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Mini Review

Vitamin D Metabolism and Tissue-Specific Activation: Systemic and Local Regulation of CYP27B1

Rosini Sergio[1], Francesco Molfetta[2], Rosini Stefano[3], Palermo Augusto[4] and Molfetta Luigi[5]*

[1]*Biomaterial Research Center, Livorno, Italy*

[2]*DINOGMI Department, School of Medical and Pharmaceutical Sciences, University of Genoa, Genoa, Italy*

[3]*Smile-Restyle, Livorno, Italy*

[4]*Head Prosthetic Surgery Unit, Istituto Clinico Humanitas Bergamo Gavazzeni, Bergamo, Italy*

[5]*DIMES Department, School of Medical and Pharmaceutical Sciences, Research Center of Osteoporosis and Osteoarticular Pathologies, University of Genoa, Genoa, Italy*

***Corresponding Author:** Molfetta Luigi, Professor, DIMES Department, School of Medical and Pharmaceutical Sciences, Research Center of Osteoporosis and Osteoarticular Pathologies, University of Genoa, Genoa, Italy.

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Abstract

Vitamin D (Vit D) metabolism involves a tightly regulated sequence of hydroxylation steps leading to the formation of the biologically active hormone 1α,25-dihydroxyvitamin D (1α,25$(OH)_2$D). While the kidney is the primary site of systemic calcitriol production, a wide range of extra-renal tissues express the activating enzyme CYP27B1, enabling local autocrine and paracrine signaling. This review examines the current understanding of Vit D metabolism, with particular emphasis on tissue-specific regulation of CYP27B1, mechanisms of cellular uptake of 25-hydroxyvitamin D (25(OH)D), and the functional implications of local versus systemic activation. The complexity of this system reflects a multilayered regulatory network rather than a single dominant pathway.

Keywords: *Vitamin D; Metabolism; Enzyme CYP27B1*

Introduction

Vit D, more appropriately considered a hormone rather than a vitamin, has long been recognized for its central role in Calcium (Ca) and phosphate homeostasis. Over the past decades, however, the discovery of widespread expression of the Vit D receptor (VDR) across multiple tissues has expanded its biological relevance far beyond skeletal metabolism [1].

A growing body of experimental and clinical evidence suggests that Vit D signaling influences a broad range of physiological processes, including immune regulation, cardiovascular function, cellular proliferation, and metabolic homeostasis. These observations have prompted increasing interest in the potential therapeutic and preventive roles of Vit D in a variety of diseases [2].

Despite this extensive research, several fundamental questions remain unresolved. In particular, there is still considerable uncertainty regarding: the optimal circulating levels of 25-hydroxyvitamin D (25(OH)D) required for biological activity; the relationship between systemic concentrations and intracellular effects; the mechanisms regulating tissue-specific activation of Vit D.

The classical model of Vit D metabolism describes a sequential process involving hepatic 25-hydroxylation followed by renal conversion to the active hormone 1α,25-dihydroxyvitamin D (1α,25(OH)$_2$D). While this endocrine pathway is well established, it does not fully explain the presence of both VDR and the activating enzyme CYP27B1 in numerous extra-renal tissues.

This apparent discrepancy raises an important conceptual issue: to what extent are the biological effects of Vit D mediated by circulating calcitriol, and to what extent do they depend on local, tissue-specific activation of its precursor, 25(OH)D?

Furthermore, the factors regulating intracellular availability of 25(OH)D, including transport mechanisms, binding proteins, and cellular uptake pathways, remain incompletely understood [3].

Aim of the Study

The aim of this review is to examine these unresolved aspects of Vit D biology, with particular focus on the relationship between systemic and local metabolism, the regulation of CYP27B1 expression, and the determinants of effective intracellular signaling.

Vitamin D metabolism

Once synthesized in the skin or obtained from dietary sources, Vit D is transported to adipose tissue for storage or reaches the liver, where the first activation step occurs through hydroxylation to 25-hydroxyvitamin D$_2$/D$_3$ (25(OH)D). This metabolite is subsequently converted in the kidney into the active form, 1α,25-dihydroxyvitamin D (1α,25(OH)$_2$D).

The conversion to the active hormone occurs primarily in the mitochondria of renal tubular cells through the action of the hydroxylase CYP27B1 (1α-hydroxylase), whose activity is regulated by multiple factors including phosphate, calcium, fibroblast growth factor 23 (FGF23), parathyroid hormone (PTH), leptin, cytokines, and 1α,25(OH)$_2$D itself [4,5].

Although CYP27B1 expression is particularly prominent in renal tissue, where it supports the synthesis of circulating 1α,25(OH)$_2$D, the same enzymatic activity, namely the 1α-hydroxylation of 25(OH)D$_3$, is also present, at varying levels, in several other tissues. These include pancreatic β-cells, vascular endothelial cells, cardiomyocytes, keratinocytes, placenta, parasympathetic ganglia, brain, gastrointestinal epithelium, and cells of the immune system, including macrophages and dendritic cells and others. In these tissues, CYP27B1 expression is generally inducible rather than constitutive and is regulated by local factors such as cytokines, growth factors, and inflammatory signals rather than systemic mineral homeostasis. Consequently, locally produced 1α,25(OH)$_2$D primarily exerts autocrine or paracrine effects [6,7].

During hepatic 25-hydroxylation of cholecalciferol, mediated primarily by CYP2R1, 25(OH)D$_3$ (calcidiol, also known as calcifediol) is produced. This metabolite remains associated with hepatic tissue for several days (approximately 7 - 19 days) before undergoing further hydroxylation at the 1α position, mainly in response to hypocalcemia and PTH.

In contrast, increased circulating levels of calcium, phosphate, and FGF23, or reduced PTH levels, promote the conversion of 25(OH)D toward 24,25(OH)$_2$D via CYP24A1. Additional metabolites such as 25,26(OH)$_2$D may also be generated, although their physiological roles remain incompletely defined.

In circulation, the half-life of Vit D metabolites is largely determined by their serum concentration and by the affinity for the Vit D binding protein (VDBP), which regulates the fraction of free hormone available for cellular uptake and biological activity. Notably, 1α,25(OH)$_2$D binds VDR with high affinity, whereas 25(OH)D exhibits significantly lower affinity. However, the much higher circulating levels of 25(OH)D raise the possibility of partial receptor activation under certain conditions [3,8,9].

$1\alpha,25(OH)_2D_3$ binds the Vit D receptor (VDR) with high affinity (KD ≈ 0.1 nM), whereas the affinity of VDR for $25(OH)D_3$ is 100 - 1000 times lower.

However, serum concentrations of $25(OH)D_3$ (50-250 nM) are approximately 1000-fold higher than those of $1\alpha,25(OH)_2D_3$ (0.05 - 0.15 nM). The higher affinity of $25(OH)D_3$ for VDBP results in lower free concentrations in circulation and consequently slower metabolic clearance [10].

The physiological actions of Vit D include genomic effects mediated by VDR activation, leading to transcription of target genes and protein synthesis. In addition, Vit D can exert non-genomic effects via membrane-associated receptors that activate intracellular signaling pathways independently of transcriptional regulation. Thus, Vit D influences cellular metabolism through both genomic and non-genomic mechanisms, either via direct receptor interaction or following intracellular conversion of 25(OH)D to $1\alpha,25(OH)_2D_3$ [8,11].

Transport and cellular uptake of 25(OH)D

The bioavailability of Vit D metabolites depends on their binding to VDBP, which determines the circulating free fraction [12,13]. While only a small proportion of 25(OH)D and $1\alpha,25(OH)_2D$ circulates in free form, this fraction is thought to be readily available for passive diffusion across cell membranes. In addition to passive diffusion, receptor-mediated endocytosis via the megalin-cubilin complex plays a critical role in specific tissues, particularly in renal proximal tubules, where it enables uptake of VDBP-bound 25(OH)D. Evidence suggests that this mechanism may also operate in other tissues, although its quantitative contribution outside the kidney remains less clearly defined.

In addition to passive diffusion across cell membranes, 25(OH)D can enter cells via receptor-mediated endocytosis involving the megalin-cubilin system. This mechanism is particularly active in the kidney, parathyroid glands, and placenta, but is also present in the adrenal glands, pituitary, mammary glands, and other tissues [14,15].

Megalin and cubilin are structurally distinct endocytic receptors that cooperate in the selective transport of ligands between compartments and play important roles in both physiology and disease.

Cellular fate of 25(OH)D

The widespread expression of VDR and CYP27B1 in tissues not directly involved in calcium homeostasis underlies the numerous extra-skeletal actions attributed to vitamin D signaling. Despite the widespread distribution of VDR, vitamin D exerts tissue-specific effects. Renally synthesized $1\alpha,25(OH)_2D$ acts as an endocrine hormone regulating calcium and phosphate homeostasis by increasing intestinal calcium absorption and renal reabsorption. In contrast, extra-renal production of $1\alpha,25(OH)_2D$ functions mainly in an autocrine or paracrine manner. This raises the question of which stimuli induce CYP27B1 expression in extra-renal tissues. These stimuli are likely related to specific cellular functions and may involve signaling pathways that promote local enzyme expression and subsequent production of $1\alpha,25(OH)_2D$.

CYP27B1 is constitutively expressed in renal proximal tubular cells and, to a certain extent, in keratinocytes, whereas in many other cell types it is inducible under specific conditions. Some tissues lack CYP27B1 but still express VDR, supporting the role of circulating calcitriol.

For extra-renal activation to occur, 25(OH)D must enter cells in sufficient amounts. While passive diffusion is possible due to its lipophilicity, circulating concentrations may limit this process. Indeed, receptor-mediated uptake via megalin-cubilin allows internalization of protein-bound 25(OH)D (e.g. VDBP-bound), ensuring adequate substrate availability [16].

Both passive diffusion and receptor-mediated uptake likely contribute to intracellular availability, although their relative importance may vary by tissue. The system appears to limit exposure to active 1α,25(OH)$_2$D, likely to prevent uncontrolled effects, with VDBP regulating bioavailability and CYP27B1 providing fine control of activation [17].

The Vit D system represents an example of "organized biological complexity", involving multiple overlapping and dynamically regulated mechanisms rather than a single dominant pathway.

Biological action

A key question concerns the intracellular concentration of 25(OH)D required for biological activity. There is likely no universal threshold, as CYP27B1 expression and enzyme kinetics are tissue-specific and influenced by local conditions such as inflammation. Nevertheless, a minimum intracellular level of 25(OH)D is probably required to sustain sufficient calcitriol production for metabolic activity, with thresholds varying across tissues.

In extra-renal tissues, 1,25(OH)$_2$D production is often substrate-dependent, meaning that increased availability of 25(OH)D can enhance local synthesis due to the absence of tight endocrine regulation. Serum concentrations generally considered sufficient (approximately 50 - 75 nmol/L, or 20 - 30 ng/mL) are typically within the range that supports enzymatic activity.

Although 25(OH)D may exert some direct biological effects, its primary physiological role is as a precursor to 1α,25(OH)$_2$D, which remains the main active mediator of vitamin D function.

Circulating 1α,25(OH)$_2$D, produced mainly by the kidney, acts as an endocrine hormone regulating calcium and phosphate homeostasis by increasing intestinal absorption and renal reabsorption.

In contrast, extra-renal production of 1α,25(OH)$_2$D supports local autocrine and paracrine signaling. This functional dichotomy highlights the importance of intracellular conversion of 25(OH)D in tissues expressing CYP27B1. The extent to which circulating 1α,25(OH)$_2$D versus locally produced hormone contributes to tissue-specific effects remains an area of ongoing investigation.

Conclusion

The Vit D system exemplifies what may be described as "organized biological complexity," characterized by multiple overlapping and partially redundant regulatory mechanisms. These include endocrine, paracrine, and autocrine pathways, variable receptor expression, and diverse modes of cellular uptake. Such complexity challenges the development of a single unified model and suggests that vitamin D biology should be interpreted within a context-dependent framework.

Vit D metabolism involves both systemic and local activation pathways, with CYP27B1 playing a central role in determining tissue-specific responses. While the kidney governs endocrine regulation, extra-renal tissues contribute to localized actions that extend beyond mineral metabolism. Further research is needed to clarify the relative contributions of passive diffusion and receptor-mediated uptake of 25(OH)D, the regulation of CYP27B1 in different cellular contexts, and the functional significance of intracellular Vit D metabolism.

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