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Vitamin D and the Intestine: Review and Update

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Abstract

The central role of vitamin D in calcium homeostasis is to increase calcium absorption from the intestine. This article describes the early work that served as the foundation for the initial model of vitamin D mediated calcium absorption. In addition, other research related to the role of vitamin D in the intestine, including those which have challenged the traditional model and the crucial role of specific calcium transport proteins, are reviewed. More recent work identifying novel targets of 1,25(OH)₂D₃ action in the intestine and highlighting the importance of 1,25(OH)₂D₃ action across the proximal/distal and crypt/villus axes in the intestine is summarized.

1. Introduction

Calcium is the fifth most abundant element in the human body and is essential to the functioning of numerous physiological processes including bone formation, nerve pulse transmission, blood clotting and hormone secretion [1]. The only source of calcium to meet these essential functions is from the diet. Even before the biochemical mechanisms were known, early studies noted an essential role for vitamin D in intestinal calcium absorption [2]. Later studies showed that vitamin D mediated intestinal calcium absorption required the transfer of calcium against a concentration gradient and that the stimulatory effect of vitamin D was inhibited by actinomycin D and required a lag time of 8 to 16 h to exert its effect [3–7]. These findings indicated that vitamin D action in the intestine requires RNA/protein synthesis. At this time a seminal discovery by the Wasserman lab was the identification of a vitamin D enhanced calcium binding protein in chick intestinal mucosa, the first known

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target of vitamin D action [8]. It was noted that the concentration of this calcium binding protein [later named calbindin-D [9] ($-D_{9k}$ (9,000 Mr) in mammalian and $-D_{28k}$ (28,000 Mr) in chick intestine] correlated to the rate of duodenal vitamin D mediated calcium absorption [8]. Subsequent studies identified the active form of vitamin D as 1,25-dihydroxyvitamin D ($1,25(OH)_2D_3$). The association of $1,25(OH)_2D_3$ with intestinal mucosa chromatin also correlated to increased intestinal calcium transport [10–14]. The identification of calbindin as the first known target of vitamin D action (its induction is still one of the most pronounced effects of $1,25(OH)_2D_3$ in the intestine) provided the foundation for building our basic understanding of the molecular mechanism of $1,25(OH)_2D_3$ action [15, 16]. This article summarizes what has been accomplished since these early studies related to our understanding of the role of vitamin D in the intestine and indicates future research directions.

2. Targets of Vitamin D and Intestinal Calcium Absorption

2.1. Active calcium transport

Calbindin served as a centerpiece in the traditional facilitated diffusion model of active intestinal calcium absorption [17]. In this model, calcium enters the intestinal epithelial cell down a concentration gradient through a calcium channel, binds to calbindin to “ferry” calcium across the cell, then is extruded from the enterocyte by the ATP-dependent calcium pump. Although calbindin was identified as the first known target of vitamin D in intestine in 1967, it was not until 1991 when the intestinal plasma membrane ATPase (PMCA1b, which is encoded by the *Atp2b1* gene) was found to be regulated by vitamin D and involved in the extrusion of calcium from the cell during active calcium transport [18]. Subsequent studies noted that *Atp2b1* was induced in response to dietary calcium and phosphate deficiencies, its induction by $1,25(OH)_2D_3$ decreased with age, and that $1,25(OH)_2D_3$ regulation of the expression of PMCA1b is at the level of transcription [19–21]. More recent studies using ChIP-seq have identified sites of vitamin D receptor (VDR) binding within the locus for *Atp2b1* [22]. Functional analysis is required to confirm that these sites are sites of active $1,25(OH)_2D_3$ regulation. The in vivo physiological importance of PMCA1 in vitamin D mediated calcium absorption was suggested in studies using mice with intestine-specific *Atp2b1* deletion [23]. Deletion of *Atp2b1* in the intestine was associated with decreased intestinal calcium absorption in response to $1,25(OH)_2D_3$ and decreased bone mineral density in the spine and femur [23]. There was no change in serum calcium. Further studies using these intestine specific *Atp2b1* knockout (KO) mice as well as further studies related to regulation of PMCA are needed.

Enhanced calcium uptake by the intestinal brush border membrane and binding of calcium to components of the brush border region in response to vitamin D had been shown in early studies [24, 25]. However, molecular basis for vitamin D dependent calcium entry into the enterocyte was only first identified in 1999 when Mathias Hediger’s group reported the cloning of the apical calcium channel, TRPV6 [26]. Although calbindin- D_{9k} and the VDR are expressed in all segments of the small and large intestine [27, 28], TRPV6 was found to be expressed in duodenum, jejunum and colon and is either not detected or present in very low levels in ileum [29]. It has been suggested that the slower rate of calcium absorption in

the ileum compared to other intestinal segments may be due to the low level of TRPV6 in the ileum [30]. Calcium transport as well as intestinal TRPV6 mRNA levels also increase in vitamin D deficient pregnant and lactating rats [31, 32], suggesting that factors in addition to $1,25(\text{OH})_2\text{D}_3$ can regulate intestinal calcium transport. Using ovariectomized VDR null mice or vitamin D deficient mice, estradiol or prolactin were each shown to induce intestinal calcium transport and TRPV6 mRNA levels [32–34]. In addition, cooperative effects of prolactin with $1,25(\text{OH})_2\text{D}_3$ in the regulation of both intestinal TRPV6 and calbindin- $\text{D}_{9\text{k}}$ have been reported [34], suggesting that prolactin and estradiol can be important modulators of intestinal calcium absorption during pregnancy and lactation.

2.2 Paracellular calcium transport

Calbindin, PMCA and TRPV6 are involved in the active, saturable transcellular process of intestinal calcium absorption that occurs when the body's demand for calcium increases during growth, pregnancy and lactation, or when serum $1,25(\text{OH})_2\text{D}_3$ levels are increased due to diets deficient in calcium. However, in addition to the transcellular pathway of calcium absorption, calcium can also flow across the intestinal barrier through a paracellular pathway. This movement occurs in direct proportion to the luminal calcium concentration, suggesting it is a passive diffusion process. As a result, when dietary calcium is high, the bulk of intestinal calcium absorption occurs by a passive diffusion process. The regulation of paracellular calcium transport by vitamin D is a matter of debate. Although the regulation of intercellular adhesion molecules (e.g. claudin-2) in the intestine by vitamin D provides some support for the existence of $1,25(\text{OH})_2\text{D}_3$ regulation of paracellular calcium transport [35, 36], early studies in rodents and Caco-2 cells reported that passive transport of calcium is not sensitive to vitamin D signaling [37, 38]. Further studies, including studies with KO and transgenic (Tg) mice, are needed to determine the physiological significance in calcium absorption of the intercellular adhesion molecules regulated by $1,25(\text{OH})_2\text{D}_3$. Recent studies have noted an essential role of tight junction regulation in enteric pathogen clearance [39]. Since the immune system is an additional target of vitamin D [40], it is possible that tight junction regulation by $1,25(\text{OH})_2\text{D}_3$ is also involved in vitamin D mediated protection from enteric infection.

3. Studies Using Genetically Modified Mouse Models

VDR null mice and mice deficient in 25-hydroxyvitamin D_3 1 α hydroxylase (CYP27B1) have rickets, hypocalcemia, hypophosphatemia, secondary hyperparathyroidism and a marked reduction in intestinal TRPV6 and calbindin- $\text{D}_{9\text{k}}$ expression [41–43]. In VDR null mice symptoms of rickets occur only after weaning, the time of onset of active intestinal calcium absorption [44]. Rickets is prevented when VDR null mice or CYP27B1 deficient mice are fed a diet which includes high calcium [45, 46], confirming that the major physiological function of $1,25(\text{OH})_2\text{D}_3$ /VDR in growing mice is to support intestinal calcium absorption. Consistent with this idea, intestine-specific transgenic expression of VDR in VDR null mice rescues abnormalities in calcium homeostasis, including an increase in intestinal TRPV6 and calbindin- $\text{D}_{9\text{k}}$ mRNA levels [27]. Paradoxically, there were no effects on calcium and bone metabolism in TRPV6 or calbindin- $\text{D}_{9\text{k}}$ KO mice compared to wild type (WT) mice under adequate calcium conditions [47–49]. In addition, $1,25(\text{OH})_2\text{D}_3$

administration to vitamin D deficient TRPV6 or calbindin-D_{9k} null mice significantly increased active duodenal calcium transport similar to WT vitamin D deficient mice [47]. These findings indicate that vitamin D mediated calcium transport can occur in the absence of these proteins and suggest that other calcium channels or calcium binding proteins can compensation for their absence. For example, calcium may also be sequestered by intracellular organelles, which could contribute to protection against calcium toxicity during enhanced calcium influx. In contrast to the studies in the KO mice, other research shows that intestine-specific transgenic expression of TRPV6 can recover calcium absorption and prevent rickets in VDR KO mice [50]. Thus, while studies in the KO mice suggest that in the absence of TRPV6 there is compensation by other proteins yet to be identified, the transgenic mouse study shows that TRPV6 is a bona fide contributor that has an important role in calcium uptake during transcellular intestinal calcium transport. In addition, this work revealed that calbindin-D_{9k} levels increased in direct proportion to the increase in transcellular calcium absorption (i.e. the elevation in calbindin-D_{9k} did not require regulation through the VDR), indicating elevated calbindin-D_{9k} levels following vitamin D treatment may be a secondary, protective response to increased cellular calcium fluxes rather than a primary driver of vitamin D regulated calcium absorption. In summary 1) Studies in VDR and CYP27B1 null mice indicate the critical role of both 1,25(OH)₂D₃ and VDR in the calcium absorptive process. 2) Although studies in transgenic mice overexpressing TRPV6 indicate the importance of calcium uptake via TRPV6 in intestinal calcium absorption, in the absence of TRPV6 this channel can be compensated by another channel yet to be identified. 3) In the cytosol calcium may be bound to calbindin to protect against toxic levels of calcium from accumulating in the intestinal cell during enhanced calcium transport. As suggested by studies in calbindin-D_{9k} KO mice, in the absence of calbindin calcium may be bound to other calcium binding proteins or may be sequestered by the endoplasmic reticulum to protect against excessively high calcium.

4. Essential role for vitamin D in distal intestinal segments and novel intestinal vitamin D targets

Most studies on 1,25(OH)₂D₃-mediated calcium absorption have focused on the proximal intestine. However, VDR, calbindin and TRPV6 are present in all segments of the small and large intestine and 1,25(OH)₂D₃ regulation of calcium absorption has also been reported in the distal intestine [27, 51–53]. To understand the role of vitamin D signaling in the distal intestine, the Christakos lab created mice with transgenic expression of VDR exclusively in the distal intestine (ileum, cecum and colon) of VDR KO mice (VDRKO-Tg). They showed that expression of VDR specifically in the distal intestine of VDR KO mice to levels equivalent to WT mice prevented the abnormalities in calcium homeostasis and bone mineralization normally seen in VDR KO mice [54]. Thus, although calcium is absorbed most rapidly in the duodenum compared to other intestinal segments, these finding provided direct evidence for the importance of 1,25(OH)₂D₃mediated calcium absorption in the distal intestine. The Christakos lab has subsequently examined the expression of VDR target genes in the intestine of the VDRKO-Tg mice using transcriptomic profiling. The classic 1,25(OH)₂D₃ target genes in the proximal intestine [*S100g* (calbindin-D_{9k}) and *Trpv6*] were also expressed, and induced by 1,25(OH)₂D₃, in the distal intestine of the transgenic mice.

These changes in gene expression correlated to the increase in serum calcium in the VDRKO-Tg mice, suggesting that active transport is involved in the rescue of VDR dependent rickets by VDR expression in the distal intestine. In addition, $1,25(\text{OH})_2\text{D}_3$ treatment suppressed expression of genes controlling drug metabolism (e.g. *Cyp2c55*, *Cyp3a25*) and cell proliferation (e.g. *Anax13*) in the distal intestine of the VDRKO-Tg mice ([55]; 22nd Workshop on Vitamin D), suggesting that vitamin D has a broader impact on the intestine beyond the control of intestinal calcium absorption. Consistent with this hypothesis, one of the genes consistently induced by $1,25(\text{OH})_2\text{D}_3$ in the distal intestine of the Tg mice, as well as in both the proximal and distal intestine of $1,25(\text{OH})_2\text{D}_3$ treated vitamin D deficient mice, was *Slc30a10*. SLC30A10 is a manganese efflux transporter localized in the apical/luminal domain of the intestine, in liver and brain which protects against manganese (Mn) toxicity [56]. Studies using *Slc30a10* KO mice (from S. Mukhopadhyay, University of Texas at Austin), which have elevated Mn levels (56), indicate a marked decrease in intestinal vitamin D target genes *Trpv6* and *S100g* (>90%) in the KO mice ([55]; 22nd Workshop on Vitamin D). Since SLC30A10 was reported to use a Ca^{2+} gradient for active counter-ion exchange [57], these findings suggest that TRPV6, calbindin- D_{9k} and SLC30A10 may work together in Mn export. Further studies are needed to determine whether $1,25(\text{OH})_2\text{D}_3$ may have a role not only in maintaining calcium homeostasis but also in the cellular homeostasis of other divalent cations.

5. VDR and $1,25(\text{OH})_2\text{D}_3$ - target gene expression along the crypts-villus axis.

$1,25(\text{OH})_2\text{D}_3$ -mediated responses are a critical function for the differentiated absorptive epithelial cells that populate the small intestine villus. However, little is known about the effect of $1,25(\text{OH})_2\text{D}_3$ in crypts and whether classical intestinal responses to $1,25(\text{OH})_2\text{D}_3$ occur in the crypts has been a matter of debate. In initial studies we examined *Cyp24a1*, which encodes the enzyme 25-hydroxyvitamin D3 24-hydroxylase (CYP24A1), as one target of $1,25(\text{OH})_2\text{D}_3$. CYP24A1 is involved in the catabolism of $1,25(\text{OH})_2\text{D}_3$ and is present in all cells that contain VDR. It has been suggested that CYP24A1 not only regulates circulating $1,25(\text{OH})_2\text{D}_3$ but may also limit the levels of $1,25(\text{OH})_2\text{D}_3$ in cells and thus control the cellular response. CYP24A1 has been used to assess cell and tissue responsiveness to $1,25(\text{OH})_2\text{D}_3$. We analyzed *Cyp24a1* in initial studies to determine if $1,25(\text{OH})_2\text{D}_3$ induced responses as well as VDR occur in not only in villi but also in crypt enterocytes. We found that in the mouse, enterocytes in both the villus and the crypts express VDR and respond to $1,25(\text{OH})_2\text{D}_3$ by inducing expression of *Cyp24a1* (Fig. 1 A–C) ([55]; 22nd Vitamin D Workshop). To capture the full breadth of the vitamin D response in the intestine, we are now conducting transcript profiling on mouse crypt and villus preparations as well as on human enteroids with either a crypt-like phenotype (i.e. high proliferation, undifferentiated cells) or a villus-like phenotype (i.e. low proliferation, differentiated cells). Our preliminary RNA-seq analysis of human enteroids shows that *VDR* is present in both crypt-like or villus-like enteroids. We have also found that $1,25(\text{OH})_2\text{D}_3$ treatment can induce some vitamin D target genes in both the villus and crypts (i.e. *TRPV6*, *CYP24A1*, *SLC30A10*) while the induction of others is limited to one compartment or another (e.g. *S100G* is strongly induced only in villus-like cultures, Fig. 2 A–C). Thus, the $1,25(\text{OH})_2\text{D}_3$

effects on intestine are conserved in humans and are also more complex than one would presume based on data from whole tissue or mucosal scrapings.

6. Conclusion.

Intestinal effects of $1,25(\text{OH})_2\text{D}_3$ are more complex than the traditional model of three regulated stages (entry of calcium, transcellular movement of calcium and energy requiring extrusion of calcium). $1,25(\text{OH})_2\text{D}_3$ affects a complex network in both proximal and distal intestine which may involve overlapping and distinct effects for calcium transport as well as for maintaining intracellular calcium homeostasis and regulation of intestinal adhesion molecules. $1,25(\text{OH})_2\text{D}_3$ calcium independent effects include a possible role in xenobiotic metabolic processes, cellular proliferation and effects on the intestinal transport of other ions. $1,25(\text{OH})_2\text{D}_3$ mediated responses are localized not only in villi but also in crypts. Effects of $1,25(\text{OH})_2\text{D}_3$ in crypts include a possible role in stem cell development and on the regulation of the Wnt signaling pathway. Future studies identifying novel vitamin D targets in both villus and crypt and in proximal and distal intestine will provide new insight on mechanisms of control by $1,25(\text{OH})_2\text{D}_3$ of various aspects of intestinal biology.

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Highlights

- The central role of vitamin D is to increase calcium absorption from the intestine
- $1,25(\text{OH})_2\text{D}_3$ affects a complex network in both proximal and distal intestine
- Some intestinal effects of $1,25(\text{OH})_2\text{D}_3$ can be calcium independent
- $1,25(\text{OH})_2\text{D}_3$ mediated responses are localized not only in villi but also in crypts

Fig. 1.

VDR and 1,25(OH)₂D₃ induced *Cyp24a1* are present in epithelial cells in villi and crypts. **A.** Isolation of intestinal epithelial cells from crypts (left panel) and villi (right panel).

Duodenum from 3 month old mice was washed in cold phosphate buffered saline (PBS).

Minced duodenal tissue was incubated in 3 mM EDTA in PBS with gentle shaking. Crypt

epithelium was depleted of contaminating villi by passage through 70 μm filters. Villus

epithelium was trapped on the 70 μm filters. **B.** Western blot analysis of VDR protein in

intestinal epithelial cells from crypts and villi prepared from 3 month old VDR KO and WT

mice. Anti-VDR (D-6) from Santa Cruz Biotechnology was used to detect mouse VDR

(mVDR). VDR was recognized as a single band at approximately 48 kDa. Anti-β actin (also

from Santa Cruz Biotechnology) was used as a control. **C.** qRT-PCR analysis of *Cyp24a1* in

epithelial cells from villi and crypts from WT 3 month old C57BL/6 mice injected ip with

vehicle (0.1 ml; 9:1 mix of propylene glycol and ethanol) (WT + Veh) or 1,25(OH)₂D₃

(1ng/g body weight; 48, 24, and 6h prior to being euthanized). Relative quantitation of

Cyp24a1 was performed using real time qPCR (Taqman analysis) using Taqman Gene

Expression probes (Applied Biosystems). qPCR reactions were performed in triplicate and

normalized to GAPDH. The 2^{-C_t} method was used to calculate relative gene expression. Data represent the results of three separate experiments. ** $p < 0.01$ compared to WT + Veh.

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Fig. 2.

RNA-seq results reveal that villus and crypt-like human enteroids exhibit a differential response to 1,25(OH)₂D₃ treatment. **A.** Venn diagram indicating genes significantly upregulated at least 1.5 fold upon 1,25(OH)₂D₃ treatment in villus (differentiated) and crypt-like (undifferentiated) human enteroids and overlap of genes. The crypt and villus compartments both show upregulation of *TRPV6*, *CYP24A1* and *SLC30A10* upon 1,25(OH)₂D₃ treatment (100 nM for 24 h). The villus compartment shows sole upregulation of *S100G* upon 1,25(OH)₂D₃ treatment. **B, C.** Expression count of *CYP24A1* and *TRPV6* after 1,25(OH)₂D₃ treatment (lanes 2 and 4) increased in both crypt and villus-like compartments. Data is from enteroids from 5 patients (3 females and 2 males).