Minireview on Regulation of Intestinal Calcium Absorption

Emphasis on Molecular Mechanisms of Transcellular Pathway

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Key Words

Intestinal absorption • Ca^{2+} • Transcellular pathway • Paracellular pathway • Vitamin D • Channels, TRPV5 and TRPV6 • $\text{Na}^+/\text{Ca}^{2+}$ exchanger • Pregnancy • Growth

Abstract

An overview of current information on the mechanisms by which intestinal calcium absorption occurs is described in this article. Both paracellular and transcellular pathways are analyzed. Special emphasis focuses on molecules participating in the latter pathway, such as TRPV5 and TRPV6 channels, located in the apical region of the enterocytes, CB_{9k} and CB_{28k} , presumably involved in the cation movement from the apical to the basolateral pole of the cell, and PMCA_{1b} and $\text{Na}^+/\text{Ca}^{2+}$ exchanger, proteins that extrude Ca^{2+} from the cells. Current concepts on the relative importance of paracellular and transcellular calcium transport and the vitamin D dependence of each pathway are referred and analyzed showing the contrasting views on this issue. More detailed information is given regarding the stimulatory effect of vitamin D on intestinal Ca^{2+} absorption either in animal models or in the human intestine. The possible mechanisms triggered by hormones such as PTH, calcitonin, estrogen, thyroid hormone, glucocorticoids and different nutritional fac-

tors on intestinal calcium absorption are also reviewed. Finally, the influence of physiological conditions such as growth, pregnancy, lactation and aging on intestinal calcium absorption are discussed.

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Introduction

Intestinal calcium (Ca^{2+}) absorption is an important process involved in the maintenance of Ca^{2+} homeostasis. It occurs by two main mechanisms: a transcellular, metabolically driven transport, and a passive non-saturable route, called the paracellular pathway [1]. These mechanisms are regulated by hormones, nutrients and other factors, which have been studied for many years due to their enormous relevance in the prevention of os-

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teoporosis and other abnormalities related to the Ca^{2+} metabolism. The transcellular pathway is mainly regulated by vitamin D, precisely by its hormonal metabolite $1,25(\text{OH})_2\text{D}_3$, via transcriptional activation of genes through binding of the ligand to the classical nuclear vitamin D receptor (VDR) [2]. Some polymorphisms of VDR such as the Fok I polymorphic site seem also to affect intestinal Ca^{2+} absorption [3] by a not well-understood mechanism.

Under physiological conditions, Ca^{2+} ions are absorbed mainly in the small intestine, responsible for about 90% of overall Ca^{2+} absorption [4]. In rat small intestine, Marcus and Lengemann [5] found that 88% of Ca^{2+} absorption occurs in the ileum, 4% in the jejunum, and 8% in the duodenum. In dogs, Cramer [6] found 80, 16 and 4% in ileum, jejunum and duodenum, respectively. The longer residence time of Ca^{2+} in the ileum as compared to the other intestinal segments favors Ca^{2+} absorption in that segment. The transit half-time in rat ileum is around 100–120 min, whereas in the duodenum it is around 2–6 min [5, 6]. Minor amounts of Ca^{2+} ions are absorbed from the stomach and large intestine; the colon accounts for less than 10% of the total Ca^{2+} absorbed [4]. The major contributors to the amount of Ca^{2+} absorbed are the residence time and the rate of absorption in the particular segment. The order of absorption rate is: duodenum > jejunum > ileum [7]. Colonic Ca^{2+} absorption is also vitamin D-responsive and it is quite possible that it becomes important in conditions such as short bowel syndrome [8].

The bioavailability of dietary Ca^{2+} affects the efficiency of intestinal Ca^{2+} absorption. Low Ca^{2+} diets increase the intestinal Ca^{2+} absorption ratio, at least in part, by altering the vitamin D endocrine system [9, 10] and the lipid composition and fluidity of intestinal membranes [11].

Variability of intestinal Ca^{2+} absorption is also related to the physiological Ca^{2+} needs, but in general when the requirements increase and/or the intake is low, the efficiency of Ca^{2+} absorption improves [11]. Growth, pregnancy and lactation stimulate intestinal Ca^{2+} absorption, whereas aging is accompanied by a decrease in the absorption of the cation.

Paracellular Pathway

The intestinal epithelium is a continuous layer of individual cells with very narrow spaces between them that allow the diffusion of small molecules and ions [12]. The

paracellular pathway must be regulated by the epithelium in order to maintain the selective permeability. Tight junctions constitute a barrier to the movement of molecules and ions through this pathway. These junctions are specialized membrane domains located in the apical region of enterocytes. They are intercellular structures where the plasma membranes of adjacent cells come into very close contact [12]. The proteins that form these structures are synthesized in the adjacent cells and they include occludin and another protein member of the claudin family [13]. Movement of Ca^{2+} through the tight junctions is a passive process that depends on the concentration and the electric gradients across the epithelium. It is a passive non-saturable process that prevails in the jejunum and ileum, mainly when Ca^{2+} intake is adequate or high [14]. It depends on the solubility of Ca^{2+} in the distal small intestine, the length of sojourn of the chyme in a particular intestinal segment and the rate of diffusion from the lumen to lymph or blood [14]. When Ca^{2+} intake is high, the paracellular pathway becomes important because the sojourn time in the intestine is short and the proteins involved in the transcellular route are down-regulated [15].

Transcellular Pathway

Epithelial Ca^{2+} Channels

The molecules involved in the apical Ca^{2+} entry step in the intestine remained unknown until the discovery of the epithelial Ca^{2+} channels TRPV5 (previously named ECaC1 or CaT2) and TRPV6 (previously named ECaC2 or CaT1) [16]. Both channels are homologous members of the transient receptor potential (TRP) superfamily, belonging precisely to the vanilloid subfamily (TRPV) to be differentiated from the canonical (TRPC) and melastatin subfamilies (TRPM). The pattern of expression of these proteins is quite variable, which can be due to differences between species or expressions below detection levels [17]. TRPV5 is the major isoform in the kidney, while TRPV6 is highly expressed in the intestine. However, TRPV5 and TRPV6 are coexpressed in human kidney and intestine, and also in other organs such as pancreas, prostate, mammary, sweat and salivary glands [16]. TRPV5 and TRPV6 have 75% homology and their main differences are located in the N and C terminal tails. Both channels permeate Ca^{2+} ions, but they also permeate other divalent cations and monovalent cations in the absence of divalent ones. These channels have three unique properties: (1) they have a constitutively activated Ca^{2+} perme-

ability; (2) the selectivity for Ca^{2+} over Na^+ is much larger than in other members of the TRP family ($P_{\text{Ca}}/P_{\text{Na}} > 100$), and (3) the current-voltage relationship of both channels shows inward rectification instead of outward rectification as shown by other TRPV channels. The structure of these channels is similar to that of other members of the TRP family, including 6 transmembrane domains, a short hydrophobic stretch between segments 5 and 6 which would be involved in the Ca^{2+} pore and large intracellular N and C terminal tails [17]. The intracellular segments contain regulatory sites involved in the regulation of channel activity and trafficking. Among them, there are phosphorylation sites, postsynaptic density protein (zona occludens) motifs, and ankyrin repeat domains. All of them participate in the maintenance of the activity of the channels, in the interaction with other proteins, and in the targeting of those channels to specific membrane regions [17].

Hoenderop et al. [18] demonstrated that channels TRPV5 and TRPV6 have a tetrameric structure and they can combine each other to form heterotetrameric channel complexes with novel properties. The tetrameric architecture of TRPV5/6 implies that 4 aspartic residues form a ring that is negatively charged and functions as a selective filter for Ca^{2+} .

Although both channels originate from two genes juxtaposed on human chromosome 7q35, the proteins share several properties and have some differences. At the transcriptional level, they are regulated by $1,25(\text{OH})_2\text{D}_3$, estrogen and dietary Ca^{2+} . Regarding the activity, both are inactivated by intracellular Ca^{2+} , but the inactivation of TRPV6 shows two phases, whereas that of TRPV5 shows only a slow inactivation phase. The affinity of TRPV5 for the inhibitor ruthenium red is 100 times higher than that of TRPV6 [19].

TRPV5 and TRPV6 are located in the brush border membrane of enterocytes and it is quite possible that they are the rate-limiting entry step of active intestinal Ca^{2+} absorption [20]. To determine the in vivo function of TRPV6, Bianco et al. [21] generated mice with targeted disruption of the TRPV6 gene. They have demonstrated that TRPV6 knockout (KO) mice were viable but showed a 60% decrease in intestinal Ca^{2+} absorption, deficient weight gain, decreased bone mineral density (BMD), and lower fertility. Their data indicate that the TRPV6 channel not only plays a role in the tissues directly involved in Ca^{2+} homeostasis, but also in other tissues.

Walters et al. [22] characterized TRPV6 transcript expression in normal human intestinal biopsies. TRPV6 transcripts were detected in the duodenum but not in the

ileum. Duodenal expression of TRPV6 was vitamin D-dependent in men; however, in elderly women TRPV6 and VDR expressions were reduced and were not vitamin D-dependent, which can explain, at least in part, the lower intestinal Ca^{2+} absorption in elderly postmenopausal women.

Calbindins

There are two calbindins (CBs) thought to be responsible for removing Ca^{2+} from the apical side of the enterocytes and carrying the Ca^{2+} transcellular movement to the basal region of the cell. In the duodenum, CB D_{28k} (CB_{28k}) is present in the avian species, while CB D_{9k} (CB_{9k}) is present in mammals [23]. Both proteins are encoded by separate genes. It has been proposed that CBs not only carry Ca^{2+} ions from the entry side to the basolateral membrane (BLM) of the enterocyte, but also buffer Ca^{2+} ions providing protection against toxic Ca^{2+} levels during high Ca^{2+} influx. In addition, CBs may function as Ca^{2+} sensors due to their biochemical properties related to their EF-hand motifs. CB_{28k} has 6 EF-hand motifs that bind Ca^{2+} in a cooperative fashion. Nuclear magnetic resonance analyses indicated that Ca^{2+} binding to CB_{28k} produce conformational changes in the protein [24]. Lambers et al. [25] have shown that renal CB_{28k} acts as a dynamic Ca^{2+} buffer, regulating the Ca^{2+} concentration in the vicinity to the TRPV5 pore by a direct association with the channel. Similar mechanisms could occur in the intestine and other tissues where CBs are abundant and where cells tolerate important fluctuations in intracellular Ca^{2+} concentration.

Genetic studies give controversial data regarding the role of CBs on Ca^{2+} homeostasis. Ablation of CB_{28k} gene in mice does not produce calcemic abnormalities [26]. VDR KO mice show hypocalcemia, secondary hyperparathyroidism, rickets and a 90% reduction in renal CB_{9k} expression with no change in CB_{28k}. VDR/CB_{28k} double KO mice develop more severe secondary hyperparathyroidism and rachitic skeletal phenotype, and the rescue diet with high calcium and lactose does not completely correct the skeletal alterations. The authors think that CB_{28k} plays an important role in Ca^{2+} homeostasis and suggest that its calcemic role might be compensated by CB_{9k} [27]. However, recent data from CB_{9k} KO mice indicate that those mice are not different from wild-type mice in phenotype and Ca^{2+} serum level [28]. In addition, the CB_{9k} null mutant mice are able to absorb Ca^{2+} from the intestine in response to $1,25(\text{OH})_2\text{D}_3$, which would mean that CB_{9k} is not required for vitamin D-dependent intestinal Ca^{2+} absorption.

Ca²⁺ Pump and Na⁺/Ca²⁺ Exchanger

The exit of Ca²⁺ ions from enterocytes to the lamina propria is performed by two molecules: the plasma membrane Ca-ATPase or Ca²⁺ pump (PMCA) and the Na⁺/Ca²⁺ exchanger (NCX1). The Ca²⁺ pump has a M_r of 130 kDa and K_m for Ca²⁺ of 0.2 μM in the presence of calmodulin [29]. It is located in small invaginations in the plasma membrane, called caveolae, which can exist in open and closed forms that control Ca²⁺ efflux from the cell [30]. It presents several isoforms but, apparently, PMCA_{1b} is the predominant form in the intestine. The expression and activity of PMCA_{1b} is higher in enterocytes from the villus tip as compared to those from the villus crypt, which supports the idea that mature enterocytes have the greatest capacity for transcellular Ca²⁺ movement [10]. Vitamin D deficiency decreases the expression and activity of PMCA_{1b} in chick intestine, which is partially reversed by a single large dose of cholecalciferol [31].

The intestinal Na⁺/Ca²⁺ exchanger, which has received little attention, is responsible for about 20% of Ca²⁺ extrusion and its activity depends on the gradient created by Na⁺/K⁺-ATPase [32]. There are several isoforms that result from three different genes [33]. The Na⁺/Ca²⁺ exchanger is present in the intestine. It has a M_r of 90 kDa and it is constituted by 11 transmembrane domains and an intracellular loop between segments 5 and 6. In this loop there is an inhibitory region involved in the inactivation of the exchanger, named exchange inhibitory peptide domain and a regulatory site with high affinity for Ca²⁺. The stoichiometry of exchange of this protein is 3 Na⁺:1 Ca²⁺ [34]. In the intestine, the Na⁺/Ca²⁺ exchanger has been detected in rats [32], mice [35] and chicks [10], but not in rabbits [36]. This transporter can operate in either a forward mode (Ca²⁺ exit) or in a reversed mode (Ca²⁺ entry), which depends on the Na⁺ and Ca²⁺ gradients and the potential across the plasma membrane [37]. The expression and activity of the Na⁺/Ca²⁺ exchanger is quite similar between mature and immature enterocytes, being slightly higher in the villus tip cells [10].

Regulation by Calcitriol or 1,25(OH)₂D₃

Calcitriol or 1,25(OH)₂D₃ induces changes in the structure and function of intestinal epithelial cells, which results in an increased intestinal Ca²⁺ absorption. As previously mentioned, calcitriol primarily stimulates the transcellular Ca²⁺ movement. Calcitriol enhances intestinal Ca²⁺ absorption through binding to VDR, nuclear

receptor and transcription factor. This binding promotes heterodimerization with the retinoid X receptor (RXR). The 1,25(OH)₂D₃-VDR-RXR complex translocates to the nucleus to bind the vitamin D-responsive element(s) (VDRE) that is (are) usually located on the DNA upstream of the transcription site. Thus, the VDR complex can regulate gene transcription [38]. The four proteins or their transcripts considered to be essential for transcellular Ca²⁺ absorption (TRPV6, CB, calcium pump and Na⁺/Ca²⁺ exchanger) are known to be enhanced by calcitriol in mouse and chick intestine [10, 31, 39–41]. However, recent data obtained in CB_{9k} null mutant mice indicate that CB_{9k} is not required by calcitriol-mediated Ca²⁺ absorption in the small intestine [42].

The sensitivity of the genes related to the intestinal Ca²⁺ absorption to the VDR levels remains unclear. VDR KO mice have a phenotype of vitamin D-resistance rickets and reduced calcium absorption [43]. Song and Fleet [2] tested the role of VDR levels in the intestinal response to calcitriol in wild-type mice and mice lacking one VDR allele (heterozygous) that have only half the intestinal VDR content. They found that a reduced intestinal VDR level causes vitamin D resistance and impairs intestinal Ca²⁺ absorption in mice. The resistance is not uniform in the vitamin D-dependent genes involved in the intestinal Ca²⁺ absorption, because apparently the effects of VDR levels have been only detected in the translation of CB_{9k}. Meyer et al. [44], by using a chromatin immunoprecipitation scanning method, have identified multiple VDREs in the distal promoter region of the TRPV6 gene. It is quite possible that the number of VDREs in each promoter, the location or the local DNA environment of each VDRE or the affinity of each VDRE for VDR could account for the differential sensitivity of each gene involved in the intestinal Ca²⁺ absorption to VDR level [2]. In addition, differences in the promoters of the genes for intestinal Ca²⁺ absorption proteins or for VDR could also explain the interspecies differences in responsiveness to calcitriol between humans and experimental animals [45].

The well-known rapid effects (minutes to an hour) of calcitriol on Ca²⁺ uptake by epithelial cells or other responses brought up the question about the possibility of a second VDR located in the plasma membrane. It now appears that the classic VDR could also be associated with caveolae present in the plasma membrane [46].

Regarding the vitamin D dependency of the paracellular pathway, the picture is less clear as compared to the transcellular pathway. However, some data indicate that vitamin D increases tight-junction conductance and paracellular Ca²⁺ transport in Caco-2 cell lines [47]. It

Fig. 1. Schematic representation of possible mechanisms involved in the intestinal Ca^{2+} absorption. The epithelial Ca^{2+} transport occurs by paracellular and transcellular pathways. The paracellular Ca^{2+} transport is carried out through tight junctions (TJ) by an electrochemical gradient (long arrow between cells). The transcellular Ca^{2+} transport consists of three steps: (1) apical entry of Ca^{2+} through epithelial Ca^{2+} channels TRPV5 and TRPV6 (the last one is the most abundant in intestine); (2) cytosolic diffusion bound to calbindins, and (3) extrusion across the basolateral membranes by the plasma membrane Ca^{2+} -ATPase (PMCA1b) and the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX1). Calcitriol ($1,25(\text{OH})_2\text{D}_3$) stimulates the individual steps of transcellular Ca^{2+} transport. Calcitriol molecules bind to their nuclear receptors (VDR), and the complex $1,25(\text{OH})_2\text{D}_3$ -VDR interacts with specific DNA sequences inducing transcription and increasing the expression levels of TRPV 5/6, calbindins and the extrusion systems.

has been reported that $1,25(\text{OH})_2\text{D}_3$ activates protein kinase C, which apparently increases paracellular permeability by affecting cytoskeleton activity [48].

Figure 1 is a schematic representation of the possible mechanisms involved in the intestinal Ca^{2+} absorption triggered by calcitriol as has been described above.

Relative Importance of Paracellular and Transcellular Calcium Transport and the Vitamin D-Dependence of Each Pathway

It is still debated whether intestinal Ca^{2+} absorption takes place predominantly through a passive or an active process and whether vitamin D constitutes a major regulator of the passive paracellular pathway. Interesting analyses and opinions regarding this issue have been reported by McCormick [49], Bronner et al. [50] and Wasserman [4]. Basically, McCormick [49] refers to evidence indicating that the contribution of passive diffusion is about 8–23% in normal adults, while in children this process is of little practical significance. The author cited two different studies on children lacking a VDR system [51, 52] that did not show improvement in their calcium status by oral calcium supplementation, but instead presented beneficial effects of calcium infusion as early as 2

weeks later. Besides, through analysis of data from Bronner et al. [53] and Pansu et al. [54], he suggested that, early in development and for several weeks after birth, intestinal Ca^{2+} absorption depends on passive diffusion and not on vitamin D-dependent processes. This would change with the age, becoming more important the saturable process that requires vitamin D in the adulthood. In addition, McCormick suggested that species differences could also complicate the interpretation of the relative importance of both pathways.

On the contrary, Bronner et al. [50] reported that the estimation of passive transport from the data of Ireland and Fordtran [55] must be calculated using a value of diffusible plasma calcium of 1.1–1.2 mmol/l instead of 1.5 mmol/l. This would almost double the slope of passive diffusion, and with the new approach that they suggested, the active transport accounted for only 27%. Bronner et al. [50] also cite the data of Sheikh et al. [56], who reported that when healthy young subjects increased their Ca^{2+} intake from 502 to 1,071 mg/day, their total absorption doubled, the increase being due to the vitamin D-independent passive transport. Although Bronner et al. [50] point out that vitamin D administration increases intestinal Ca^{2+} absorption, they consider that increasing Ca^{2+} intake is more effective and involves less risk for normal adults.

Contrary to the aforementioned views, Wasserman [4] presented evidence that both paracellular and trans-cellular pathways are positively affected by vitamin D in the ileum, the site where most of the dietary Ca^{2+} is absorbed. The author mentions several papers [57, 58] demonstrating that the ileum can absorb Ca^{2+} by an active process which is stimulated by vitamin D. Wasserman [4] suggests that the slower rate of absorption in the ileum as compared to the duodenum might be the absence of an entry Ca^{2+} channel in the rat ileum (but present in the human and mouse ileum) and the lower CB and Ca^{2+} pump concentrations compared with the duodenum, but the long transit time of the cation in the ileum would have a considerable effect on overall Ca^{2+} absorption. Wasserman [4] also suggests that calcitriol might control the paracellular permeability as was previously demonstrated in other systems by different hormones.

Regulation by Other Hormones

Parathyroid Hormone

It is well known that parathyroid hormone (PTH) plays an important role in the maintenance of the extracellular Ca^{2+} concentration, sensing minute by minute changes in the blood Ca^{2+} . When the extracellular Ca^{2+} is low, the parathyroid glands secrete PTH to the circulation and then PTH binds to the PTH/PTHrP receptor protein (PTHrP) receptor, mainly in the bone and kidney stimulating bone resorption and Ca^{2+} reabsorption, respectively. At the intestinal level, PTH seems to act indirectly on intestinal Ca^{2+} absorption by stimulation of renal 1α -hydroxylase and, thereby, increasing $1,25(\text{OH})_2\text{D}_3$ -dependent absorption of Ca^{2+} from the intestine. However, direct effects of PTH on Ca^{2+} uptake by enterocytes from rat duodenum have been demonstrated. PTH stimulates enterocyte Ca^{2+} influx, which could be blocked by the Ca^{2+} channel antagonists verapamil and nitrendipine [59], and modulates intracellular Ca^{2+} concentration [60]. Furthermore, PTH/PTHrP receptors have been localized by immunocytochemistry in intestinal epithelial cells along the villus [61]. Aside from its rapid signal transduction mechanism, PTH also promotes nuclear effects such as regulation of gene transcription and cell proliferation [61, 62]. Studies that demonstrated the direct effect of PTH on the global process of intestinal Ca^{2+} absorption have not been reported yet.

Calcitonin

Although calcitonin is considered one of the three major regulating hormones of plasma calcium, little information is available about its effect on intestinal calcium absorption. Jaeger et al. [63] have studied the effect of chronic calcitonin infusion in thyroparathyroidectomized animals. Calcitonin increased plasma calcium levels in animals on a regular diet by 50%, which was most likely due to an enhancement of intestinal calcium absorption since removal of calcium from the diet markedly blunted this effect. The authors concluded that calcitonin stimulates intestinal calcium absorption through an increase in $1,25(\text{OH})_2\text{D}_3$ circulating levels. Afterwards, Yoshida et al. [64] demonstrated that calcitonin stimulates the 25-hydroxyvitamin D_3 1α -hydroxylase mRNA level in LLC-PK₁ cells via the protein kinase C pathway. So far, there have been no reports of pathologies caused by either calcitonin deficiency or excess [65].

Thyroid Hormone

Thyrotoxicosis is quite often accompanied by hypercalcemia in either humans or animals [66, 67]. However, this issue has received little attention. Cross et al. [68, 69] reported that thyroid hormone and vitamin D have a co-operative effect not only on intestinal calcium transport but also on intestinal phosphate movement. The authors also demonstrated that thyroid hormones increase the genomic action of calcitriol in the intestine. Recently, it has been shown that hyperthyroid rats show higher Ca^{2+} uptake by brush border membrane vesicles and Ca^{2+} efflux from the BLMs of enterocytes than hypothyroid rats. Ca^{2+} -ATPase activity was not changed by the thyroid hormones, while the $\text{Na}^+/\text{Ca}^{2+}$ exchanger activity was highly increased possibly via the cAMP-mediated pathway [67].

Estrogen

Several studies have reported that the global Ca^{2+} absorption in normal postmenopausal women is decreased, an effect that is more pronounced in postmenopausal osteoporotic women [70]. Estrogen therapy corrects the decline in Ca^{2+} absorption efficiency at the onset of menopause as indicated by cell culture studies [71]. However, the mechanisms that underlie this effect are not clear. Some studies indicate that estrogen acts independently of $1,25(\text{OH})_2\text{D}_3$ in the intestine [72], whereas others suggest that estrogen modifies intestinal Ca^{2+} absorption through the vitamin D endocrine system [71]. Most of the estrogen studies were performed in ovariectomized animals. This ablation significantly reduces endogenous estrogen

production, but not completely due to the fact that adrenal androgens can be aromatized to estrogen [70]. Because of that, KO animals for estrogen receptor- α (ER α) and ER β were obtained to totally abolish the genomic actions of estrogen mediated by their receptors. ER α KO mice showed a decrease in the duodenal ECaC2 mRNA expression, while CB $_{9k}$, PMCA $_{1b}$ and VDR levels were unchanged. ER β KO mice did not change the genes for intestinal calcium transporter. Apparently, in mice the genomic effects of estrogen are mainly mediated by the ER α . This concept must not be extrapolated to humans because it has been demonstrated that in normal colon and cancer colon cells the subtype β is the predominant form of the ER [73].

Van Abel et al. [74] supplemented ovariectomized rats with estradiol and found increased duodenal gene expression of TRPV5, TRPV6, CB $_{9k}$ and PMCA $_{1b}$. In addition, they used 25-hydroxyvitamin D $_3$ 1 α -hydroxylase KO mice to study the 1,25(OH) $_2$ D $_3$ dependency of the stimulatory effects of estradiol on intestinal Ca $^{2+}$ absorption. Under this condition of undetectable levels of 1,25(OH) $_2$ D $_3$, the estradiol treatment increased mRNA levels of duodenal TRPV6. During pregnancy or lactation, estrogen or hormonal changes produce vitamin D-independent effects at the genomic level on duodenal calcium absorption. Estrogen seems to upregulate the calcium influx channel CaT1, which apparently is only mediated by ER α [75]. Cotter and Cashman [76] have investigated the effect of two dietary phytoestrogens (coumestrol and apigenin) as well as ipriflavone, a synthetic phytoestrogen, on Ca absorption in the human Caco-2 cell line. The authors did not find a direct effect of these compounds on intestinal Ca absorption. These equivocal results evidence that further studies are needed to clarify the mechanism(s) triggered by estrogen in intestine.

Glucocorticoids

Glucocorticoids (GCs) are extensively used as anti-inflammatory drugs. Osteoporosis is one of the most important side effects after long-term GC treatments. Reduced intestinal Ca $^{2+}$ absorption seems to be part of the pathogenesis of GC-induced osteoporosis [77]. However, the mechanisms triggered by GCs on the intestine are not clear. Some data have shown that short-term GC excess in young animals affects bone metabolism but not the expression of genes involved in intestinal Ca $^{2+}$ absorption such as TRPV6, CB $_{9k}$ and PMCA $_{1b}$ [78]. In contrast, sustained dexamethasone suppresses mouse duodenal CB $_{9k}$ expression via the GC receptor pathway [79]. Re-

cently, it has been reported that 10 mg/kg body weight prednisolone for 10 days decreases rat intestinal Ca $^{2+}$ absorption through diminished expression of the active calcium transporters such as the channel TRPV6 and CB $_{9k}$, independently of 1,25(OH) $_2$ D $_3$ [80]. A similar finding was also reported by Scholz-Ahrens et al. [81]. These authors used adult Göttingen miniature pigs treated orally with prednisolone at a dose of 1 mg·kg body weight $^{-1}$ ·day $^{-1}$ for 8 weeks (short-term) and thereafter at 0.5 mg·kg body weight $^{-1}$ ·day $^{-1}$ (long-term). These animals are omnivore and thus have more similarities with humans than other animals. By looking at the parallel decline in calcium balance and BMD and the parallel lower bone ash content, the authors concluded that GC-induced osteoporosis was the consequence of reduced intestinal mineral absorption. Scholz-Ahrens et al. [81] think that GCs may directly alter the vitamin D-independent transmucosal absorption of calcium because they did not find large effects on plasma vitamin D metabolites or on PTH. Their conclusion is that alterations in vitamin D metabolites associated with the GC-induced osteoporosis might be the consequence of preexistent disorders.

Regulation by Nutrients

Dietary Ca $^{2+}$

About 70% of dietary Ca $^{2+}$ is provided by milk and dairy products, 16% by green vegetables and fruits, and 6–7% by drinking water including mineral water [82]. Milk provides large amounts of calcium and phosphorus and other components such as lactose and casein phosphopeptides that may increase calcium absorption and mineral retention. This must be emphasized because intestinal absorption does not completely reflect the bioavailability of calcium. The bioavailability of calcium has been defined as the fraction of dietary Ca $^{2+}$ that is potentially absorbable by the intestine and can be used for physiological functions, mainly bone mineralization, or to limit bone loss [82]. In brief, the bioavailability of Ca $^{2+}$ depends on absorbability, urinary excretion and fecal loss of endogenous Ca $^{2+}$. The effect of several factors affecting the bioavailability of Ca $^{2+}$ has been extensively examined in several reviews [82–85].

The amount of dietary Ca $^{2+}$ influences the process of intestinal absorption of the cation. Low Ca $^{2+}$ diets enhance the efficiency of intestinal Ca $^{2+}$ absorption [39]. This is an adaptation process performed as a compensation mechanism in order to cover the cation needs of the organism. When dietary Ca $^{2+}$ is low, serum levels of

1,25(OH)₂D₃ are enhanced by stimulation of renal 1 α -hydroxylase activity in chicks [9]. Consequently, intestinal CB_{28k} and its mRNA are increased, in addition to the protein expression and activity of PMCA_{1b} and the Na⁺/Ca²⁺ exchanger [10, 39]. Low Ca²⁺ diets also provoke alteration in lipid composition and increment in the fluidity of intestinal BLM [11]. It appears, then, that the Ca²⁺ exit through BLM from enterocytes of animals adapted to a low Ca²⁺ diet is greater than that of control animals because of the increment in PMCA_{1b} and Na⁺/Ca²⁺ exchanger activities and changes in lipid composition and fluidity of BLM, which might alter the microdomains of cation transporters and, hence, increase their activities [86]. The gene expression of the epithelial Ca²⁺ channels CaT1 and CaT2 has also been shown to increase by dietary Ca²⁺ deficiency either in young or adult animals [87]. In other words, the activities of all genes involved in the transcellular pathway (Ca²⁺ channels, CBs, PMCA_{1b} and Na⁺/Ca²⁺ exchanger) are enhanced by the low Ca²⁺ diet, probably by activation of the vitamin D endocrine system.

Other Nutritional Factors

In general, carbohydrates increase intestinal Ca²⁺ absorption, but lactose contained mainly in dairy food seem to be the most efficient one [85]. However, studies done in lactose-tolerant healthy adults did not show an effect of lactose on intestinal Ca absorption by using a stable strontium test under controlled metabolic conditions [88]. Lately, the use of lactosucrose in humans and animals seems to be very important in order to increase intestinal Ca²⁺ absorption due to the fact that it promotes a decrease in intestinal pH increasing the solubility of Ca²⁺ salts [89, 90]. Data regarding protein intake and intestinal Ca²⁺ absorption indicate that dietary protein does not alter the intestinal Ca²⁺ absorption and, hence, intestinal calcium absorption does not explain hypercalciuria induced by high protein intake [91]. For many years, steatorrhea has been associated with decreased intestinal Ca²⁺ absorption [92]. However, the data related to the effects of dietary lipids on intestinal Ca²⁺ absorption are not clear [85]. Human intestinal Caco-2 cells were exposed to conjugated linoleic acid (CLA) and medium chain fatty acids to measure transepithelial and transcellular transport of ⁴⁵Ca, fluorescein transport (a marker of paracellular Ca transport) and transepithelial electrical resistance (an indicator of permeability). Overall Ca transport and transepithelial electrical resistance in Caco-2 cells were unaffected by exposure to any of the fatty acids for 24 h or 22 days, whereas paracellular (but not total

transepithelial and transcellular) Ca²⁺ transport across Caco-2 cells was significantly increased after 22 days [93]. Later, the same authors reported that both isomers of CLA increased overall transepithelial Ca²⁺ transport as well as transcellular and paracellular Ca²⁺ transport in monolayers of Caco-2 cells, an effect that could be related to changes in the zona occludens-1 (a tight junction protein) [94]. Recently, Murphy et al. [95] investigated the molecular mechanisms underlying CLA-induced stimulation of Ca transport using microarray data together with quantitative reverse transcriptase-PCR analysis. They found that zona occludens-1, occludin, and claudin-4 were all upregulated and claudin-1 downregulated by trans-10, cis-12 CLA, which might explain the increase in the paracellular route [95]. However, since they did not find alterations in the genes involved in the transcellular pathway, the mechanism/s involved in this route remain unknown.

The effect of probiotics on stimulation of intestinal Ca²⁺ absorption has been proposed to be produced by the increased production of short-chain fatty acids by bacteria. In a recent review, it was suggested that prebiotics are the most promising and also the best investigated substances with respect to bone health-promoting potential, as compared to probiotics and synbiotics [96].

Other components of the diet such as phytates, oxalates and tannins can form insoluble complexes with Ca²⁺, which reduce the cation absorption, this effect being very important when the diets are unbalanced, for example diets lacking dairy products and enriched in fibers [82].

Intestinal Ca²⁺ Absorption under Different Physiological Conditions

Intestinal Ca²⁺ absorption varies according to the age and physiological conditions of individuals. In general, when the needs are high and/or dietary Ca²⁺ is low, intestinal Ca²⁺ absorption is found to be more efficient. Growth, gender, pregnancy and lactation and high physical activity increase the Ca²⁺ demands and, therefore, promote Ca²⁺ absorption from the intestine. Ca²⁺ absorption is high during pregnancy. This increment is evident in early to mid pregnancy and precedes the increased Ca²⁺ demand from the fetus for skeletal growth. This alteration in Ca²⁺ absorption during pregnancy may be due to an increase in serum calcitriol with little alteration in PTH and calcitonin concentrations [97]. Kent et al. [98], in contrast, demonstrated that the efficiency of

Table 1. Hormones, factors and physiological conditions affecting intestinal Ca^{2+} absorption in different species

Ca^{2+} uptake	Ca^{2+} transport	Ca^{2+} exit
<i>Humans</i>		
↑ D [45, 71]	↓ A [22]	↑ D [71, 122]
↑ CH [90, 107]	↑ CLA [95]	
↑ ITF [106]	↑ PL [123]	
↑ PB [121]		
<i>Dog</i>		
↑ CH [108]	↓ 24D [109]	
<i>Rat</i>		
↑ CT [63]	↓ GC [80]	↑ PL [111, 112]
↑ T3 [67]	↓ A [87]	↑ E [74]
↑ PL [111, 112]		↑ LCa^{2+} [86]
↑ E [74]		
↑ PTH [59, 60]	↓ A [87]	
↑ IF [110]	↓ GC [80]	
↑ CH [89]		
↑ LCa^{2+} [86]		
<i>Mouse</i>		
↑ D [41, 113]	↓ A [114]	↑ αD [113]
↑ 24D [113]		↓ GC [79]
↑ E [75]		
↑ P [75]		
<i>Chick</i>		
↑ D [23, 31, 39]	↑ LCa^{2+} [11]	↑ D [31, 39]
↑ LCa^{2+} [10]	↑ PTH [116]	↑ LCa^{2+} [10]
	↓ GC [115]	
<i>Fish</i>		
↑ PTHrP [117]		
↑ E [118]		
↑ D [119, 120]		
↓ 24D [119]		

A = Aging; CH = carbohydrates; CLA = conjugated linoleic acid; CT = calcitonin; D = $1,25(\text{OH})_2\text{D}_3$; αD = $1\alpha\text{-OHD}_3$; 24D = $24,25(\text{OH})_2\text{D}_3$; E = estrogen; GC = glucocorticoids; IF = ipriflavone; ITF = inulin-type fructans; LCa^{2+} = low Ca^{2+} ; P = pregnancy; PB = probiotic bacteria; PL = prolactin; PTH = parathyroid hormone; PTHrP = parathyroid hormone-related protein.

intestinal Ca^{2+} absorption is increased in late pregnancy but not during lactation. Zhu et al. [99] found in rats that at 21 days of gestation both Ca^{2+} pump and CB mRNA levels increased 2- to 3-fold. They also demonstrated that the Ca^{2+} pump and CB mRNA remained elevated at 7 days of lactation. Intestinal Ca^{2+} absorption in pregnant adolescents has also been reported to be significantly higher in the third trimester of pregnancy than in the early postpartum period [100]. In colon biopsies from

dairy goats and sheep, it was found that VDR immunohistochemistry revealed a lower staining in early lactation as compared to late lactation, which would mean that VDR seems to be involved in the metabolic changes related to intestinal Ca^{2+} absorption during the lactation period [101]. Yamagishi et al. [102] found in rats that vitamin D deficiency during pregnancy produces severe hypocalcemia due to reduced intestinal Ca^{2+} absorption and elevated fetal demand for the cation.

Braun et al. [103] recently presented data showing that Ca^{2+} retention in boys is higher than in girls at different Ca^{2+} intakes, and this difference is attributed to a higher intestinal Ca^{2+} absorption and lower urinary excretion in boys as compared to girls. To optimize Ca^{2+} intake it is crucial in adolescents to maximize calcium retention, acquire a good peak of bone mass and prevent osteoporosis later in life. A Spanish study in boys aged 11–14 years on their usual diets revealed that the adolescents absorbed 31% of dietary intake, retained 20% of the total intake, but dietary Ca^{2+} intake failed to meet the recommended values [104]. It has also been demonstrated in adolescents that polymorphisms of the Fok I site in the VDR gene are significantly associated with Ca^{2+} absorption and BMD; individuals carrying the genotype ff have deficit in whole Ca^{2+} accretion compared to those with the genotype FF [3].

Advancing age has been associated with lower intestinal Ca^{2+} absorption [105]. This decrease occurs, at least in part, because of a declination in the serum calcitriol levels, but in addition, a resistance to the actions of calcitriol in the intestine has been reported [22]. Although the mechanisms are poorly known, in women the lack of estrogen during menopause could partially explain this age-associated intestinal resistance to calcitriol. It has also been reported that duodenal TRPV6 expression is not vitamin D-dependent in postmenopausal women [22]. Recent data have shown that in mice, heterozygotes for the VDR gene KO, low levels of VDR produce resistance to intestinal Ca^{2+} absorption of $1,25(\text{OH})_2\text{D}_3$. This resistance appears to be generated by the low translation of CB_{9k} that is mediated by binding of VDR to the ligand [2].

Table 1 summarizes the effects of hormones, factors and physiological conditions that participate in the regulation of uptake, transport and extrusion of Ca^{2+} in the intestine from different species.

Conclusion

The intestine is the gate of Ca^{2+} entry to the entire body. Due to the enormous participation of the cation in different important physiological processes such as muscle contraction, neuronal activity, strength of the skeleton, etc., the mechanisms of intestinal Ca^{2+} absorption have been studied for many years. The transcellular pathway seems to be highly regulated mainly by calcitriol, which acts through activation of intestinal channels TRPV6 and TRPV5, CBs and PMCA_{1b} and the $\text{Na}^+/\text{Ca}^{2+}$ exchanger. PTH, estrogen, GC, thyroid hormone and other hormones also regulate these proteins or their genes by mechanisms under investigation. Dietary Ca^{2+} and many nutritional factors have been studied in order to elucidate their role in cation absorption. Growth, pregnancy and lactation promote intestinal Ca^{2+} absorption, while aging and many pathological conditions occur

with deterioration in the Ca^{2+} absorption. Although many findings have been achieved during the last decade on the regulation of intestinal Ca^{2+} absorption, there are many open questions that need further investigation, for instance: (1) is the Ca^{2+} pump the main molecule to extrude Ca^{2+} from the intestinal cell in humans? (2) how is the intestinal $\text{Na}^+/\text{Ca}^{2+}$ exchanger regulated by calcitriol and other hormones that stimulate intestinal Ca^{2+} absorption? (3) does PTH stimulate intestinal Ca^{2+} absorption or simply trigger rapid responses in enterocytes that finally do not promote the global process of Ca absorption? (4) what are the mechanisms by which Ca^{2+} diffuses between enterocytes? The responses to these and other basic questions on the regulation of intestinal Ca absorption could give some clues to improve cation absorption in the elderly and in some pathologies, and also to develop nutritional or medical strategies to stimulate the efficiency of intestinal Ca^{2+} absorption.

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