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REVIEW

Vitamin D endocrine system and the intestine

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Calcium and phosphate regulate numerous biological processes and they are essential for bone mass and bone quality. The calcium and phosphate balance largely depends on intestinal absorption, and the dietary content of these ions determines the type of transport. High dietary intake of calcium and phosphate enables absorption by passive transport, but often the dietary content of these ions is in the low–normal range, especially for calcium. In this condition, the contribution of active intestinal calcium transport will increase to maintain normal serum levels. This adaptation is mainly regulated by the active form of vitamin D, 1,25 dihydroxyvitamin D, and requires normal concentrations of the precursor 25-hydroxyvitamin D. When intestinal calcium absorption is insufficient, hormonal adaptations will release calcium from bones to secure normocalcemia, not only by increasing bone loss but also by decreasing bone mineralization. These data underline the fact that adequate calcium intake is critical to secure skeletal integrity. Despite the insights that sufficient dietary calcium intake and normal 25-hydroxyvitamin D levels are critical for calcium and bone homeostasis, surprisingly little is known on the proteins that mediate intestinal calcium transport. Also, the interaction between the intestine and the kidney to control serum phosphate levels is still incompletely understood.

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Introduction

Serum ionized calcium levels (in this chapter calcium refers to the ionized form) are maintained within a narrow range (1.0–1.3 mM) because they regulate a wide range of biological processes including muscular contraction, secretion, cell division and blood clotting. Phosphate concentrations, on the other hand, are less tightly controlled (0.8–1.5 mM), but similar to calcium, phosphate exerts an important physiological role ranging from cellular energy metabolism and biological information transfer to enzymatic activities or protein interactions.

Body calcium and phosphate are ultimately derived from the diet and adequate intestinal absorption is thus critical for the calcium and phosphate balance. Adaptations in renal calcium and phosphate reabsorption aid in maintaining constant serum levels. In addition, the skeleton forms a major reservoir of these ions, and can buffer the serum concentrations by taking up or releasing calcium and phosphate. On the other hand, calcium and phosphate have an important structural role in the skeleton; constituting the mineral compartment of bone, they are essential for bone mass and bone quality. Preservation of serum calcium levels may thus occur at the expense of skeletal integrity.

Intestinal calcium and phosphate absorption occurs through two defined mechanisms: (1) the paracellular transport pathway, which results from passive diffusion and (2) the active transcellular pathway. In a standard diet the calcium content is in the low–normal range, whereas phosphate is abundantly present. In these dietary conditions calcium absorption already requires the contribution of the active transcellular pathway, whereas phosphate absorption occurs primarily through the passive process.

Calcium and phosphate homeostasis is mainly regulated by three hormones, parathyroid hormone (PTH), 1,25 dihydroxyvitamin D [1,25$(OH)_2$D] and fibroblast growth factor (FGF) 23, which together control several processes in the different target tissues. As intestinal absorption is critical for the calcium and phosphate balance, in this chapter we will focus on the role of 1,25$(OH)_2$D in regulating intestinal calcium and phosphate absorption (see Sections 2 and 4) and on the indirect influence of this process on serum calcium levels and bone homeostasis (Section 3). Most of these insights are obtained through preclinical studies in mice. In Section 5, we will discuss the impact of this knowledge on calcium and vitamin D supplementation in humans.

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Molecular Mechanisms of $1,25(OH)_2D$-mediated Intestinal Calcium Transport

Transcellular calcium transport. Active, $1,25(OH)_2D$-mediated, transcellular calcium absorption is a saturable process that involves the transfer of calcium across the luminal brush border membrane, its transfer through the cell interior and the active extrusion of calcium from the basolateral membrane (**Figure 1**). Early studies using ion microscopic imaging of calcium showed that, upon vitamin D repletion, the absorbed calcium interacted transiently with elements within the microvillar region of the brush border membrane before diffusing through the cell.[1] Recent studies have suggested that the molecular basis of $1,25(OH)_2D$-dependent calcium entry involves uptake of calcium through the $1,25(OH)_2D$-inducible epithelial calcium-selective channel TRPV6.[2] TRPV6 is present in all segments of the mouse and rat intestine, is expressed in villi tips and not in villi crypts and is strongly calcium selective.[2] TRPV6 has been reported to associate with other proteins including calmodulin, which is involved in TRPV6 inactivation. In addition, TRPV6 can interact with the S100A10-annexin 2 complex and Rab11a, which have a role in the trafficking of TRPV6 to the plasma membrane and in the recycling of TRPV6, respectively.[3–5] These TRPV6-associated proteins may represent additional components of the regulation of calcium entry into the intestinal cell by $1,25(OH)_2D$. TRPV6 and the calcium-binding protein Calbindin-D_{9k} are colocalized in the intestine and both proteins are similarly regulated; more specifically, they are induced at weaning or under low calcium conditions and after $1,25(OH)_2D$ injection.[6] TRPV6 and Calbindin-D_{9k} are induced before the peak of intestinal calcium absorption.[6] Vitamin D receptor (*Vdr*)–null mice display decreased intestinal calcium absorption, which largely contributes to the development of rickets. In these mice, intestinal *Trpv6* mRNA is reduced by more than 90% and there is a 50% reduction in intestinal *Calbindin-D_{9k}*.[7–9] These findings provide indirect evidence for a role of TRPV6 and Calbindin-D_{9k} in the process of $1,25(OH)_2D$-mediated calcium absorption. However, studies in *Trpv6*- or *Calbindin-D_{9k}*-null mice indicate that active calcium transport still occurs in these mice, suggesting compensation by other calcium channels or calcium-binding proteins.[10–12] Consistent herewith, bone mass is comparable in *Trpv6*-null and wild-type (WT) mice under normal calcium intake. On the other hand, a low dietary calcium intake induces a more pronounced bone turnover and osteoid abundance in *Trpv6*-null mice compared with WT mice. These data suggest a role for TRPV6 under low dietary calcium conditions to maintain proper bone mineralization.[13] Recent studies have shown that transgenic mice overexpressing *Trpv6 in vivo* develop hypercalcemia and tissue calcification, indicating a direct role for TRPV6 in the process of intestinal calcium absorption.[14] On the other hand, the increase in intestinal calcium absorption in response to $1,25(OH)_2D$ is similar in WT mice and in *Trpv6*- and *Calbindin-D_{9k}* single–null mice, but the $1,25(OH)_2D$-induced intestinal calcium transport is 60% lower in *Trpv6*/*Calbindin-D_{9k}* double–null mice. These findings suggest that TRPV6 and Calbindin-D_{9k} can act together in certain aspects of the intestinal absorptive process.[11] Early studies from Dr Robert Wasserman's lab showed the association of calbindin with intestinal brush borders.[15] It is indeed possible, but has not as yet been investigated, that Calbindin-D_{9k} associates with TRPV6 and that a principal function of Calbindin-D_{9k} is to facilitate TRPV6-mediated calcium influx by preventing calcium channel inactivation. Calbindin may also act as a calcium buffer preventing the levels of intracellular calcium from accumulating in intestinal cells. In the cytosol, calcium may be bound to calbindin and to other calcium-binding proteins. Calcium may also be sequestered by the endoplasmic reticulum. At the basolateral membrane calcium is extruded against a concentration gradient by the intestinal plasma membrane ATPase (PMCA1b), which has been reported to be upregulated by $1,25(OH)_2D$ and under conditions of low dietary calcium.[16,17]

Paracellular calcium transport. In addition to transcellular calcium transport, calcium is also absorbed from the intestine by a non-saturable diffusional process that occurs through intercellular tight junctions. During the first weeks after birth, intestinal calcium absorption depends on passive non-saturable diffusion and not on a $1,25(OH)_2D$-mediated process,[18] a finding that was confirmed in suckling rats.[19] The lack of response of the intestine to $1,25(OH)_2D$ can be explained by the relative absence of the VDR during early neonatal life.[20] The paracellular pathway is gradually replaced by a $1,25(OH)_2D$-dependent saturable component, which is fully active by the time of weaning of rats. The time of weaning also correlates with the development of hypocalcemia in *Vdr*-null mice.[7] These data indicate that high dietary calcium content together with lactose, such as in mother's milk, is sufficient for calcium and bone homeostasis and encompasses paracellular calcium transport.

Early studies indicated that $1,25(OH)_2D$ not only regulates the saturable intestinal process but also the non-saturable process.[19,21] However, paracellular calcium transport and its regulation by $1,25(OH)_2D$ are much less defined compared with $1,25(OH)_2D$-mediated transcellular calcium transport (**Figure 1**). Claudins are major transmembrane components of tight junctions that may function as paracellular cation

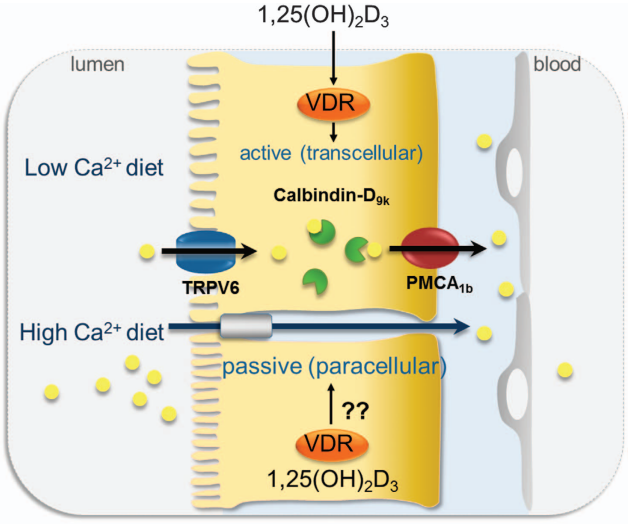

Figure 1 Intestinal calcium absorption. Model of intestinal calcium transport comprising a transcellular, active mechanism that transports calcium when dietary calcium intake is normal/low and a paracellular, passive pathway that functions under high calcium intake. Recent studies indicate that in the transcellular pathway other, still unknown, calcium transporters are involved beside the depicted TRPV6, Calbindin-D_{9k} and $PMCA_{1b}$.

channels. In *Vdr*-null mice Claudin-2 and -12 are downregulated in the jejunum, ileum and colon compared with WT mice.[22] In addition, these claudins are induced by $1,25(OH)_2D$ in Caco-2 colon adenocarcinoma cells,[22] further suggesting $1,25(OH)_2D$ regulation of these proteins. $1,25(OH)_2D$ has also been reported to downregulate Cadherin-17 and Aquaporin-8 in the intestine.[11,23] Cadherin-17 is important for cell-to-cell contact and its downregulation may increase intestinal permeability, whereas the decrease in the tight junctional channel Aquaporin-8 may influence the tight junction selectivity toward cations. These findings indicate that the transjunctional movement of calcium occurs in a regulated fashion and these data support the regulation of paracellular calcium transport by $1,25(OH)_2D$. It will be of interest, using intestine-specific null mice, to determine the contribution of specific claudins as well as that of specific intra- and intercellular matrix proteins to the calcium absorption mediated by $1,25(OH)_2D$. Although the intestine has a major role in $1,25(OH)_2D$ action on calcium homeostasis, these recent studies suggest that our understanding of the mechanisms involved in $1,25(OH)_2D$ stimulation of intestinal calcium absorption is still incomplete. Future studies are needed to determine the role of the distal as well as the proximal intestine in $1,25(OH)_2D$-mediated intestinal calcium transport and to identify novel $1,25(OH)_2D$-regulated proteins involved in transcellular and paracellular calcium transport.

Effect of pregnancy on intestinal calcium transport. $1,25(OH)_2D$ levels increase during pregnancy.[24] Calcium transport has been reported to increase during pregnancy in vitamin D-replete as well as vitamin D-deficient animals.[24] These early findings strengthened the relationship between $1,25(OH)_2D$ and active intestinal calcium transport and suggested that factors independent of $1,25(OH)_2D$ can stimulate intestinal calcium transport during pregnancy. More recent studies showed that pregnancy results in an induction of *Trpv6* in the duodenum of *Vdr*-null mice.[25–27] In addition, in estrogen receptor null mice *Trpv6* is reduced, suggesting that estrogen, independent of $1,25(OH)_2D$, may be an important regulator of calcium influx during pregnancy.[27]

Impact of VDR Signaling in the Intestine on Calcium and Bone Homeostasis.

The contribution of $1,25(OH)_2D$-mediated intestinal calcium absorption to calcium and bone homeostasis depends on the dietary calcium content. When dietary calcium levels are high, calcium transport in the intestine comprises a passive, paracellular pathway, which is independent of intestinal VDR action. Consistent herewith and further proving this concept, several studies show that feeding *Vdr*-null mice a calcium-rich rescue diet completely prevented the calcium, endocrine and bone abnormalities that are associated with *Vdr* deficiency.[28,29]

During normal to low calcium intake, on the other hand, stimulation of intestinal calcium transport by $1,25(OH)_2D$ becomes essential to support normal calcium homeostasis. Indeed, we found that merely the inactivation of VDR activity in the intestine (intestinal-specific *Vdr*-null mice, Vdr^{int-} mice[30]) leads to a pronounced defect in intestinal calcium absorption, even during normal calcium intake, with a negative calcium balance as a consequence. Surprisingly, intestinal-specific *Vdr*-null mice are able to maintain serum calcium levels within a normal range due to compensatory mechanisms that are activated by a small rise in serum PTH and a massive increase in $1,25(OH)_2D$ levels. Physiologically, the high PTH and $1,25(OH)_2D$ levels contribute to the maintenance of normocalcemia by stimulating renal calcium reabsorption and by promoting calcium release from the skeleton with a positive flux to the serum calcium pool. The latter involves two, separate, mutually exclusive mechanisms, that is, a PTH- and $1,25(OH)_2D$-mediated increase in bone resorption, and a $1,25(OH)_2D$-mediated suppression of bone matrix mineralization that is evoked by the increased generation of mineralization inhibitors by the osteoblasts. These compensatory mechanisms are essential to maintain normal serum calcium levels during calcium restriction (or lack of intestinal VDR activity), but consequently and unfortunately lead to osteopenia, hyperosteoidosis and a profound reduction in the mineral content of the mineralized bone, with bone fractures as a consequence (**Figure 2**).[30] Moreover, we confirmed that osteoblastic VDR signaling contributes to the maintenance of normocalcemia when intestinal calcium acquisition is insufficient, by showing a more marked reduction in serum calcium levels with less pronounced defects in bone matrix mineralization in calcium-deprived osteoblast-/osteocyte-specific *Vdr*-null (*Dmp1*-CRE) mice, compared with calcium-deprived WT mice.[30] Thus, when dietary calcium intake is in the normal–low range, intestinal VDR activity is crucial for bone homeostasis, as it avoids the need for a shift of calcium from the bone to serum. Importantly, the finding that combined dietary calcium restriction and intestinal VDR inactivity results in early death associated with severe bone loss and hypocalcemia[30] underscores the fact that $1,25(OH)_2D$-mediated intestinal calcium transport is essential to survive calcium restriction and that the skeletal responses to secure serum calcium are limited.

The above findings also imply that $1,25(OH)_2D$ controls calcium homeostasis not only by acting on the intestine, but also by affecting renal and osteoblastic calcium handling. However, several observations suggest that the impact of intestinal VDR action on calcium homeostasis dominates, and that the $1,25(OH)_2D$-mediated shift of calcium from bone only functions as a backup mechanism, when the intestinal calcium acquisition does not suffice (**Figure 3**). First, just the genetic reintroduction of the *Vdr* in the intestine, though at supraphysiological levels, rescues the hypocalcemia that is associated with *Vdr* deficiency without the need for renal or skeletal VDR activity.[31] Second, the genetic inactivation of VDR signaling in mature osteoblasts and osteocytes via two different targeting strategies (*Collagen1*-CRE[32] and *Dmp1*-CRE[30] mice) does not affect calcium homeostasis during normal calcium intake, whereas the inactivation of intestinal VDR activity in similar conditions does. Notably, genetic targeting of subphysiological *Vdr* levels to the intestine of *Vdr*-null mice almost completely rescues intestinal calcium transport to the levels observed in WT mice, but does not completely prevent hypocalcemia (unpublished results). This observation may imply that renal VDR activity does contribute to serum calcium in normal circumstances, yet verification of this hypothesis requires the generation of kidney-specific *Vdr*-null mice.

In conclusion, intestinal VDR activity is essential for maintaining normal calcium and bone homeostasis during normal–low calcium intake. When this process fails, due to insufficient dietary calcium intake or lack of intestinal VDR signaling,

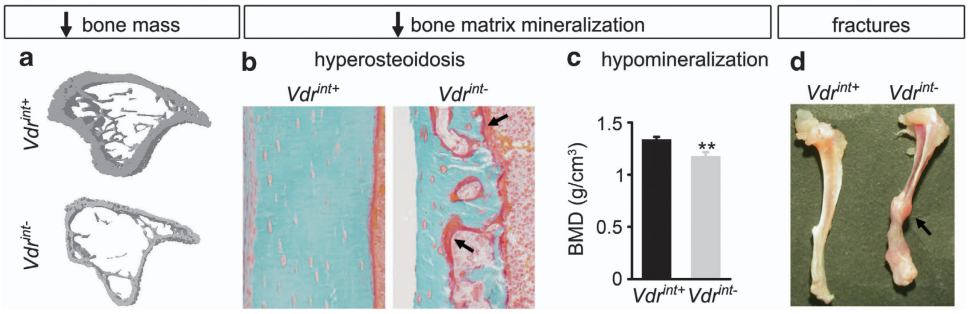

Figure 2 Impaired 1,25(OH)$_2$D-mediated intestinal calcium transport negatively affects bone homeostasis. (**a**) Cross-sectional 3D-reconstruction of the tibial midshaft by μCT illustrates the reduction in bone mass in Vdr^{int-} compared with WT (Vdr^{int+}) mice. (**b**) Goldner staining of the tibial cortex shows that abundant unmineralized bone matrix (osteoid, arrow) lines the endocortical bone surface and resorption cavities in Vdr^{int-} mice. (**c**) Reduction in bone mineral density (BMD), analyzed in a defined region in the tibial cortex by μCT, depicts the reduction in mineral content of the mineralized bone matrix. (**d**) Representative image of the spontaneous bone fractures that are observed in Vdr^{int-} mice, but never in WT littermates. Notably, mice were fed a diet containing normal calcium content from weaning onwards. Figure adapted from Lieben *et al.*[30]

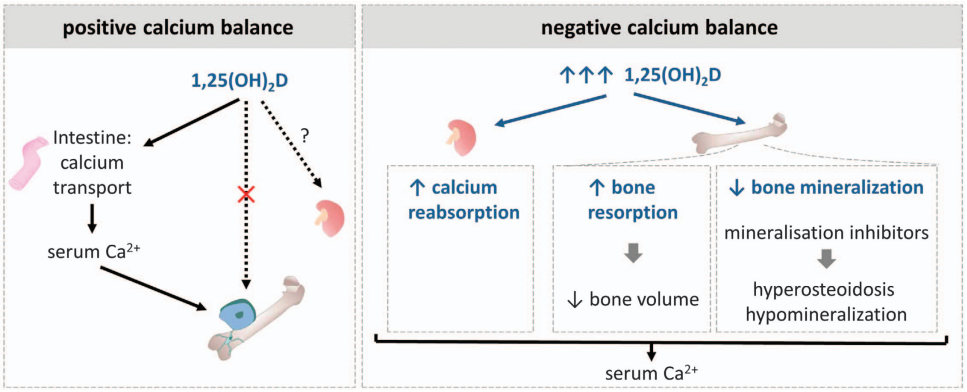

Figure 3 Impact of VDR signaling on calcium homeostasis. (Left panel) During low–normal dietary calcium intake, 1,25(OH)$_2$D supports calcium homeostasis by stimulating intestinal calcium transport. The contribution of renal calcium reabsorption in these circumstances remains to be determined. (Right panel) When stimulation of intestinal calcium absorption does not suffice to maintain normocalcemia, increased 1,25(OH)$_2$D levels will aim to preserve serum calcium levels within a normal range by acting on the kidney to stimulate renal calcium reabsorption, and on the bone to shift calcium from the skeleton to serum by increasing bone resorption and suppressing bone matrix mineralization. Figure adapted from Lieben *et al.*[80]

increased 1,25(OH)$_2$D levels will aim to maintain normocalcemia by acting on the kidney and osteoblasts with negative consequences for bone mass and quality.

Mechanisms of Phosphate Absorption in the Intestine

Serum phosphate levels are not as tightly regulated as serum calcium, and they rise with a high phosphorus diet, particularly after meals. Phosphate is absorbed along the entire intestine, with the small intestine having a significantly higher absorption capacity compared with the colon. Approximately 70% of dietary phosphate is absorbed in the small intestine. The fact that fractional phosphate absorption is virtually constant across a broad range of dietary intakes suggests that the bulk of phosphate absorption occurs by a passive, concentration-dependent diffusion process. Even in the face of severe hyperphosphatemia, phosphate continues to be absorbed from the diet with an efficiency that is only slightly lower than normal.[33] However, a fraction of phosphorus is absorbed through a saturable, active transport, which is facilitated by 1,25(OH)$_2$D.[34–37] It has been suggested that this active phosphate transport becomes important under conditions of hypophosphatemia or low phosphate intake.[35]

Transepithelial transport by phosphate transporters. The transcellular mechanism of phosphate transport depends on active transport primarily through sodium-dependent phosphate cotransporters. Members of one of these protein families, the type-III transporters PiT1 and PiT2, are expressed ubiquitously. By supplying phosphate to the cells they support numerous cellular functions, but the contribution of these transporters to intestinal phosphate transport seems to be limited.[38–41] The most important family consists of the type-II transporters that are mainly involved in phosphate transport in the kidney through NPT2a (NaPi-IIa, SLC34a1) and NPT2c (NaPi-IIc, SLC34a3),[42] and in the intestine through NPT2b (NaPi-IIb, SLC34a2).[43]

NPT2b is a membrane glycoprotein with eight membrane-spanning domains and long intracellular C- and N-terminal domains. It is expressed on the apical surface of intestinal epithelial cells, and aids in the transport of sodium and phosphate ions from the intestinal lumen into the epithelial cells. NPT2b is electrogenic, transports phosphate with a stoichiometry of 3:1 Na:Pi, and generates an intestinal lumen negative voltage potential.[44,45] NPT2b is suggested to be mainly important for intestinal phosphate transport when dietary phosphate intake is low.[43] Genetic studies have

confirmed the significance of NPT2b for intestinal phosphate transport and its influence on phosphate homeostasis. Systemic *Npt2b* deletion is embryonic lethal,[46] but inducible inactivation in adult mice resulted in decreased intestinal phosphate absorption.[47] Similarly, phosphate uptake through the brush border membrane of intestinal cells was decreased in *Npt2b* heterozygous mice.[48] Despite the decreased absorption of intestinal phosphate caused by *Npt2b* heterozygosity or inducible *Npt2b* deficiency, serum phosphate levels were not altered in adult mice. Consistent herewith, patients harboring inactivating *Npt2b* mutations display pulmonary alveolar microlithiasis (as NPT2b is also expressed in the lung), but do not show any abnormalities in serum phosphate levels.[49,50] The normal serum phosphate levels observed with *Npt2b* inactivation are considered to rely on renal compensatory mechanisms resulting in increased phosphate reabsorption, which is primarily controlled within the proximal tubule by NPT2a and NPT2c. Indeed, genetic ablations of these transporters in mice have demonstrated that NPT2a and NPT2c accomplish most of the total renal phosphate reabsorption.[51,52] The likely mechanism for the renal adaptations in *Npt2b*-deficient mice is the decrease in serum levels of the phosphaturic hormone FGF23 and consequently an increase in renal expression of NPT2a leading to reduced urinary phosphate excretion.[47] These data clearly indicate that NPT2b is important for intestinal phosphate transport and that it indirectly contributes to systemic phosphate homeostasis.

Adaptation of intestinal phosphate absorption. Not only calcium homeostasis but also phosphate homeostasis is compromised in vitamin D deficient–[53] and systemic *Vdr*–null mice, as illustrated by the hypophosphatemia.[7,8] However, the relative contribution of the intestine versus the kidney and bone in the development of the hypophosphatemia is likely different from their role in calcium homeostasis. Moreover, the influence of $1,25(OH)_2D$ on intestinal phosphate absorption is still not fully elucidated (**Figure 4**). Treatment of rats with $1,25(OH)_2D$ increases intestinal *Npt2b* expression and Na–phosphate uptake by intestinal brush border membrane vesicles.[54] However, studies in mice show that the regulation of intestinal phosphate transport in response to $1,25(OH)_2D$ may be site- and age-dependent. Indeed, compared with WT mice, adult *Vdr*-null mice show a reduction in Na–phosphate cotransport and in NPT2b protein but not in *Npt2b* mRNA levels in the proximal intestine.[35] In contrast, NPT2b protein expression and Na–phosphate cotransport has been equally detected in brush border membrane vesicles isolated from the distal intestine of young WT and *Vdr*-null mice.[55] In addition, apparent phosphate absorption in the intestine is not decreased in young *Vdr*-null mice.[56,57] Furthermore, as demonstrated by Segawa *et al.*,[35] NPT2b expression is not only influenced by $1,25(OH)_2D$ signaling, but it is also upregulated by dietary phosphate restriction through a $1,25(OH)_2D$-independent pathway. These data indicate that the intestinal epithelial cell is capable of responding to the luminal concentration of phosphate and controls NPT2b expression to increase phosphate transport. On the other hand, when intestinal phosphate absorption is high in response to a high phosphate diet,[58] a feed-forward mechanism between the intestine and kidney is proposed. Intestinal cells would sense changes in luminal phosphate concentration (or somehow in the phosphate transport in the intestine) and release a phosphaturic factor to regulate renal phosphate reabsorption. The nature of the phosphate sensor and phosphaturic factor remains yet to be identified. Taken together, evidence has been provided for a vitamin D-dependent and -independent pathway in intestinal cells that regulates phosphate handling and homeostasis with intensive communication to the kidney.

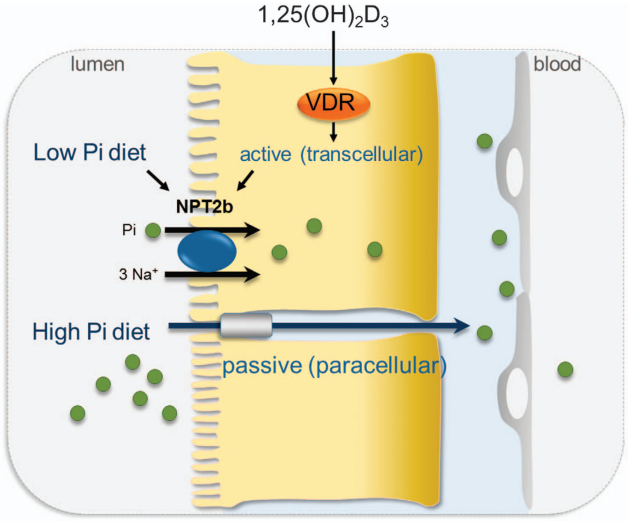

Figure 4 Intestinal phosphate absorption. A large fraction of dietary phosphate intake is considered to be transported by a passive, paracellular pathway. The transcellular pathway consists of NPT2b localized at the intestinal brush border membrane. The expression of this transporter is increased by $1,25(OH)_2D$ and when dietary phosphate intake is low.

Human Studies

The important role of $1,25(OH)_2D$-regulated intestinal calcium absorption for calcium and bone homeostasis in humans is evidently shown by the observation that the bone and endocrine abnormalities of patients with *Vdr*-inactivating mutations are prevented when these patients are treated with high oral doses of calcium.[59,60] Thus, sufficient $1,25(OH)_2D$ activity is required when calcium intake is normal/low to guarantee adequate calcium absorption. Indeed, at normal calcium intake, $1,25(OH)_2D$-dependent transport accounts for the majority of calcium absorption, whereas as little as 8–23% of overall calcium absorption is caused by passive diffusion.[61] However, the ability to adapt to a low calcium diet decreases with age and is significantly compromised from 60 years onwards in humans.[62,63]

Vitamin D deficiency is an important factor leading to reduced intestinal calcium absorption. Indeed, several cross-sectional studies observed a correlation between intestinal absorption and $1,25(OH)_2D$ serum levels.[64–67] In vitamin D-deficient subjects, a positive correlation between calcium absorption and 25-OHD was found, but the correlation with serum $1,25(OH)_2D$ was still stronger. Moreover, intervention studies showed that intestinal calcium absorption was decreased when 25-OHD serum levels were very low ($<4\,ng\,ml^{-1}$ or $10\,nmol\,l^{-1}$), but that the calcium absorption plateaus when serum 25-OHD levels are $8\,ng\,ml^{-1}$ ($20\,nmol\,l^{-1}$) or higher.[68] These data suggest that calcium absorption in vitamin D

deficiency is reduced secondary to substrate deficiency of 25-OHD and reduced $1,25(OH)_2D$ production.[69]

Poor dietary calcium intake is a second cause of reduced intestinal absorption, which is common in the elderly and often associated with vitamin D deficiency. This situation leads to a negative calcium balance, which may contribute to osteoporosis and osteoporotic fractures. The importance of sufficient dietary calcium intake is also evidenced by the success of therapies that add calcium supplementation to reduce fracture risk. Indeed, meta-analyses comparing the effect of vitamin D alone with placebo showed that vitamin D in monotherapy is insufficient for fracture prevention.[70–72] In contrast, several meta-analyses have shown that a combination of vitamin D and calcium is more effective in reducing fracture risk than calcium alone or vitamin D alone.[70–74] Notably, the effects of these clinical trials depend on baseline calcium intake, baseline vitamin D status, age and residence.[75]

Not only the elderly, but also children, particularly in tropical countries, are exposed to inadequate dietary calcium intake, which is now considered an important cause of nutritional rickets.[76,77] Dietary calcium intake in African children is low ($<250\,mg\,d^{-1}$) because dairy products are not frequently included in the African diet. The serum levels of $1,25(OH)_2D$ are increased in these children, suggesting compensatory mechanisms to increase intestinal calcium absorption, which, however, remain insufficient. Similar to the mice with intestinal *Vdr* inactivation (see Section 3;[30]), the increased $1,25(OH)_2D$ and PTH levels in these children likely contribute to the bone defects that are characterized by impaired bone mineralization and are clinically manifested as rickets. Different from the mouse study,[30] these children are slightly hypocalcemic and the low serum calcium levels may also hinder bone mineralization. Other conditions considered to diminish intestinal calcium absorption are reduced gastric acidification[78] and gastrectomy[79] and they may lead to a negative calcium balance.

Taken together, adequate intestinal calcium transport is important to guarantee normal calcium and bone homeostasis, and both sufficient calcium intake and $1,25(OH)_2D$ serum levels are critical determinants of this process.

Conclusions

Mouse as well as human studies clearly indicate that $1,25(OH)_2D$-regulated intestinal calcium transport is critical to preserve normal serum calcium levels and to secure skeletal integrity. Thus, these basic as well as clinical studies strongly advocate the use of vitamin D combined with calcium supplementation to halt the osteoporotic process. However, further studies are required to fully characterize the molecular mechanisms that mediate active intestinal transport and the specific contribution of the different intestinal segments. The contribution of active intestinal phosphate transport to phosphate homeostasis may be of less importance compared with the renal adaptations of phosphate handling. However, a strong interaction seems to exist between the intestine and the kidney in order to control serum phosphate levels, but the factors responsible for this communication remain to be identified.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

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