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Vitamin D: Metabolism

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Synopsis

The biologically active metabolite of vitamin D, 1,25(OH)₂D₃, affects mineral homeostasis and has numerous other diverse physiological functions including effects on growth of cancer cells and protection against certain immune disorders. This chapter reviews the role of vitamin D hydroxylases in providing a tightly regulated supply of 1,25(OH)₂D₃. The role of extrarenal 1α(OH)ase in placenta and macrophages is also discussed as well as regulation of the hydroxylases and vitamin D hydroxylases in aging and chronic kidney disease. Understanding specific factors involved in regulating the hydroxylases may lead to the design of drugs that can selectively modulate the hydroxylases. The ability to alter levels of these enzymes would have therapeutic potential for the treatment of various diseases including bone loss disorders and certain immune diseases.

Keywords

vitamin D metabolites; vitamin D hydroxylases; vitamin D binding protein; FGF-23; kidney; placenta

I. SYNTHESIS OF 1,25(OH)₂D₃ FROM VITAMIN D₃

Vitamin D₃ (cholecalciferol) is taken in the diet (from fortified dairy products and fish oils) or is synthesized in the skin from 7-dehydrocholesterol by ultraviolet irradiation. The vitamin D produced by 7-dehydrocholesterol depends on the intensity of UV irradiation which varies with season and latitude (1). Sunscreen and clothing have been reported to prevent the conversion of 7-dehydrocholesterol to vitamin D₃ (2,3). In order to be biologically active and affect mineral

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metabolism and to have effects on numerous other diverse physiological functions including inhibition of growth of cancer cells and protection against certain immune mediated disorders, vitamin D must be converted to its active form (4,5). Vitamin D is transported in the blood by the vitamin D binding protein (DBP, a specific binding protein for vitamin D and its metabolites in serum) to the liver. In the liver vitamin D is hydroxylated at C-25 by one or more cytochrome P450 vitamin D 25 hydroxylases (including CYP2R1, CYP2D11 and CYP2D25), resulting in the formation of 25-hydroxyvitamin D₃ (25(OH)D₃). It has been suggested that CYP2R1 is the key enzyme required for 25 hydroxylation of vitamin D since a homozygous mutation of the CYP2R1 gene was found in a patient with low circulating levels of 25(OH)D₃ and classic symptoms of vitamin D deficiency (6). 25(OH)D₃, the major circulating form of vitamin D, is transported by the DBP to the kidney. In the kidney, megalin, a member of the LDL receptor superfamily, plays an essential role in endocytic internalization of 25(OH)D₃ (7). In the proximal renal tubule 25(OH)D₃ is hydroxylated at the position of carbon 1 of the A ring, resulting in the hormonally active form of vitamin D, 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) which is responsible for most, if not all of the biological actions of vitamin D (Fig. 1). The cytochrome P450 monooxygenase 25(OH)D 1 α hydroxylase (CYP27B1; 1 α (OH)ase) which metabolizes 25(OH)D₃ to 1,25(OH)₂D₃ is present predominantly in kidney. This enzyme is also found in extrarenal sites including placenta, monocytes and macrophages (8–11). As with all mitochondrial P450 containing enzymes, during the 1 α (OH)ase reaction electrons are transferred from NADPH to NADPH-ferredoxin reductase through ferredoxin. Inactivating mutations in the 1 α (OH)ase gene result in vitamin D dependency rickets (VDDR) type 1 in spite of normal intake of vitamin D, indicating the importance of the 1 α (OH)ase enzyme (12). Type 1 vitamin D dependent rickets is characterized by growth failure, hypocalcemia, elevated PTH, muscle weakness and radiologic findings typical of rickets (12). The 1 α (OH)ase null mutant mouse has provided a mouse model of VDDR type 1 (13, 14). It is of interest that in these mice, in addition to rickets, reproductive and immune defects have been noted (13). Further studies are needed to test the role of 1 α (OH)ase in extra renal sites that has been a matter of debate.

II. ROLE OF THE VITAMIN D BINDING PROTEIN (DBP) IN VITAMIN D METABOLISM AND ACTION

Studies using mice deficient in DBP have resulted in new insight into the role of DBP in vitamin D metabolism and action. Although DBP null (–/–) mice have markedly lower total serum levels of 25(OH)D and 1,25(OH)₂D₃ than wild type (WT) mice, the levels of serum calcium and PTH are normal in the DBP –/– mice (15). In patients with reduced levels of circulating DBP, serum calcium levels have also been reported to be normal (16). More recent studies using DBP null mice have shown that DBP is important for total circulating 1,25(OH)₂D₃ but DBP does not influence the pool of 1,25(OH)₂D₃ that enters cells and affects the synthesis of vitamin D target proteins (17). Thus direct measurement of 1,25(OH)₂D₃ may not, in all cases, reflect the biologically active 1,25(OH)₂D₃ (16). This may be, in part, why 25(OH)D₃, which is also more stable than 1,25(OH)₂D₃, is used to assess clinical vitamin D status. It has been suggested that the maintenance of normal serum calcium levels in the DBP null mice may be due to the ability of the vitamin D receptor to concentrate 1,25(OH)₂D₃ in tissues due to its high affinity for 1,25(OH)₂D₃, resulting in transcriptional regulation of genes involved in maintenance of calcium homeostasis (17).

III. 24-HYDROXYLASE (24(OH)ase)

In addition to 1,25(OH)₂D₃, the kidney can also produce 24, 25 dihydroxyvitamin D₃ (24, 25(OH)₂D₃), a relatively inactive metabolite when compared to 1,25(OH)₂D₃. 25-Hydroxyvitamin D₃ 24 hydroxylase (CYP24), also a mitochondrial P450 enzyme, can hydroxylate both 25(OH)D₃ and 1,25(OH)₂D₃ (5). It has been suggested that the preferred

substrate for 24(OH)ase is 1,25(OH)₂D₃ (18). Thus, 24(OH)ase limits the amount of 1,25(OH)₂D₃ in target tissues both by accelerating the catabolism of 1,25(OH)₂D₃ to 1,24,25(OH)₃D₃ resulting in calcitroic acid or by producing 24,25(OH)₂D₃ thus decreasing the pool of 25(OH)D₃ available for 1 hydroxylation (Fig. 1). Studies using 24(OH)ase null mutant mice provided the first direct evidence for a role for 24(OH)ase in the catabolism of 1,25(OH)₂D₃. 24(OH)ase null mutant mice are unable to clear 1,25(OH)₂D₃ from the bloodstream after both chronic and acute treatment with 1,25(OH)₂D₃ (19). Lack of 24(OH)ase resulted in impaired mineralization in intramembranous bones. This defect was normalized by crossing 24(OH)ase deficient mice to VDR ablated mice, indicating that elevated 1,25(OH)₂D₃ levels, and not the absence of 24,25(OH)₂D₃, was responsible for the abnormalities in bone. Thus, the main function of 24(OH)ase is vitamin D inactivation.

IV. REGULATION OF RENAL VITAMIN D HYDROXYLASES

A. by calcium, phosphate, PTH and 1,25(OH)₂D₃

The wide spread effects of 1,25(OH)₂D₃ necessitate a tight regulation of its bioavailability and a process of activation and deactivation that occurs through a series of negative and positive feedbacks resulting in changes in the expression of the hydroxylase enzymes depending on the physiological state (20,21). With regard to regulation of vitamin D metabolism, low dietary calcium and phosphate result in enhanced activity of 1α(OH)ase (5) (Fig. 1). Elevated PTH resulting from hypocalcemia is a primary signal mediating the induction of 1,25(OH)₂D₃ synthesis in the kidney (22–24). PTH stimulates the transcription of 1α(OH)ase (25–27). Recent studies have shown that the nuclear receptor 4A2 (NR4A2) is a key factor involved in the induction of 1α(OH)ase transcription by PTH (26). 1,25(OH)₂D₃ in turn suppresses PTH production at the level of transcription (28). The 1α(OH)ase gene is also negatively regulated by 1,25(OH)₂D₃ (29,30). When compared to the regulation of 1α(OH)ase, 24(OH)ase is reciprocally regulated (stimulated by 1,25(OH)₂D₃ and inhibited by low calcium and PTH) (5). The marked induction of 24(OH)ase by 1,25(OH)₂D₃ results in an autoregulatory suppression of 1,25(OH)₂D₃ when gene transcriptional effects of 1,25(OH)₂D₃ need to be attenuated to protect against hypercalcemia. Various factors that cooperate with the vitamin D receptor (VDR) in the transcriptional regulation of 24(OH)ase have been identified including the transcription factor C/EBPβ, SWI/SNF (complexes that remodel chromatin using the energy of ATP hydrolysis) and histone methyltransferases (CARM1 and G9) (31–33). Recent studies have suggested a synergy between acetylated and methylated histones to disrupt histone/DNA binding resulting in enhanced VDR activation of 24(OH)ase transcription (33).

B. by FGF23

In addition to calcium, phosphate, PTH and 1,25(OH)₂D₃, fibroblast growth factor 23 (FGF23), a phosphaturic factor that promotes renal phosphate excretion by decreasing its reabsorption in the proximal tubule, is also a physiological regulator of vitamin D metabolism (34). Unlike classical FGFs that function via paracrine mechanisms, FGF23 belongs to the FGF19 subfamily that acts in an endocrine fashion (35). From its identification as a causative factor in autosomal dominant hypophosphatemic rickets (ADHR), X-linked hypophosphatemic rickets (XLH), and tumor-induced osteomalacia (TIO), FGF23 has been shown to be a significant regulator of phosphate homeostasis and vitamin D biosynthesis (36–39). 1,25(OH)₂D₃ stimulates the production of FGF23 in bone (40). Administration of 1,25(OH)₂D₃ to mice results in increased serum levels of FGF23 prior to elevations in serum phosphate, suggesting that 1,25(OH)₂D₃ induces FGF23 expression independent of changes in serum phosphate (40). Increased FGF23 in turn suppresses the expression of 1α(OH)ase and induces 24(OH)ase in kidney (41) (Fig. 1,2). By inhibiting synthesis and promoting catabolism of 1,25(OH)₂D₃, FGF23 functions to reduce levels of 1,25(OH)₂D₃, which in turn decreases FGF23 expression in bone, forming a negative feedback circuit between the FGF23 and the vitamin D endocrine system (41).

Overactivity of FGF23 has been suggested to be a common pathogenic mechanism of phosphate wasting disorders that may explain their shared clinical characteristics including hypophosphatemia, low serum $1,25(\text{OH})_2\text{D}_3$, and rickets/osteomalacia (41).

It has been reported that FGF23 requires klotho (a multifunctional protein involved in phosphate and calcium homeostasis) as a cofactor for FGF signaling (42). Expressed predominantly in the kidney and also in parathyroid gland and choroid plexus (43), the klotho gene was first identified when mice homozygous for the gene mutation developed a syndrome resembling human premature-ageing, including a short lifespan, infertility, arteriosclerosis, skin atrophy, osteoporosis and emphysema (44). $1,25(\text{OH})_2\text{D}_3$ up-regulates klotho gene expression in kidney and the loss of klotho results in induction of $1\alpha(\text{OH})\text{ase}$, suggesting that klotho participates in $1,25(\text{OH})_2\text{D}_3$ autoregulatory suppression (45). Klotho can bind to several FGF receptor isoforms and can convert the canonical FGF receptor to a receptor specific for FGF23 (46). The cooperation of klotho and FGF23 in a common signal transduction pathway (Fig 2) may explain why klotho-deficient mice and FGF23-deficient mice exhibit identical phenotypes including premature ageing and metabolic disturbances such as hyperphosphatemia and increased synthesis of $1,25(\text{OH})_2\text{D}_3$ (45,47,48).

C. by other hormones (sex hormones, calcitonin, prolactin)

In avian species estrogens alone or when combined with androgens or progesterone have been reported to stimulate $1,25(\text{OH})_2\text{D}_3$ production (49,50). In addition, estrogens have been reported to suppress $24,25(\text{OH})_2\text{D}_3$ synthesis (49). However whether this relationship exists in mammalian species has been a matter of debate (51).

Although calcitonin is known to have a role in shrinking osteoclasts under high calcium conditions, under conditions where serum calcium levels are normal calcitonin has been reported to stimulate $1,25(\text{OH})_2\text{D}_3$ production (52-54). The stimulation of $1,25(\text{OH})_2\text{D}_3$ production by calcitonin may have physiological significance during lactation when calcitonin levels as well as $1,25(\text{OH})_2\text{D}_3$ levels are elevated and when the need for calcium is increased (55,56). Recent studies have shown a direct effect of calcitonin on renal $1\alpha(\text{OH})\text{ase}$ transcription (57). The transcription factor C/EBP β and the SWI/SNF chromatin remodeling complex were found to mediate the calcitonin regulation of $1\alpha(\text{OH})\text{ase}$ transcription, indicating a mechanism responsible, at least in part, for the increase in plasma $1,25(\text{OH})_2\text{D}_3$ during these times of increased calcium requirement (57).

It has been suggested that prolactin, which is also elevated during lactation, can stimulate $1,25(\text{OH})_2\text{D}_3$ production. Bromocriptine, which inhibits pituitary prolactin secretion, has been reported to significantly reduce plasma $1,25(\text{OH})_2\text{D}_3$ levels in lactating animals and recent studies have shown that prolactin also has a direct effect on the transcription of the $1\alpha(\text{OH})\text{ase}$ gene (58,59). Clinical studies have also suggested that factors besides PTH stimulate $1\alpha(\text{OH})\text{ase}$ during lactation. For example, lactating hypoparathyroid women treated with the usual dose of calcitriol have been reported to develop hypercalcemia. In the absence of treatment, serum $1,25(\text{OH})_2\text{D}_3$ levels in the hypoparathyroid women remain within the normal range during lactation (60,61). Thus, it is likely that prolactin and calcitonin have a physiological function to increase $1,25(\text{OH})_2\text{D}_3$ levels during lactation to protect the maternal skeleton.

V. EXTRARENAL 1-HYDROXYLASE ($1\alpha(\text{OH})\text{ase}$)

A. Placenta

In pregnancy, the placenta regulates communication and transport between mother and fetus, with the placental trophoblasts and maternal decidua serving as the functional interface for exchange. $1\alpha(\text{OH})\text{ase}$ is expressed in both fetal trophoblast and maternal decidual cells beginning early in gestation (62). $1\alpha(\text{OH})\text{ase}$ is most abundant in decidua (62). Synthesis of

$1\alpha(\text{OH})\text{ase}$ is 8 fold higher in first trimester decidual cells than in third trimester cells (63). This trend indicates an important role for $1,25(\text{OH})_2\text{D}_3$ in early pregnancy. It has been suggested that the immunosuppressive effects of $1,25(\text{OH})_2\text{D}_3$ are crucial for allowing proper trophoblast invasion of the uterus without triggering a maternal immune response (62). Decidual natural killer cells isolated from the first trimester decidua show decreased synthesis of cytokines such as tumor necrosis factor and interleukin 6 in response to $1,25(\text{OH})_2\text{D}_3$ (63). A role of $1,25(\text{OH})_2\text{D}_3$ as an activator of innate immunity in the placenta has also been suggested. It has been shown that in trophoblasts $1,25(\text{OH})_2\text{D}_3$ increases the expression of cathelicidin, an antimicrobial peptide (64). In addition to upregulation of $1\alpha(\text{OH})\text{ase}$ expression, recent findings indicate that the increased bioavailability of $1,25(\text{OH})_2\text{D}_3$ at the fetomaternal interface may also be partially attributed to a decrease in activity of the catabolic $24(\text{OH})\text{ase}$ enzyme in placenta (65). Taken together, these findings suggest the importance of the local production of $1,25(\text{OH})_2\text{D}_3$ in the placenta to regulate both acquired and innate immune responses and a possible role for $1,25(\text{OH})_2\text{D}_3$ in the immunoregulation of implantation.

B. Monocytes/macrophages

Monocytes and macrophages express $1\alpha(\text{OH})\text{ase}$ and produce $1,25(\text{OH})_2\text{D}_3$. However, monocyte/macrophage $1\alpha(\text{OH})\text{ase}$ is regulated differently than renal $1\alpha(\text{OH})\text{ase}$ (10,11). Clinical evidence for a different regulation of macrophage $1\alpha(\text{OH})\text{ase}$ is observed in patients with sarcoidosis. In sarcoidosis patients there is increased production of $1,25(\text{OH})_2\text{D}_3$ despite hypercalcemia (66). The disordered calcium homeostasis in sarcoidosis is due to dysregulated production of $1,25(\text{OH})_2\text{D}_3$ by activated macrophages (66). Unlike renal $1\alpha(\text{OH})\text{ase}$, $1\alpha(\text{OH})\text{ase}$ produced by macrophages is not suppressed by elevated calcium or $1,25(\text{OH})_2\text{D}_3$ and is upregulated by immune stimuli such as interferon gamma ($\text{IFN-}\gamma$) and lipopolysaccharide (LPS) (10,11). Multiple pathways have been reported to be involved in this upregulation, including JAK/STAT, MAPK, and NF κ B (10). Activation of $1\alpha(\text{OH})\text{ase}$ transcription by immune stimuli also requires the binding of C/EBP β to its recognition sites in the $1\alpha(\text{OH})\text{ase}$ promoter region (10,11). These findings suggest mechanisms responsible for the hypercalcemia of granulomatous disorders in which activated macrophages constitutively express $1\alpha(\text{OH})\text{ase}$ in the presence of elevated calcium and $1,25(\text{OH})_2\text{D}_3$.

VI. VITAMIN D P450s IN AGING AND CHRONIC KIDNEY DISEASE

The capacity of the kidney to convert $25(\text{OH})\text{D}_3$ to $1,25(\text{OH})_2\text{D}_3$ has been reported to decline with age. An increase in $24(\text{OH})\text{ase}$ gene expression and an increase in clearance of $1,25(\text{OH})_2\text{D}_3$ with aging have been reported (67–69). These findings suggest that the combined effect of a decline in the ability of the kidney to synthesize $1,25(\text{OH})_2\text{D}_3$ and an increase in renal metabolism of $1,25(\text{OH})_2\text{D}_3$ may contribute to age related bone loss.

Chronic kidney disease (CKD) has been shown to result in decreased vitamin D metabolism through multiple mechanisms (70). The loss of functional renal mass leads to decreased production of $1\alpha(\text{OH})\text{ase}$, specifically in the later stages of CKD (70). However, studies have also shown a suppression of enzyme activity due to associated aspects of the disease, including metabolic acidosis (71), hyperphosphatemia (72) and uremic toxins that accumulate in CKD (73). Elevated levels of FGF23 have also been shown in early CKD, resulting in decreased activity of $1\alpha(\text{OH})\text{ase}$. (74).

In summary, understanding vitamin D metabolism is of fundamental importance in understanding mechanisms involved in the maintenance of calcium homeostasis. The vitamin D hydroxylases have an important role in providing a tightly regulated supply of $1,25(\text{OH})_2\text{D}_3$. Understanding specific factors involved in regulating the hydroxylases may lead to the design of drugs that can selectively modulate the hydroxylases. The ability to alter levels

of these enzymes would have therapeutic potential for the treatment of various diseases including bone loss disorders and certain immune diseases.

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Figure 1.
The metabolic pathway for vitamin D

Fig. 2.

Regulation of vitamin D hydroxylases by FGF23-Klotho. 1,25(OH)₂D₃ binds to VDR. The ligand-bound VDR forms a heterodimer with nuclear retinoid X receptor (RXR) resulting in increased the expression of FGF23 in osteocytes. Secreted FGF23 activates FGFR bound by klotho in renal tubular cells. FGF signaling suppresses expression of 1α(OH)ase and induces 24(OH)ase thereby inhibiting synthesis and promoting catabolism of 1,25(OH)₂D₃. Thus, the FGF23-Klotho results in decreased levels of 1,25(OH)₂D₃.

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