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MINIREVIEWS

Molecular aspects of intestinal calcium absorption

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Abstract

Intestinal Ca^{2+} absorption is a crucial physiological process for maintaining bone mineralization and Ca^{2+} homeostasis. It occurs through the transcellular and paracellular pathways. The first route comprises 3 steps: the entrance of Ca^{2+} across the brush border membranes (BBM) of enterocytes through epithelial Ca^{2+} channels TRPV6, TRPV5, and $Ca_v1.3$; Ca^{2+} movement from the BBM to the basolateral membranes by binding proteins with high Ca^{2+} affinity (such as CB_{9k}); and Ca^{2+} extrusion into the blood. Plasma membrane Ca^{2+} ATPase (PMCA1b) and sodium calcium exchanger (NCX1) are mainly involved in the exit of Ca^{2+} from enterocytes. A novel molecule, the 4.1R protein, seems to be a partner of PMCA1b, since both molecules co-localize and interact. The paracellular pathway consists of Ca^{2+} transport through transmembrane proteins of tight junction structures, such as claudins 2, 12, and 15. There is evidence of crosstalk between the transcellular and paracellular pathways in intestinal Ca^{2+} transport. When intestinal oxidative stress is triggered, there is a decrease in the expression of several molecules of both pathways that inhibit intestinal Ca^{2+} absorption. Normalization of redox status in the intestine with drugs such as quercetin, ursodeoxycholic acid, or melatonin return intestinal Ca^{2+} transport to control values. Calcitriol [$1,25(OH)_2D_3$] is the major controlling hormone of intestinal Ca^{2+} transport. It increases the gene and protein expression of most of the molecules involved in both pathways. PTH, thyroid hormones, estrogens, prolactin, growth hormone, and glucocorticoids apparently also regulate Ca^{2+} transport by direct action, indirect mechanism mediated by the increase of renal $1,25(OH)_2D_3$ production, or both. Different physiological conditions, such as growth, pregnancy, lactation, and aging, adjust intestinal Ca^{2+} absorption according to Ca^{2+} demands. Better knowledge of the molecular details of intestinal Ca^{2+} absorption could lead to the development of nutritional and medical strategies for optimizing the efficiency of intestinal Ca^{2+} absorption and preventing osteoporosis and other pathologies related to Ca^{2+} metabolism.

Key words: Intestinal Ca^{2+} absorption; Transcellular pathway; Paracellular route; $1,25(OH)_2D_3$; PTH; Prolactin; Estrogen; Lactation; Pregnancy; Aging

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Core tip: Intestinal Ca^{2+} absorption occurs through transcellular and paracellular pathways. Apparently, novel proteins, such as $Ca_V1.3$ and 4.1R, are involved in the Ca^{2+} transcellular pathway. Proteins involved in tight junction structures, such as claudins 2, 12, and 15, participate in the paracellular pathway. There is evidence of crosstalk between the transcellular and paracellular pathways. Better knowledge of the molecular details of intestinal Ca^{2+} absorption could lead to the development of nutritional and medical strategies for optimizing the efficiency of intestinal Ca^{2+} absorption and preventing osteoporosis and other pathologies related to Ca^{2+} metabolism.

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INTRODUCTION

The Ca^{2+} ion plays an important role during human life. The accumulation of Ca^{2+} in the first decades of life allows for the achievement of optimal peak bone mass and, later in life, the maintenance of bone mass. It is a key constituent of many essential physiological processes, from intracellular signals to the mineralization of bone[1]. It is well known that Ca^{2+} is involved in nerve impulse transmission, muscle contraction, blood coagulation, secretory activity, cell death, cell differentiation, immune response, and enzyme activation[2]. The dysregulation of Ca^{2+} homeostasis is associated with bone disorders, metabolic diseases[3], and an increase in the risk of epithelial cancers[4].

The intestine, kidney, bone, and parathyroid glands work together to maintain serum Ca^{2+} within a narrow range. Intestinal Ca^{2+} absorption is a crucial process for the maintenance of Ca^{2+} balance and bone health. It occurs through two main mechanisms: transcellular, metabolically driven transport, and a passive non-saturable route called the paracellular pathway[5]. Both pathways are regulated by hormones, nutrients, and other factors. There has been much research work dedicated to ascertaining the regulation mechanisms of these factors due to their high relevance in the prevention of osteoporosis and other pathologies related to Ca^{2+} metabolism.

The transcellular pathway implicates Ca^{2+} movement from the mucosal-to-serosal side of the intestinal barrier that occurs against a concentration gradient. It is an active saturable process that predominates in the duodenum and jejunum, and is regulated by nutritional and physiological factors, mainly vitamin D. The paracellular mechanism is a non-saturable and passive transport that occurs across the majority of the intestine and is a linear function of luminal Ca^{2+} concentration[6]. Recently, it has been demonstrated that Ca^{2+} transport through the paracellular shunt is also significantly regulated[1].

The total Ca^{2+} absorbed depends on the amount of Ca^{2+} consumed, the sojourn time in the various segments of the small and large intestine, and the amount of soluble Ca^{2+} available for absorption, which is mainly determined by the pH in each segment. The acidic environment of the stomach dissolves calcium salts into Ca^{2+} ions. However, the pH of stomach is less relevant than that of the other segments, as Ca^{2+} is absorbed in the small and large intestine. The average pH is 7.3 in the small intestine and 6.6 in the colon. When pH increases, Ca solubility decreases. Nevertheless, the duodenum seems to be the site with the maximum solubility of Ca, as the average pH is 6.0, which is the lowest in the entire gut[7].

TRANSCELLULAR PATHWAY

The transcellular pathway of intestinal Ca^{2+} absorption comprises 3 steps: the entrance of Ca^{2+} across the brush border membranes (BBM) of enterocytes through epithelial Ca^{2+} channels; Ca^{2+} movement from the BBM to the basolateral membranes (BLM) by binding to proteins with high Ca^{2+} affinity [(calbindins (CB)]; and Ca^{2+} extrusion into the blood *via* plasma membrane Ca^{2+}-ATPase (PMCA1b/Ca^{2+} pump) and the Na^+/Ca^{2+} exchanger (NCX1)[5].

Epithelial Ca^{2+} channels

Two epithelial Ca^{2+} channels seem to be involved with Ca^{2+} entry to enterocytes: the epithelial Ca^{2+} channel transient receptor potential vanilloid 6 (TRPV6; previously named ECaC2 and CaT1) and TRPV5 (previously named ECaC1 and CaT2). Both molecules are co-expressed in the human kidney and intestine, but TRPV6 is highly expressed in the intestine and TRPV5 is the major isoform in the kidney. High levels of TRPV6 have been detected in the duodenum and colon of humans, rats, and mice[8-10]. Although TRPV6 is one of the key players in human intestinal Ca^{2+} absorption, its precise role needs to be investigated. In $TRPV6^{-/-}$ mice, a considerable amount of Ca^{2+} transport still occurs, which suggests that some other channels or molecules contribute significantly to intestinal Ca^{2+} absorption[11]. TRPV6 and TRPV5 are also present in other organs, such as the pancreas, prostate, mammary glands, sweat, and salivary glands[11]. Both channels can be combined to form heterotetrameric channel complexes with different properties[12]. They originate from two genes juxtaposed on human chromosome 7q35, have 75% homology, but differ in the N and C terminal tails. They are regulated by calcitriol, estrogen, and dietary Ca^{2+}. However, the inactivation of both by intracellular Ca^{2+} shows

different kinetics, and the response to ruthenium red is also different. Ruthenium red is a potent blocker of epithelial Ca^{2+} channel activity. However, TRPV6 has a 100-fold lower affinity for ruthenium red (IC_{50} 9 ± 1 μm) than TRPV5 (IC_{50} 121 ± 13 nm)[12]. *In vitro* studies demonstrate that a negatively charged amino acid (D) within the putative pore region of mouse TRPV6 (position 541 in mice; position 542 in humans) is critical for Ca^{2+} permeation of the channel. Woudenberg-Vrenken *et al*[13] analyzed the role of TRPV6 in transepithelial Ca^{2+} transport *in vivo* by using a $TRPV6^{D541A/D541A}$ knock-in mouse model. $TRPV6^{D541A/D541A}$ mice showed significantly impaired intestinal Ca^{2+} uptake compared with wild-type mice, and duodenal TRPV5 expression was increased, but insufficient to correct the diminished Ca^{2+} absorption. Since intestinal Ca^{2+} absorption was not totally abolished in the $TRPV6^{D541A/D541A}$ mice, the authors suggest that other transport mechanisms, either paracellular or an as yet unidentified transcellular transport mechanism, were functioning.

$Ca_v1.3$ is an L-type channel, located in the apical membrane, capable of active, transcellular Ca^{2+} absorption in the intestine. It has been hypothesized that TRPV6 and $Ca_v1.3$ have complementary roles in Ca^{2+} entry. TRPV6 plays a dominant role under the polarizing conditions between meals. Overnight or during starvation, the BBM repolarize and the intestine gradually atrophies, so the Ca^{2+} lost into the lumen by desquamation must be recovered. TRPV6 is activated by apical membrane repolarization and upregulated by vitamin D to avoid massive loss of Ca^{2+} from the body. In contrast, $Ca_v1.3$ plays a dominant role under depolarizing conditions, such as during digestion, mainly when diet and Ca^{2+} are plentiful. Glucose, amino acids, and peptides may activate $Ca_v1.3$ and inhibit TRPV6. The general distribution of TRPV6 and $Ca_v1.3$ is in line with the aforementioned hypothesis. TRPV6 levels are higher in the duodenum, which has a polarizing environment and decreases through the jejunum to the ileum. In contrast, $Ca_v1.3$ levels are low in the duodenum, but high from the proximal jejunum to the mid-ileum. The interplay of TRPV6 and $Ca_v1.3$ maintains the tight control of free Ca^{2+} concentration in the extracellular space at any time of day. TRPV6 and $Ca_v1.3$ would have independent and complementary actions through activation by repolarization or depolarization between digestive periods or during digestion, respectively[14].

TRPV6 transcripts were detected in the duodenum, but not in the ileum of human intestinal biopsies. The duodenal expression of TRPV6 in men was vitamin D dependent, whereas in elderly women TRPV6 and vitamin D receptor (VDR) expressions were low and not vitamin D dependent. This might explain, at least in part, the lower intestinal Ca^{2+} absorption in elderly post-menopausal women[15]. In mice, the basal levels of TRPV6 protein in the duodenum, ileum, and colon have been found in the rank order of duodenum > colon (72% of duodenum) > ileum (25% of duodenum)[16].

Calbindins

Traditionally, calbindins (CB) were proteins considered to be responsible for carrying Ca^{2+} from the apical side of the enterocyte to the BLM of the cell. CB_{9k} (human gene symbol: *S100G*) is present in the intestine of mammals and CB_{28k} (M_r ≈ 29 kDa; human gene symbol: *CALB1*) in the intestine of avian species[17]. CB_{9k} is the smallest protein with four alpha-helical regions, which form an EF-hand pair consisting of a canonical and non-canonical/pseudo EF-hand domain, joined by a linker region. Two Ca^{2+} ions bind the EF-hand domains with positive cooperativity[18]. CB_{28k} has six EF-hand domains, but only the four medium/high affinity sites are considered Ca^{2+}-specific[19].

CBs not only carry Ca^{2+} from the apical side to the BLM of enterocytes, but also buffer Ca^{2+} maintaining intracellular Ca^{2+} concentrations below 10^{-7} mol/L, which prevents premature cell death by apoptosis. Excess Ca^{2+} resulting from a downregulation of CBs may trigger apoptosis in the epithelial cells[20]. It is known that a high concentration of free Ca^{2+} provokes apoptosis in many different cell types. CB_{28k} is able to inhibit apoptosis in osteoblastic cells[21] and in germ cells from Robertsonian mice[22,23]. In the kidney, CB_{28k} acts as a dynamic buffer which regulates Ca^{2+} concentration in the vicinity to the TRPV5 pore by direct association with the channel[24]. The possibility that these mechanisms occur in the intestine and other tissues with important fluctuations of intracellular Ca^{2+} should be explored.

The regulation of human intestinal CB_{9k} is not completely elucidated. CB_{9k} is actively expressed in enterocytes, which are the predominant cells of the duodenal mucosa, and its expression decreases along the gastrointestinal tract until finally reaching undetectable levels in the distal ileum and large intestine[25]. It is quite intriguing that intestinal CB_{9k} expression increases with age in the bulb and 2nd portion of the duodenum, whereas the plasma Ca^{2+} levels decrease[26]. In rodents, CB_{9k} is regulated at the transcriptional and post-transcriptional levels by 1,25-dihydroxyvitamin D3 [$1,25(OH)_2D_3$ (calcitriol)], with the hormonal form derived from vitamin D[27-29]. It has been observed in mice that TRPV6 and CB_{9k} are similarly regulated. Both proteins are induced at weaning or under low Ca diet and after $1,25(OH)_2D_3$ administration. It is important to note that the induction of these proteins occur before the peak of intestinal Ca^{2+} absorption[30]. Christakos *et al*[31] have suggested that CB_{9k} and TRPV6 are associated, with the function of CB_{9k} being to facilitate TRPV6-mediated Ca^{2+} influx by preventing Ca^{2+} channel inactivation, but this requires further evidence to be proven.

Hwang *et al*[32] have demonstrated that an ablation

of CB_{9k} alters the expression of paracellular tight junction (TJ) genes. The compensatory expression of paracellular TJ genes in the duodenum was associated with transcellular CB_{9k}, but not CB_{28k}. This crosstalk between the transcellular and paracellular pathways might partially explain the variety of gut responses to the absorption of Ca^{2+} under different pathophysiological conditions.

Ca^{2+} pump and Na^+/Ca^{2+} exchanger

Ca^{2+} extrusion from enterocytes is performed by two proteins: PMCA1 and NCX1. PMCA1 was first detected in erythrocyte membranes and found to have a high affinity for Ca^{2+}[33]. There are four PMCA isoforms (PMCA1 to 4) that are in turn divided into several subtypes by alternative splicing. In mammals, four different genes encode PMCA, which in humans are located on four different chromosomes. For the four genes, two splice sites have been characterized, which are positioned either close to or within the regulatory regions of the pump, giving rise to many spliced isoforms[34]. PMCA1 is known as the housekeeping isoform because its mRNA is found in all tissues, but this concept is now questionable due to several factors involved in its regulation[35]. The predominant form in the intestine is the isoform PMCA1b, the expression and activity of which is higher in enterocytes from the villus tip in comparison with those from the villus crypt[36]. This finding agrees with the idea that mature enterocytes have the greatest capacity for transcellular Ca^{2+} movement. In chick intestine, vitamin D deficiency decreases the expression and activity of PMCA1b, which can be partially reversed by a single large dose of cholecalciferol[37].

PMCAs can be activated by Ca^{2+}/calmodulin, acidic phospholipids, and serine/threonine phosphorylation, and can interact with numerous molecules[38]. The novel protein 4.1R seems to be a partner of PMCA1b, which could have a crucial role in the transcellular Ca^{2+} pathway. The protein 4.1R was first identified in the erythrocyte membrane skeleton, and is expressed in the epithelia of the intestine and other epithelia. So far, its physiological function is not well known. Liu *et al*[39] found that 4.1R co-localizes with PMCA1b. They also found that 4.1R KO mice exhibit impairment in intestinal Ca^{2+} absorption and decreased expression of PMCA1b in enterocytes. The association between PMCA1b and 4.1R involves the membrane-binding domain of 4.1R, as well as the second intracellular loop and C-terminus of PMCA1b. The finding that the protein 4.1R binds to PMCA1b suggests that protein 4.1R may regulate the function of PMCA1b and, consequently, intestinal Ca^{2+} absorption.

PMCA1b is the main protein involved in the exit of Ca^{2+} from enterocytes[40]. In contrast, NCX1 is only responsible for about 20% of Ca^{2+} extrusion from the intestine to plasma[41]. Because of that, this exchanger has received little attention, with some reviews ignoring it as another molecule involved in the exit of Ca^{2+} from the intestine. In contrast, NCX1 is critical for Ca^2 regulation in cardiac muscle, vascular smooth muscle, and nerve fibers, and most of the literature about NCX1 refers to these organs[42-44]. NCX electrogenically exchanges Na^+ and Ca^{2+} across the plasma membrane, depending on membrane potential and ion gradients[45]. This exchanger has a stoichiometry of 3 Na^+:1 Ca^{2+}, and can operate in either a forward mode (Ca^{2+} extrusion) or in a reversed mode (Ca^{2+} entry), which depends on the Na^+ and Ca^{2+} gradients and the potential across the plasma membrane[46,47]. The expression and activity of NCX1 are quite similar between mature and immature enterocytes from chick duodenum, but are slightly higher in the villus tip cells[36].

PARACELLULAR PATHWAY

The movement of molecules and ions through this pathway is regulated by the TJ, which are specialized membrane domains mostly positioned in the apical region of enterocytes. TJ are intercellular structures where plasma membranes of adjacent cells have very close contact. These junctions are composed of transmembrane proteins, cytoskeleton components, and cytoplasmic plaques[48]. The transmembrane proteins of TJ structures are synthesized in adjacent cells and include occludin (Ocln) and claudins (Cldns). These proteins close intercellular junctions and restrict the free movement of materials through the paracellular space. Cldn 2, 12, and 15 are responsible for transporting Ca^{2+} in the intestine[49,50]. Cldn 1 and Cldn 5 have clear sealing functions that might also affect Ca^{2+} transport, as they influence general paracellular permeability[51,52]. The involvement of Ocln in intestinal Ca^{2+} absorption remains to be determined. Ocln is a tetraspan transmembrane protein, but its precise function is not well established[32]. Cytoplasmic plaques, such as zona occludens (ZO) proteins, contain a binding domain for transmembrane proteins[53]. ZO-1, a cytoplasmic protein with the ability to bind with both occludin and claudins[54], is associated with the structure and formation of the TJ, and possibly with paracellular ion transport[55]. Although it is not clear whether ZO-1 has a physiological role in intestinal Ca^{2+} transport, the increase in ZO-1 expression in the intestine of rats with 21-d chronic metabolic acidosis suggests that this protein might be implicated in intestinal Ca^{2+} absorption[56].

Ca^{2+} transport through the TJ is a passive process which depends on the concentration and electric gradient across the epithelium. The process is non-saturable transport that predominates in the jejunum and ileum when Ca^{2+} intake is adequate or high[57]. This route becomes important when Ca^{2+} intake is high, because the sojourn time in the intestine is short and there is a downregulation of proteins involved in

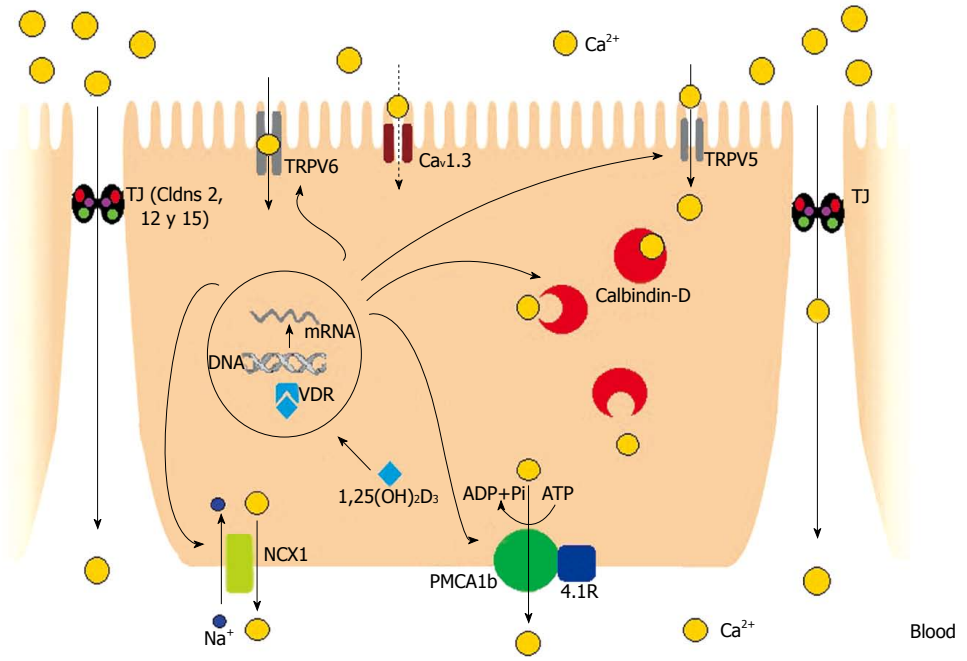

Figure 1 Schematic representation of intestinal Ca^{2+} absorption. ADP: Adenosine monophosphate; ATP: Adenosine triphosphate; Cldns: Claudins; NCX1: Intestinal Na^{+}/Ca^{2+} exchanger; PMCA1b: Plasma membrane Ca^{2+}- ATPase 1b; TJ: Tight junctions; TRPV6: Transient receptor potential vanilloid 6; TRPV5: Transient receptor potential vanilloid 5; VDR: Vitamin D receptor; $1,25(OH)_2D_3$: Calcitriol.

the transcellular pathway[58] (Figure 1).

As previously mentioned, it seems that there is crosstalk between the transcellular and paracellular pathways involved in intestinal Ca^{2+} absorption. The expression of most TJ genes in the duodenum was significantly increased in CB_{9k} KO mice compared to wild-type animals under a normal diet. A deficiency in dietary Ca^{2+} or vitamin D decreased TJ gene expression in CB_{9k} KO mice. The data indicate that the expression of paracellular TJ genes is regulated by transcellular CB proteins, which suggests that active and passive Ca^{2+} transport pathways may function cooperatively[32]. More research work is necessary to clarify the network of transcellular and paracellular pathways for intestinal Ca^{2+} absorption.

Intestinal redox status is critical for both transcellular and paracellular pathways of intestinal Ca^{2+} absorption. Xiao *et al*[59] have demonstrated that a duodenal oxidation state induced by a high fat diet could significantly downregulate the expression of CB_{9k}, PMCA1b, and NCX, as well as inhibiting intestinal calcium absorption. We have recently demonstrated that type I diabetes mellitus transiently inhibits intestinal Ca^{2+} absorption. Inhibition is accompanied by oxidative stress, which alters the gene and protein expression of molecules involved in the transcellular and paracellular pathways. When insulin is administered, the duodenal redox state returns to the control values, while intestinal Ca^{2+} absorption normalizes[60]. Similarly, oxidant drugs such as menadione, DL-buthionine-S,R-sulfoximine, and sodium deoxycholate decrease intestinal glutathione content, thereby affecting mainly the gene and protein expression of molecules involved in the transcellular pathway of intestinal Ca^{2+} absorption. Normalization of redox status with drugs such as quercetin, ursodeoxycholic acid, and melatonin returns intestinal Ca^{2+} transport to control values[61-65].

Molecular mechanisms of $1,25(OH)_2D_3$-mediated intestinal Ca^{2+} absorption

$1,25\ (OH)_2D_3$ is the major controlling hormone of intestinal Ca^{2+} absorption. It causes changes in the structure and function of enterocytes[66,37], which enhance Ca^{2+} transport across the intestine. The action of calcitriol is mediated by genomic and non-genomic mechanisms after binding VDR. This receptor, located mainly in the nucleus, is a transcription factor that mediates the cellular effects of vitamin D by binding the vitamin D response elements of target genes[67]. The critical role of VDR and its ligand in intestinal Ca^{2+} absorption was confirmed in VDR KO mice in the third week of life. At birth, VDR KO mice are indistinguishable from their normal littermates. Alterations in growth and mineral ion homeostasis begin later[68], which is consistent with the observation that intestinal Ca^{2+} absorption is vitamin D independent in rodents in the first weeks of life[69]. Ten-week-old VDR KO mice showed a dramatic decrease in duodenal Ca^{2+} absorption, which was associated with impaired expression of TRPV6, TRPV5, and CB_{9k}[70]. Beyond VDR function in intestinal Ca^{2+} absorption, a recent proteomic approach has revealed that VDR is also an important factor for controlling cell proliferation, migration, and stress response in the small intestine[71].

Most studies related to the effect of calcitriol on intestinal Ca^{2+} absorption have been focused on the transcellular Ca^{2+} pathway. All molecules presumably involved in this route are increased by calcitriol in experimental animals, and even in humans[72-75].

Cyp24a1 is a major VDR-responsive gene that metabolizes $1,25(OH)_2D_3$ into 1,24,25-trihydroxyvitamin D_3 and $25(OH)D_3$ into 24,25-dihydroxyvitamin D_3[76], while *Cyp27b1* is the gene involved in the synthesis of $1,25(OH)_2D_3$, which is mainly located in the kidney[77], but is also expressed in other tissues, such as the intestine[78] and parathyroid gland[79]. Both genes are regulated by $1,25(OH)_2D_3$ levels. Their temporal profiles and those from other VDR responsive genes were analyzed in the intestine and other tissues of mice after single and multiple dosing of $1,25(OH)_2D_3$. Due to the lipophilic nature of the compound, a rapid distribution of $1,25(OH)_2D_3$ into tissues was observed, regardless of variation in VDR abundance in different tissues. The maximal induction of VDR target genes such as TRPV6 and *Cyp24a1* mRNA expression in the intestine were similar after single *vs* multiple dosing, with a peak between 3 and 9 h post-injection, whereas the peak of $1,25(OH)_2D_3$ concentration in the ileum occurred at 0.5-1 h. This lag time was the result of the time required for translocation of the VDR into the nucleus in order to heterodimerize with the RXR to initiate the transcription. An increase in ileal VDR levels was also observed, as well as an attenuation of serum PTH and a decrease in renal *Cyp27b1* expression after a time delay in VDR activation. The data revealed that exogenous $1,25(OH)_2D_3$ enters the intestine, rapidly equilibrates, and then VDR target genes respond quickly. Consequently, plasma Ca^{2+} levels increase as a result of enhanced intestinal Ca^{2+} absorption[16].

$1,25(OH)_2D_3$-enhanced Ca^{2+} transport in mice was reported to be inhibited by fibroblast growth factor-23 (FGF-23), as well as Ca^{2+} transport in colon cancer Caco-2 cells. FGF-23 produced an abolishment of enhanced transcellular active Ca^{2+} fluxes and a modest downregulation of the paracellular Ca^{2+} route[80].

VDR null mice adapt to pregnancy by the upregulation of duodenal TRPV6 and intestinal Ca^{2+} absorption. These mice lactate normally and fully restore bone mineral content after weaning. Therefore, VDR seems not to be required for skeletal adaptation during pregnancy, lactation, and after weaning[81]. In the elderly, there is a decrease in intestinal Ca^{2+} absorption, and thus higher Ca^{2+} intake is needed. Increasing Ca^{2+} intake *via* dairy products and Ca^{2+}-fortified food is a much better option than supplements. It has been estimated a 30% reduction in fractures for elderly individuals is possible by using the simple and inexpensive strategy of a daily vitamin D intake of 800 IU, together with a total Ca^{2+} intake of 1000 mg/d[82].

The paracellular pathway of intestinal Ca^{2+} absorption has been demonstrated to also be increased by $1,25(OH)_2D_3$, predominantly in the jejunum and ileum[83]. It has been found that $1,25(OH)_2D_3$ significantly enhanced Cldn- 2 and Cldn-12 mRNA levels in colon cancer Caco-2 cells. The mRNA and protein levels for these proteins were lower at 12 wk in the jejunum of VDR KO mice in comparison with wild-type mice, and siRNA against these Cldns diminished Ca^{2+} permeability in Caco-2 cells[84]. Cadherin-17 and aquaporin-8 have been reported to be downregulated by $1,25(OH)_2D_3$ in the intestine[85,86]. Cadherin-17 is involved in cell-to-cell contact, and its decrease might in turn increase intestinal permeability. A decrease in channel aquaporin-8 might influence TJ selectivity towards cations. The data indicate that Ca^{2+} movement through TJ is regulated and supports the regulation of the paracellular Ca^{2+} transport route by $1,25(OH)_2D_3$[31].

Evidence for the regulation of Ca^{2+} absorption by other hormones

PTH acts indirectly on intestinal Ca^{2+} absorption by the stimulation of renal CYP27B1 and, therefore, increases $1,25(OH)_2D_3$-dependent Ca^{2+} absorption. A direct effect of PTH on intestinal Ca^{2+} absorption has not been demonstrated, but some direct effects of PTH on Ca^{2+} uptake by enterocytes from rat duodenum were reported. PTH/PTHrP receptors have been localized in intestinal epithelial cells along the villus[87]. It has been suggested that an *in vivo* model would need to be generated with targeted deletion of intestinal PTH receptor 1 in order to test if PTH directly affects intestinal Ca^{2+} absorption[88].

With regard to thyroid hormones, it has been reported that they produce a cooperative effect with vitamin D for intestinal Ca^{2+} transport. Apparently, thyroid hormones increase the genomic action of $1,25(OH)_2D_3$ in the intestine[89]. Kumar *et al*[90] demonstrated that hyperthyroid rats show higher Ca^{2+} uptake and Ca^{2+} efflux from enterocytes than hypothyroid rats. They have also observed that NCX1 activity was highly increased by thyroid hormones, presumably *via* the cAMP-mediated pathway. Orihuela[91] has analyzed the effect of different statuses of thyroid hormones on the inhibitory effect of aluminum (Al) on intestinal Ca^{2+} absorption by using a rodent model. Mucosa-to-serosa Ca^{2+} fluxes in Al-exposed rats declined as thyroid hormones levels increased, thereby showing a trend opposite to that seen in non-Al-treated control rats.

Growth hormone (GH) has a major role in linear bone growth and bone Ca^{2+} deposition during childhood and adolescence. GH has proliferative effects upon the intestinal epithelium[92], and can also stimulate intestinal Ca^{2+} absorption, which would occur indirectly by increasing serum $1,25(OH)_2D_3$ concentration[93].

However, it has also been shown that GH treatment increases intestinal Ca^{2+} absorption and duodenal CB_{9k} levels in aged rats without increasing serum $1,25(OH)_2D_3$ levels[94]. In adult men, Ca^{2+} absorption has been shown to be positively correlated with IGF-1, and age-related declines in IGF-1 have a negative

impact on Ca^{2+} absorption that could not be explained by a decrease in serum $1,25(OH)_2D_3$[95].

Most estrogen studies related to intestinal Ca^{2+} absorption were performed in ovariectomized (OVX) animals. This ablation significantly decreases endogenous estrogen, but not totally, since adrenal androgens can be aromatized to estrogen[96]. An estradiol replacement in OVX rats has been reported to increase intestinal Ca^{2+} absorption without stimulation of circulating $1,25(OH)_2D_3$ levels[97]. van Abel *et al*[98] found increased duodenal gene expression of TRPV5, TRPV6, CB_{9k}, and PMCA1b in OVX rats treated with estradiol. They used *Cyp27b1* KO mice to analyze the calcitriol dependency of the stimulatory effects of estradiol on intestinal Ca^{2+} absorption, and found that estradiol treatment increased mRNA levels of duodenal TRPV6. Cell culture studies suggest that estrogen corrects the decline in the efficiency of intestinal Ca^{2+} absorption at the onset of menopause[99], but the mechanisms that underlie this effect remain unknown. Estrogen receptor alpha ($ER\alpha$) KO mice showed a decrease in duodenal TRPV6 mRNA expression, while CB_{9k}, PMCA1b, and VDR levels were not modified. Therefore, it seems that the genomic effects of estrogen on mice are mainly mediated by $ER\alpha$[100]. In addition to estrogen, prolactin, a hormone that is elevated during pregnancy and lactation, has been shown to stimulate active intestinal Ca^{2+} transport in vitamin D deficient rats[101]. A direct effect of prolactin on active duodenal Ca^{2+} transport was shown[102]. Some data indicate that prolactin can regulate intestinal TRPV6 and cooperate with $1,25(OH)_2D_3$ in regulating TRPV6 and CB_{9k}. Prolactin also has a direct effect on the transcription of the *Cyp27b1* gene, thus enhancing CYP27B1 protein expression and increasing levels of $1,25(OH)_2D_3$ during lactation when there is an increased Ca^{2+} requirement for the neonate[103]. It has been suggested that prolactin also has an effect on the paracellular pathway of intestinal Ca^{2+} absorption through an upregulation of Cldn 15[104]. Some authors indicate that there is enough evidence that prolactin could be considered the cardinal calciotropic hormone in pregnancy and lactation[105].

Although reduced intestinal Ca^{2+} absorption seems to be part of the pathogenesis of glucocorticoid-induced osteoporosis[106], the mechanisms triggered by GCs in the intestine remain unclear. Short-term GC treatment in young animals does not alter the expression of genes involved in intestinal Ca^{2+} absorption, such as TRPV6, CB_{9k} and PMCA1b[107], but sustained dexamethasone suppresses mouse duodenal CB_{9k} expression[108]. Kim *et al*[109] found that gene regulation in the intestine by dexamethasone is complex in mice. It provokes an increase of duodenal TRPV6, CB_{9k}, and PMCA1b 24 h after administration, which was followed by a decrease in a 5-d treatment. Ten days of prednisolone treatment decreases rat intestinal Ca^{2+} absorption through a diminished expression of the active Ca^{2+} transporters, which is independent of $1,25(OH)_2D_3$[110] (Figure 2).

Intestinal calcium absorption under different physiological conditions

Intestinal Ca^{2+} absorption changes according to the physiological conditions of individuals. When needs are high and/or dietary Ca^{2+} is low, intestinal Ca^{2+} absorption becomes more efficient. Growth, pregnancy, lactation, dietary Ca^{2+} deficiency, and high physical activity enhance the Ca^{2+} demands that promote intestinal Ca^{2+} absorption. During pregnancy, Ca^{2+} absorption is higher than before conception or after delivery. The enhancement occurs in early-to-mid pregnancy, and precedes the increased Ca^{2+} demand from the fetus for skeletal growth. This alteration in Ca^{2+} absorption during pregnancy may be due to increased serum calcitriol, with little alteration in serum PTH or calcitonin[111].

The maternal adaptation for the enhancement of intestinal Ca^{2+} absorption in pregnancy and lactation is very important for fetal development and lactogenesis. Intestinal Ca^{2+} absorption in pregnant adolescents has been shown to be higher in the third trimester of pregnancy than in the early postpartum period[112].

Vitamin D seems to play an important role during pregnancy. Yamagishi *et al*[113] found in pregnant rats that vitamin D deficiency produces severe hypocalcemia due to reduced intestinal Ca^{2+} absorption and elevated fetal demand for cation. It has been shown in mice that serum calcitriol was enhanced five-fold during pregnancy, whereas vitamin D binding protein levels were unchanged. A 30-fold higher expression of *Cyp27b1* in maternal kidneys *vs* placenta suggests that the increase in calcitriol comes from the kidneys. Apparently, PTH is not required to upregulate *Cyp27b1* expression during pregnancy[114]. A custom-designed cDNA microarray validated by quantitative real time PCR has found in rats that several duodenal transporters, such as TRPV6, are upregulated during pregnancy[115]. In addition, when the plasma estradiol levels are increased in pregnancy, intestinal CB_{9k} gene expression is concomitantly enhanced, which suggests that the CB_{9k} gene is involved in the compensatory induction of other Ca^{2+} transporter genes in duodenal epithelial cells[116]. Zhu *et al*[117] have found that both PMCA1 and CB mRNA levels were increased 2- to 3-fold in rats at 21 d of gestation. These levels of PMCA1 and CB mRNA remained elevated at 7 d of lactation.

Recently, in an animal model of preeclampsia, disturbance of Ca^{2+} metabolism in the placenta, intestine, and kidney has been observed. A decrease in Ca^{2+} transporting genes (TRPV5, TPRV6, PMCA1, and CB_{9k}) has been detected in all these organs. In the duodenum, there was a slight recovery after calcium supplementation, whereas, in the kidney, these alterations were reverted to the control levels by the supplements[118].

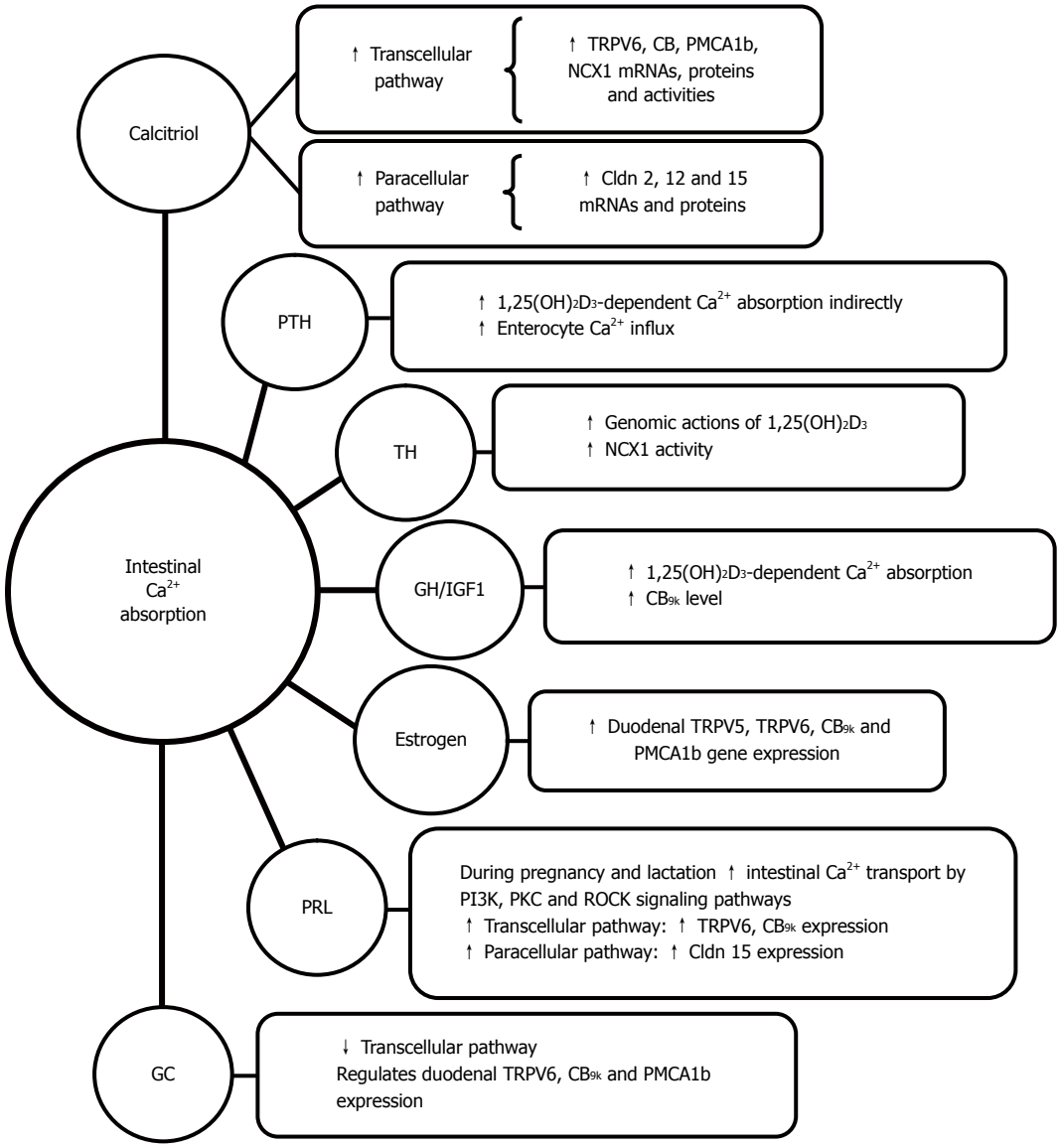

Figure 2 Hormonal regulation of intestinal calcium absorption. CB: Calbindin; Cldn: Claudin; GC: Glucocorticoids; GH: Growth hormone; IGF1: Insulin-like growth factor 1; NCX1: Intestinal Na^+/Ca^{2+} exchanger; PI3K: Phosphoinositide 3-kinase C; PKC: Protein kinase C; PMCA1b: Plasma membrane Ca^{2+}- ATPase 1b; PRL: Prolactin; PTH: Parathyroid hormone; ROCK: RhoA-associated coiled-coil-forming kinase; TH: Thyroid hormones; TRPV6: Transient receptor potential vanilloid 6.

The mechanisms by which intestinal Ca^{2+} absorption is induced in lactation are not quite clear. One possible adaptive mechanism includes hypertrophy and hyperplasia of the intestinal villi, as shown in lactating mammals such as rodents, pigs, and sheep[119,120]. Wongdee *et al*[121] have also demonstrated that intestinal hypertrophy in lactating rats is associated with an upregulation of Cldn 15 protein expression. It has been shown in rats that the suckling-induced transient prolactin surge increases intestinal Ca^{2+} transport during lactation, an effect that occurs through signaling pathways involving phosphoinositide 3-kinase C (PI3K), as well as two serine/threonine kinases, such as protein kinase C (PKC) and RhoA-associated coiled-coil-forming kinase (ROCK)[122].

In newborn rats, intestinal Ca^{2+} absorption is largely passive non-saturable, and not dependent on calcitriol[123]. This lack of responsiveness to calcitriol at earlier stages is explained by the undetectable levels of VDR within enterocytes at 7 and 14 d after birth[69]. Human data indicate that, in neonates, intestinal absorption is initially a passive process, which is favored by milk lactose. Later, it becomes a calcitriol dependent active process, but the hormone's role can be bypassed by high dietary Ca^{2+} content or parenteral Ca^{2+} administration[124].

Ca^{2+} supplied in human milk during infancy is primarily derived from maternal bone, which is rapidly replenished during and after weaning[125]. The optimization of Ca^{2+} intake is crucial in adolescents to maximize calcium retention, acquire a good peak of bone mass, and prevent osteoporosis later in life. At early puberty, there is an association between an increase in both Ca^{2+} absorption and bone Ca^{2+} deposition. In girls, bone

Ca^{2+} deposition reaches a maximum shortly before menarche, with the deposition rate being approximately five times that of adulthood. After menarche, bone Ca^{2+} deposition, as well as intestinal Ca^{2+} absorption, gradually declines[125]. In boys aged 11-14 years on their usual diets, it has been shown that adolescents absorbed 31% of their dietary Ca^{2+} intake and retained 20% of their total Ca^{2+} intake, but their dietary Ca^{2+} intake failed to meet recommended values[126]. During puberty, polymorphisms of the Fok I site in the VDR gene have been significantly associated with Ca^{2+} absorption and bone mineral density[127].

Aging has been associated with lower intestinal Ca^{2+} absorption[128], with an additional decrease at the time of menopause that is reversible with estrogen therapy[129]. This decrease might occur by a declination in serum calcitriol levels and a resistance to the actions of calcitriol in the intestine[130]. It has been reported that low levels of VDR in mouse heterozygotes for the VDR gene KO produce a resistance of intestinal Ca^{2+} absorption to $1,25(OH)_2D_3$. This resistance seems to be generated by the low translation of CB_{9k}, which is mediated by binding VDR with the ligand[131]. A *post hoc* analysis of dual isotope studies in post-menopausal women has identified associations of several factors with intestinal Ca^{2+} absorption. The data indicate that age, $1,25(OH)_2D_3$, and dietary calcium and fat are associated with Ca^{2+} absorption, whereas serum 25(OH)D levels are not. The authors claim that this study has unique findings, as the dietary intake of kilocalories, carbohydrates, and potassium are also associated with intestinal Ca^{2+} absorption, suggesting that beyond the traditional focus on Ca^{2+} and vitamin D, some other factors also influence intestinal Ca^{2+} absorption in post-menopausal women[129].

Concluding remarks

Since Ca^{2+} ions are involved in most physiological processes, it becomes important to know the molecular details of intestinal absorption of cation, as the intestine is the only entrance for Ca^{2+} to the organism. Absorption occurs through two different pathways, transcellular and paracellular, which apparently interact with each other depending on physiological conditions. Calcitriol [$1,25(OH)_2D_3$] is the major regulating hormone, which clearly operates through VDR signaling, but the exact mechanism by which Ca^{2+} moves from the lumen to the serosa remains elusive. The role of other hormones, such as PTH, GC, GH, estrogens, thyroid hormones, and prolactin, is under investigation due to controversial data. Some of them act indirectly through the regulation of renal $1,25(OH)_2D_3$ production, others by direct action, and still others by both methods. Different physiological conditions, such as growth, pregnancy, lactation, and aging, significantly alter intestinal Ca^{2+} absorption, according to Ca^{2+} requirements. Further research should be carried out to improve the current knowledge concerning the regulation of intestinal Ca^{2+} absorption in order to develop nutritional or medical strategies to optimize the efficiency of intestinal Ca^{2+} absorption and prevent osteoporosis and other pathologies related to Ca^{2+} metabolism.

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