in fat metabolism in the liver, such as constitutive androstane receptor (CAR), pregnane X receptor (PXR), or peroxisome proliferator-activated receptor (PPAR) binding sites in the proximal promoter of mouse or human CYP2R1 (http://www.cbrc.jp/htbin/nph-tfsearch), but several binding sites for NFkB were identified.[2]

Both studies thus clearly demonstrate that the major (CYP2R1) and global hepatic 25-hydroxylase activity is under tight control of metabolic signals induced by fasting, diabetes, or exposure to high-dose glucocorticoids. PGC1α and ERRα as well as the GR are involved in the regulation of CYP2R1, but additional mechanisms may also be involved. A recent abstract demonstrates that a high-fat diet induces an epigenetic downregulation of CYP2R1 in the mouse liver, thereby causing decreased serum 25OHD, whereas CYP24A1 was upregulated.[6] These observations are fully in line with previous studies cited above. However, these authors now add another mechanism by hypermethylation of the promotor regions of these CYP2R1 and CYP27B1 genes and hypomethylation of the CYP24A1 promoter. In addition, they observed a decreased expression of glutathione, and treatment of such mice with glutathione precursors could partially correct the abnormal expression of vitamin D regulatory genes. They concluded that high-fat diet caused glutathione deficiency, changing the methylation pattern of vitamin D regulatory genes and causing low serum 25OHD concentrations. All these studies failed to report blood glucose levels in their obese animals so that the separate effects of obesity and diabetes cannot be fully estimated. There are many other remaining questions such as: 1) Are CYP2R1 and CYP24A1 expression in other tissues also under the same metabolic control? 2) Is DBP, the major transport protein of all D metabolites, also regulated by metabolic factors? DBP is indeed decreased in diabetic subjects or animals.[7,8] Apart from obesity, fasting, and diabetes, many other diseases are associated with poor vitamin D status compared with healthy controls. Therefore, the question arises whether patients with chronic renal failure, liver cirrhosis, and acute illness also have low serum 25OHD due to metabolic control of CYP2R1. If so, it could at least partially explain why such patients and especially patients admitted to intensive care units require so much vitamin D (10 to 100 times the normal doses) to generate serum 25OHD concentrations above 20 ng/mL.[9,10] On the other hand, DBP levels often drop in these circumstances as an acute phase reactant, and this is associated with reduced 25OHD concentrations.

Finally, the short- and long-term effects (harm or benefit) of this metabolic regulation of CYPs involved in vitamin D metabolism are not known. The PGC1α-ERRα pathway is known to play a major role in hepatic gluconeogenesis, in energy homeostasis in general and in fat tissue in particular. Indeed, because PGC1α is a strong positive regulator of mitochondrial function (and energy production), one might wonder whether the new observations are linked to overall energy balance and may help to clarify why VDR or CYP27B1 null mice are resistant to high-fat-diet-induced obesity (by activating energy expenditure),[11,12] whereas in humans a low vitamin D status is strongly associated with obesity. Knockdown of CYP2R1 in zebrafish did not affect bone homeostasis but generated a phenotype of abnormal visceral fat accumulation.[13] A similar phenotype of increased visceral and subcutaneous fat accumulation was observed in zebrafish raised on a vitamin D–deficient diet, probably related to the increased expression of adipogenic and lipid processing markers in their liver.[14] These data clearly indicate that the link between vitamin D metabolism and energy homeostasis already occurred early in the evolution of vertebrates.[15]

Variations in hepatic (or extra-hepatic) CYP2R1 expression may also play a role in the great variability of serum concentrations of 25OHD in healthy populations with similar food and lifestyle attitudes. Indeed, there is widespread variability in the response of serum 25OHD to comparable amounts of dietary vitamin D and/or vitamin D supplementation[16–18] and no compelling mechanism has been able to explain this variability.

## 25-Hydroxylase Activity (CYP2R1)

The studies just reviewed dealt with mice and need to be confirmed in humans. Human and mouse CYP2R1 are structurally and functionally very similar[19] and in both species, serum 25OHD is lower in case of type 1 or type 2 diabetes. Therefore, these studies clearly demonstrate that the general belief of constitutive expression of liver 25-hydroxylase activity no longer holds true (Fig. 1). Indeed, the major 25-hydroxylase, CYP2R1, is highly regulated by a variety of "clinical" conditions (obesity, starvation, type 1 or type 2 diabetes) and a number of regulatory factors are now clearly identified, albeit there are still major missing links. Genetic silencing mutations in CYP2R1 can cause rickets or osteomalacia,[20,21] but no activating mutations are so far described. Null mutations of the same gene cause the same phenotype in cats.[22] Polymorphisms in CYP2R1 have the greatest effect on interindividual variations in serum 25OHD when comparing with other known polymorphisms.[23] If confirmed in humans, serum 25OHD is not only reflecting access to vitamin D of nutritional and skin-produced vitamin D but is also reflecting a complex metabolic regulation of its hepatic synthesis and the likely involvement of many hormones.

These studies may also have practical implications for correcting a poor vitamin D status in obese or diabetic subjects. Intervention studies have shown that obese subjects need more vitamin D than normal-weight subjects to achieve similar serum 25OHD concentrations as based on a comparison between an overview of such studies.[5,16] However, vitamin D supplementation of vitamin D–replete prediabetic subjects did not decrease their risk of progression to type 2 diabetes.[24] Whether

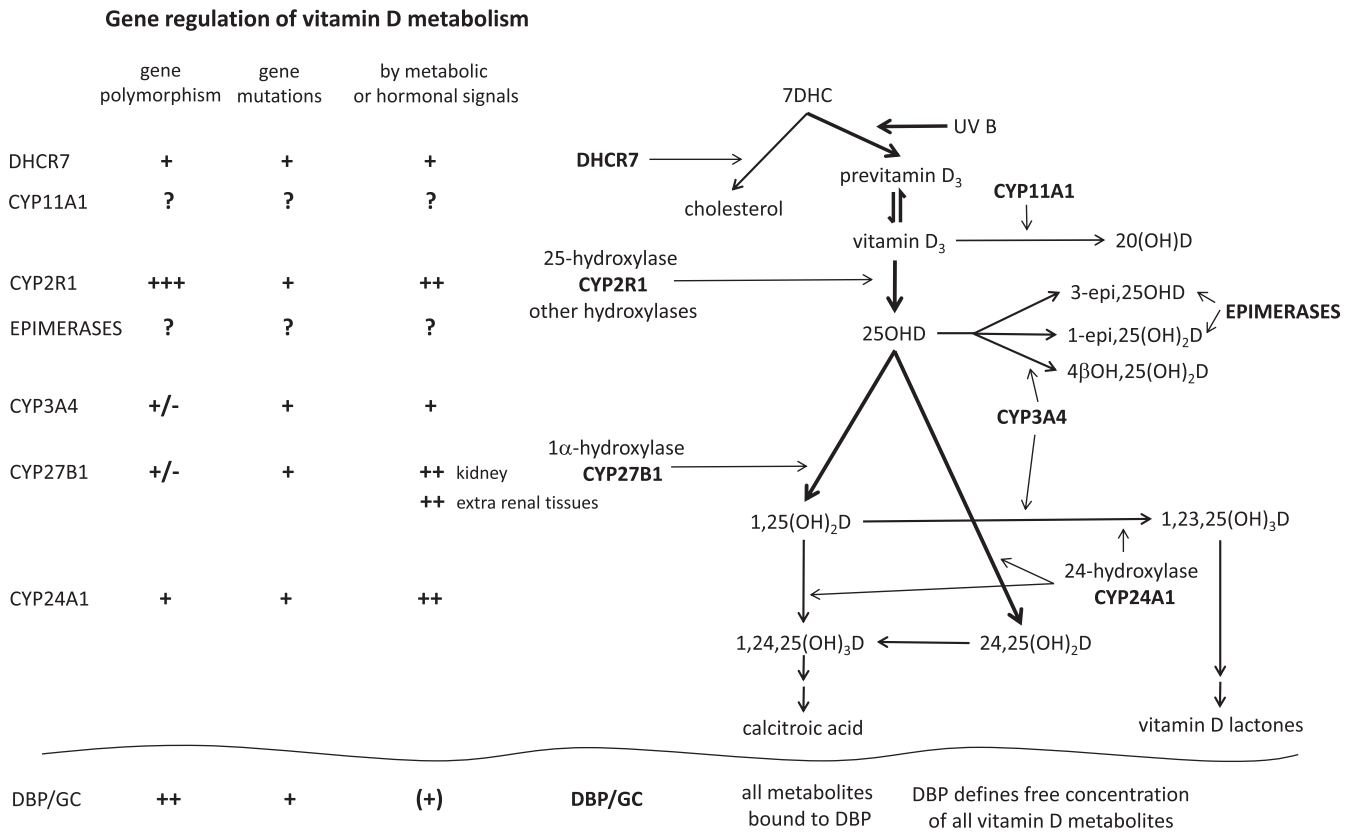


**Fig. 1.** Overview of the origin, metabolism, and transport of vitamin D and its most important "old" and newly discovered metabolites and the major enzymes involved. The polymorphisms, mutations, and metabolic or hormonal regulation of these major genes involved in vitamin D metabolism are also depicted.

supplementation of more vitamin D–deficient subjects may generate better results is yet unclear.

Now that the dogma of a nonregulated CYP2R1 has been challenged, one may also question other "dogmas" regarding vitamin D metabolism (Fig. 1).

## 7-Dehydrocholesterol Reductase (DHCR7)

DHC-7a-reductase (DHCR7) is a key determinant of the amount of the vitamin D precursor, 7-dehydrocholesterol (7-DHC), in the skin. Most reviews mention that older subjects may have lower 7-DHC concentrations in the skin but do not include regulation of DHCR7 as an important regulator of vitamin D status. DHCR7 is the last step in the Kandutsch-Russell pathway of cholesterol synthesis, converting 7DHC to cholesterol. As such, DHCR7 is essential for the presence or absence of 7-DHC in skin cells. In case of overexpression of this enzyme, as in the skin of the members of the feline species (including cats and dogs), the near absence of 7-DHC makes these animals unable to synthesize vitamin D, so that vitamin D is a true vitamin in these species.[1] The opposite condition, genetic absence of DHCR7, causes Lemli-Smith-Opitz disease,[25] mainly characterized by the consequences of too little cholesterol, steroids, or bile acids. However, this disease increases the accumulation of 7DHC and thereby increases the effect of UVB on the synthesis of vitamin D. Therefore these patients usually have higher serum 25OHD concentrations than normal subjects.[26] In humans, polymorphisms in DHCR7 have been associated with either reduced[27,28] or increased[29] 25OHD levels. However, the impact of these polymorphisms on enzyme function has not been demonstrated. The regulation of DHCR7 is incompletely understood. Cholesterol and vitamin D (but not $1,25(OH)_2D$) increase proteasomal degradation of DHCR7, as does UVB, leading to increased vitamin D production.[30] AMPK, a key sensor and regulator of cellular energy homeostasis, and protein kinase A are potent inhibitors of DHCR7, whereas CaMKII has a lower inhibitory effect.[31,32] Most textbooks and reviews clearly state that the photochemical production of vitamin D in the skin is a nonenzymatic reaction. Although this remains technically correct, recent data suggest that the activity of DHCR7 is under (cellular) metabolic and genetic control. By controlling substrate (7DHC) availability, these factors thus can influence interindividual variations in photosynthesis of vitamin D. To what extent this has implications for the vitamin D status of humans is, however, unknown.

## CYP27B1

When Fraser and Kodicek[33] first identified the kidney as the source of $1,25(OH)_2D$ in 1971, it was thought to be the sole source. However, anephric pregnant rats can produce $1,25(OH)_2D$.[34] Similarly a case report of a woman with chronic kidney disease showed an increase in serum $1,25(OH)_2D$ during pregnancy,[35] and the human placenta was shown to be capable of $1,25(OH)_2D$ production.[36] Moreover, in nonpregnant anephric humans[36,37] and pigs[38,39] detectable levels of 1,25(OH)2D were found at baseline and could be further increased with vitamin D or 25OHD administration. A report by Barbour et al.[40] of

an anephric patient with sarcoidosis with clearly detectable 1,25$(OH)_2$D levels demonstrated a disease state in which extrarenal 1,25$(OH)_2D_3$ production occurred. The source was soon discovered to be the activated pulmonary alveolar macrophages from the involved lungs.[41] At about the same time, a number of investigators were finding 1,25$(OH)_2$D production by bone cells,[42] melanocytes,[43] and epidermal keratinocytes in vitro[44] and many other cells and tissues.[45] With the cloning of the 25OHD-1α hydroxylase (CYP27B1) in 1997 by 4 groups[46–49] came the demonstration that there is only one gene and protein such that the renal and extrarenal enzyme is the same.[46,50] The cloning enabled the development of molecular probes and antibodies to CYP27B1,[51] facilitating the demonstration of its expression in many other tissues. However, it soon became apparent that the regulation of CYP27B1 activity in non-renal tissues differed from that in the kidney. This difference in regulation is clearly demonstrated in diseases such as sarcoidosis and other disorders that lead to unregulated increases in circulating 1,25$(OH)_2$D and hypercalcemia. Four examples of CYP27B1 regulation in non-renal tissues follow a discussion of its regulation in the kidney.

### Kidney

CYP27B1 in the renal proximal convoluted tubule (PCT) is controlled principally by three hormones, parathyroid hormone (PTH), having a positive effect and FGF23 as well as 1,25$(OH)_2$D itself (both having an inhibitory effect), responding at least in part to changes in ambient calcium and phosphate levels (review in[52]). Calcitonin can stimulate CYP27B1 activity in the proximal straight tubule.[53] PTH and FGF23 act by binding to their respective receptors and activating their signaling pathways. Meyer et al.[54] identified a region in the enhancer region of CYP27B1 in renal DNA that was responsive to PTH, FGF23, and 1,25$(OH)_2$D regulation. However, this region was not accessible to such regulation in the extrarenal tissues they tested including skin and immune cells. In these non-renal tissues, a different region of the CYP27B1 enhancer region was regulated by inflammatory factors, consistent with different regulatory mechanisms in non-renal tissues by the cytokines interferon-γ and tumor necrosis factor-α. Leptin may also (negatively) regulate CYP27B1 but probably mainly by its stimulatory effect on FGF23 production.[55,56] These feedback loops provide very tight regulation of 1,25$(OH)_2$D production by the PCT of the kidney, control that differs from that of CYP27B1 in other cell types including that of distal renal tubule cells where PTH has little effect.[57]

### Keratinocytes

1,25$(OH)_2$D has very little effect on CYP27B1 activity in keratinocytes.[58] Rather, 1,25$(OH)_2$D regulates its own levels in the keratinocyte by inducing CYP24A1, the catabolic enzyme for 1,25$(OH)_2$D.[58] Tumor necrosis factor-α (TNFα)[59] and interferon-γ (IFNγ),[60] on the other hand, are potent inducers of CYP27B1 activity in the keratinocyte as is TGFβ1.[61] 1,25$(OH)_2$D induces the expression TLR2 and CD14 in keratinocytes, and activation of TLR2, but not TLR4 (by LPS), induces CYP27B1.[61]

### Macrophages and monocytes

The production of 1,25$(OH)_2$D by pulmonary alveolar macrophages is activated by IFNγ and TNFα, but not by IFNα and IFNβ, and is inhibited by dexamethasone,[62,63] but not by 1,25$(OH)_2$D. IL-1, IL-2, and IL-15 also stimulate CYP27B1 activity in peripheral blood mononuclear cells (PBMC), whereas IL-4 is suppressive.[64,65] In contrast to Th1 cells, which produce IFNγ and IL-2, Th2 cells produce not only IL-4 but IFNβ that increases IL-10 to decrease CYP27B1 activity.[66] Mononuclear cells express FGF receptors and αKlotho and respond to FGF23 with a reduction in CYP27B1 expression.[67]

### Bone

CYP27B1 in human mesenchymal stem cells from bone marrow is stimulated by PTH through mechanisms involving both the phosphorylation of CREB (an acute response) and through the expression of IGF1 and the activation of its receptor (longer-term response).[68] 25OHD increases CYP27B1 expression in these cells, but that appears to be due to a combination of increased expression of the PTH/PTHrP receptor[69] and IGF1[70] as 1,25$(OH)_2$D decreases the expression of CYP27B1 in these cells.[70] However, not all studies have found that PTH stimulated CYP27B1 in human osteoblasts.[71]

### Parathyroid gland

The parathyroid gland expresses both FGF receptors and αKlotho.[72] Unlike the kidney, FGF23 stimulates CYP27B1 expression in the parathyroid gland.[73,74] Activation of the calcium-sensing receptor in the parathyroid gland either by calcium or cinacalcet also increases CYP27B1 expression.[74] Both FGF23[72] and cinacalcet[74] reduce PTH secretion, suggesting a link between PTH secretion and CYP27B1 expression.

These data clearly show that the production of 1,25$(OH)_2$D is much more complex than the original dogma of the kidney being the single source of the active vitamin D hormone, regulated by two key hormones, PTH and FGF23. Moreover, recent data demonstrate that the renal and especially the extrarenal production of this hormone is extremely complex and regulated by a wide variety of mechanisms. The contribution of extrarenal 1,25$(OH)_2$D production in normal physiology and disease states is a matter of debate. Extrarenal tissues can contribute to the serum concentration of 1,25$(OH)_2$D in case of inflammatory diseases and pregnancy, but this is disputed in other situations, although as noted earlier, 1,25$(OH)_2$D levels can be increased with vitamin D or 25OHD supplementation in anephric or end-stage renal failure patients. That said, the prevailing view is that extrarenal 1,25(OH)2D production serves primarily a paracrine function in the tissue where it is produced rather than an endocrine function.

## CYP24A1

CYP24A1 is the main enzyme responsible for the catabolism of all vitamin D metabolites (Fig. 1). It creates a multistep pathway resulting in a large number of metabolites with side chain modifications ultimately leading to calcitroic acid. It also plays an essential role (albeit species specific) in the formation of 25OHD lactones. Absence of this unique 24-hydroxylase (in contrast with multiple 25-hydroxylases) results in accumulation of 1,25$(OH)_2$D and neonatal hypercalcemia.[75] This is potentially lethal in mice and infants (infantile hypercalcemia). In addition, absence of this enzyme may first demonstrate its consequences by nephrocalcinosis or multiple kidney stones in adulthood.[76,77]

CYP24A1 null mice also have a problem with fracture repair as 24R,25$(OH)_2$D is able to bind to a GPCR, Fam57B2, and thereby

stimulates lactosylceramide production and fracture repair.[78] Whether this also applies to humans with bi-allelic mutations has, however, so far not been reported.[77] Polymorphism of the CYP24A1 gene is responsible for modest genetic variability of serum 25OHD (as one of the 8 genes known so far to result in genetically predisposed higher or lower serum 25OHD concentrations). CYP24A1 is under control of many hormones but mainly by $1,25(OH)_2D$ (very strong upregulation) and FGF23 (also stimulatory effect) or calcium.[79] Even 5α-dihydrotestosterone, by using the progesterone receptor, seems to be able to stimulate CYP24A1.[80]

Although incompletely understood, there must be other mechanisms to eliminate vitamin D metabolites, as serum 25OHD is only modestly increased in animals or humans with bi-allelic null mutations. The most likely candidates are CYP3A4 and a variety of enzymes capable of esterification of all vitamin D metabolites.

## CYP11A1

This enzyme is well known as the rate-limiting enzyme in steroid synthesis, converting cholesterol to pregnenolone, the side chain cleavage reaction. However, Slominski and colleagues[81] have demonstrated that CYP11A1 also metabolizes vitamin $D_3$ to $20(OH)D_3$ with subsequent further metabolism to a variety of metabolites including $1,20(OH)_2D_3$, which have biologic activity comparable in some cases to $1,25(OH)_2D_3$. 25OHD is not a substrate (Fig. 1). The efficiency of $1,20(OH)_2D$ production presumably by CYP27B1 acting on 20(OH)D is much lower than that of $1,25(OH)_2D$ production from 25(OH)D. CYP11A1 is expressed in the skin and cultured keratinocytes[82] as well as better-known steroid-producing tissues such as the adrenals, ovary, testes, and placenta. At this point, little is known about how this enzyme is regulated in the skin and elsewhere with respect to its vitamin D–metabolizing activity.

## CYP3A4

CYP3A4 is the major drug-metabolizing enzyme.[83] It is primarily expressed in the liver and intestinal mucosa. $1,25(OH)_2D$ induces this enzyme in both liver and intestinal cells,[84] although in vivo there is probably little induction in the liver given the low levels of VDR in that tissue. The enterohepatic circulation of $1,25(OH)_2D$ and 25OHD may increase the levels of CYP3A4 more than would be expected based on serum levels.[85,86] Lithocholic acid can also function as a ligand for VDR inducing CYP3A4 in the intestine.[87] CYP3A4 can metabolize both 25OHD and $1,25(OH)_2D$ as well as other vitamin products such as 1αOHD and $D_2$. These hydroxylations occur in the 24 and 25 positions of the side chains[88] as well as the 23 position for 1,25(OH)2D.[89] The induction of CYP3A4 by $1,25(OH)_2D$ was at least as great as the induction of CYP24A1 in the intestine.[90] Rifampin is a potent inducer of CYP3A4, and its use results in lower levels of 25OHD and $1,25(OH)_2D$. This could lead to drug-induced osteomalacia.[91] The major circulating product of CYP3A4 activity is $4β,25(OH)_2D$, which can reach levels comparable to $1,25(OH)_2D$ after rifampin therapy[92] (Fig. 1). Its biologic activity is not known.

Recently a publication has appeared describing two unrelated subjects with early onset of rickets for which none of the known mutations in the enzymes involved with vitamin D metabolism or VDR could be found.[93] Both 25(OH)D and $1,25(OH)_2D$ levels were low, whereas $4β,25(OH)_2D$ levels were elevated. The authors used whole exome sequencing to find the same *activating* missense mutation in the CYP3A4. The authors labeled this mutation as vitamin D–dependent rickets type 3. It can be treated with very large doses of vitamin D.[93]

## 25OHD-3-epimerase

The enzyme catalyzing the 3β-epimerization of (3α)25OHD remains poorly studied. This reaction does not appear to be reversible. The gene has yet to be identified. The enzymatic activity is broadly distributed and resides in the microsomal fraction of cells.[94] Circulating levels of 3-epi-25OHD can be substantial, ranging from 3.5% to 22% of the 25OHD levels in adults[95] and 8.7% to 61.1% in children.[96] LC/tandem mass spectroscopy methods have been developed to separate the 3-epi form from 25OHD itself.[97] The 3-epi-25OHD can be further metabolized by CYP27B1 to 3-epi-$1,25(OH)_2D$.[98] 3-epi-$1,25(OH)_2D$ has biologic activity, although in most studies its activity is less than $1,25(OH)_2D$,[99] although its affinity for the VDR appears to be substantially less.[100] Moreover, its ability to stimulate intestinal calcium absorption, differentiation of UMR 106 cells, or CYP24A1 induction is markedly reduced. Thus the 3-epi forms of 25OHD and $1,25(OH)_2D$ cannot be ignored, but their biologic roles need further study.

## 1β-epimerase

Substantial amounts of $1β,25(OH)_2D$ are detectable in serum of normal subjects (about 16% to 33% of the concentration of $1α,25(OH)_2D$). Its concentration shows a high correlation with serum 25OHD ($r = 0.85$) but a lower correlation with $1,25(OH)_2D$. The origin (tissue?) or enzyme(s) involved have not yet been defined.[101]

## Vitamin D Esterification

The conversion of vitamin D into 25OHD is far from complete. Based on clinical supplementation trials (reviewed in Quesada-Gomez and Bouillon(16)), only one of three to 10 molecules of vitamin D ultimately is converted into 25OHD. The same is true for the conversion of 25OHD into $1,25(OH)_2D$. The other 25OHD molecules can be converted by CYP24A1 into $24,25(OH)_2D$ and a number of other metabolites (Fig. 1). The fate of the other vitamin D (or 25OHD) molecules is unclear, but esterification is most likely involved as part of the degradation pathway. This involves conjugations with sulfate (into vitamin D/25-hydroxyvitamin D3-3-sulfate), glycosides (eg, vitamin D and 25-hydroxyvitamin D3-3-glucuronide), taurine, or long-chain fatty acids.[102,103] The esterification of vitamin D is already found early in evolution as some glycosides of vitamin D are even found as toxic agents in plants[104] and most vitamin D found in fish liver is in the form of fatty acid esters.[105] The regulation of these esterifications and the potential recovery of vitamin D metabolites by de-esterification (eg, hepato-biliary-intestinal recycling) are largely unexplored.

## Vitamin D Binding Protein

The serum DBP is responsible for the transport of all vitamin D metabolites due to its high affinity for all metabolites and especially for 25OHD. It thereby regulates the free concentration of

these metabolites as is best demonstrated by the extremely low serum concentrations of 25OHD and $1,25(OH)_2D$ in animals or the single human subject with bi-allelic mutations in the DBP/GC gene.[106] Up to now, most experts considered DBP as being stably expressed by hepatocytes with little or no regulation, apart from the stimulatory effects of estrogens.[107] DBP concentrations, however, are slightly (~10%) lower in homozygous DBP/GC2-2 carriers with a similar decrease in total 25OHD concentrations. Polymorphisms in DBP are responsible for part of the genetic variability of serum 25OHD concentrations in all populations tested so far. DBP in serum can be measured by mono- and polyclonal antibodies and more recently also by mass spectroscopy, whereby careful attention must be given to assure equal measurements of all isoforms of DBP.[108]

DBP concentrations are markedly decreased in liver diseases, nephrotic syndrome, and in patients with very severe acute illness or acute trauma due at least in part to its actin scavenging function.[109] Therefore, DBP is not a passive but an active player in the overall vitamin D homeostasis and is probably under control of various metabolic signals (Fig. 1).

## Summary and Perspective

The dual origin of vitamin D, discovered about a century ago, first evolved into a rather simple metabolic schema of constitutive 25-hydroxylation of vitamin D in the liver to produce 25OHD, followed by a tightly regulated 1α-hydroxylation by a unique CYP27B1 in a unique organ (kidney) to generate $1,25(OH)_2D$ as ligand of a nuclear receptor, VDR. All these metabolites are transported by a single serum binding protein and are finally catabolized by a unique nearly ubiquitous CYP24A1. The present picture is much more complex with a large number of enzymes expressed in a variety of cells. Most of these genes contain genetic polymorphisms that may alter their functionand are regulated by hormones and/or metabolic signaling that can vary in different tissues of the body. Finally, the vitamin D endocrine system regulates a large number of vertebrate genes. These recent findings reveal that the vitamin D endocrine system is much more complex than initially thought and remains still incompletely understood.

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