Commentary

The absorbing life of bile acids

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Hyperphosphatemia increases cardiovascular complications and all-cause mortality rate in patients with chronic kidney disease. Targeting the processes involved in dietary phosphate absorption is an attractive means for reducing this phosphate burden. We do not, however, fully understand this process and how it is regulated. This commentary describes recent findings regarding the novel role of bile acids in regulating paracellular phosphate (and calcium) absorption by the small intestine and the potential cellular mechanisms involved.

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Although it is accepted that calcium and phosphate homeostasis are intrinsically linked, the study of calcium homeostasis, and, in particular, the mechanisms governing intestinal calcium absorption, has received more attention than phosphate. Both dietary ions are absorbed from the gastrointestinal tract via transcellular and paracellular routes, with 1,25-dihydroxyvitamin D_3 ($1,25[OH]_2D_3$) recognized as a major regulator of transcellular calcium and phosphate absorption. Interestingly, studies have also reported that paracellular calcium absorption is regulated by $1,25(OH)_2D_3$ and that this involves altered expression of the cation-selective pore-forming claudins, claudin 2 and 12.[1] Based on these findings, it has been speculated that claudins may also be involved in paracellular phosphate absorption, although to date no specific isoform(s) have been shown to mediate phosphate flux. This commentary discusses recent findings regarding bile acids as novel regulators of the paracellular pathways for calcium and phosphate absorption and the potential regulators of these processes, including the involvement of the gut microbiome.

As reviewed by Marchionatti *et al.*, several studies describe a role for bile acids in the regulation of intestinal calcium absorption.[2] These are, however, often conflicting, with different bile acids inducing different responses (potentially via different mechanisms) depending on the type of bile acid investigated, the concentration used, and the receptor target. In comparison, the regulation of phosphate absorption by bile acids has not been investigated. The study by Hashimoto *et al.*[3] in this issue of *Kidney International* has for the first time investigated the impact of 4 major bile acids on intestinal phosphate handling. They showed that the primary bile acids cholic acid and chenodeoxycholic acid did not influence intestinal phosphate absorption, whereas the secondary bile acid, lithocholic acid (LCA), but not deoxycholic acid, enhanced absorption. Comparison of the effect of LCA in 25-hydroxyvitamin D-1α-hydroxylase (Cyp27b1) knockout mice, and systemic and intestinal-specific vitamin D receptor (VDR) knockout mice, demonstrated that changes in intestinal phosphate absorption occurred via activation of intestinal VDR. Surprisingly, given the involvement of VDR in the regulation of transcellular phosphate absorption, LCA did not alter sodium-dependent phosphate transport or increase mRNA or protein levels of the main sodium-dependent sodium-phosphate cotransporter, type IIb. Instead, LCA treatment of normal mice decreased protein levels of claudin 3, an effect that was absent in VDR knockout mice, leading the authors to speculate that changes in this barrier-forming, tight-junction protein are responsible for the altered intestinal phosphate absorption. The novel role for claudin 3 in paracellular phosphate absorption was subsequently confirmed with claudin 3 knockout mice, which had increased phosphate absorption in the jejunum, ileum, and colon.

In this study, and as previously described,[2] LCA also increased intestinal calcium absorption. This was independent of alterations in transient receptor potential vanilloid subfamily member 6 (TRPV6) mRNA and protein levels, indicating the involvement of the paracellular pathway. Interestingly, although claudin 2 and 12 have been shown to elicit $1,25(OH)_2D_3$-sensitive paracellular calcium absorption,[1] these proteins did not change in response to LCA. Importantly, claudin 3 knockout mice had normal levels of intestinal calcium absorption, suggesting that LCA-induced changes in claudin 3 levels and the subsequent increase in paracellular phosphate flux are not simply a result of the breakdown of the epithelial barrier function. Finally, using the 5/6 nephrectomy and adenine diet mouse models of chronic kidney disease (CKD), the authors demonstrated that the LCA-induced increase in paracellular calcium and phosphate absorption exacerbated medial vascular calcification.

These novel findings add to our understanding regarding the mechanisms of intestinal calcium and phosphate transport; in particular, demonstrating for the first time a role of claudin 3 in paracellular

phosphate flux. They also raise several interesting questions concerning the regulation of these processes and their physiological and pathophysiological relevance. It appears that activation of VDR by different agonists can selectively, and differentially, regulate the pathways for calcium and phosphate absorption, with $1,25(OH)_2D_3$ playing a major role in regulating transcellular transport, and with LCA impacting the paracellular pathway (Figure 1). How this occurs in terms of agonist binding and downstream signaling is unclear, however. Activation of VDR occurs in a ligand-dependent manner, whereby VDR forms a heterodimer with the retinoid X receptor (RXR), and in turn VDR/RXR heterodimers bind to vitamin D response elements (VDREs), resulting in altered transcription of target genes. The VDR/RXR-induced transcription of target genes is also modulated by other transcriptional coactivators and corepressors, which are recruited to the VDREs. Although it is recognized that of the numerous bile acids present in the gastrointestinal tract, only LCA and its major metabolites are agonists for VDR, binding to the same ligand-binding pocket as $1,25(OH)_2D_3$, this binding occurs in the opposite orientation.[4] Whether the altered binding orientation induces distinct VDR/RXR complexes and cofactors that result in different downstream events compared with $1,25(OH)_2D_3$ requires clarification.

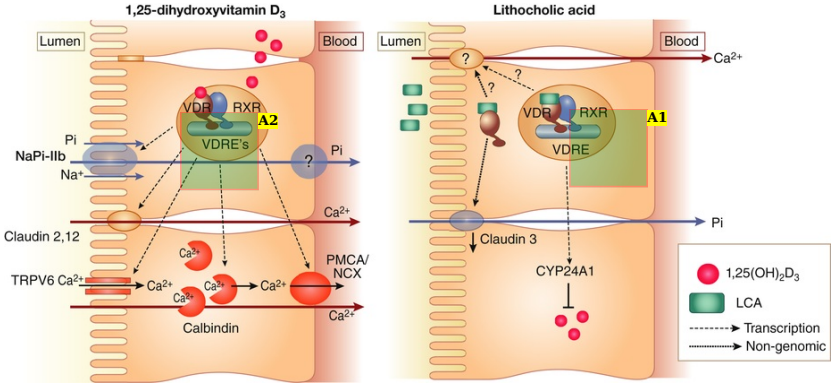

Figure 1 Vitamin D receptor-dependent regulation of intestinal phosphate and calcium absorption. 1,25-dihydroxyvitamin D_3 enhances transcellular phosphate (Pi) and calcium (Ca^{2+}) absorption via increased transcription of genes encoding for the sodium-phosphate cotransporter type IIb (NaPi-IIb), transient receptor potential vanilloid subfamily member 6 (TRPV6), calbindin, plasma membrane Ca^{2+} ATPase (PMCA), and sodium-calcium exchanger (NCX), and also increases paracellular calcium absorption as a result of increased claudin 2 and 12. In contrast, lithocholic acid (LCA) decreases claudin 3 protein levels via nongenomic vitamin D receptor (VDR) signaling to enhance paracellular phosphate absorption. It also increases paracellular calcium absorption, but the mechanisms are unknown.

Annotations:

A1. Could VDRE have a 's' included to be the same as the other panel = VDREs

A2. could the ' be removed from the VDRE's so that it matches with the text above

In addition, although LCA binds to VDR with a significantly lower affinity than $1,25(OH)_2D_3$, a recent study by Kollitz *et al.* has shown that LCA can effectively compete with the native ligand for the VDR binding site.[4] This raises the question as to whether competition between the 2 ligands for the same receptor has a physiological role in switching between transcellular and paracellular absorption to maintain calcium and phosphate homeostasis. It is also interesting to note that in the mouse ileum, preferential binding of LCA to VDR activates CYP24A1, the gene-encoding 25-hydroxyvitamin D-24-hydroxylase, whose catabolic enzyme activity controls intracellular levels of $1,25(OH)_2D_3$.[5] Whether this function of LCA-dependent VDR activation also plays a role in switching between the 2 pathways for absorption warrants investigation. Importantly, given that Hashimoto *et al.*[3] showed that the deleterious effect of LCA was potentiated in their mouse models of CKD, understanding exactly how LCA induces alterations in paracellular calcium and phosphate flux makes for an attractive novel therapeutic target for treating the vascular calcification commonly seen in patients with CKD.

Apart from their recognized role in lipid digestion and absorption, bile acids also play a role in intestinal fluid homeostasis, affecting either fluid absorption or secretion depending on their type and concentration.[6] Studies have started to establish the cellular transport mechanisms involved and suggest that in the distal colon bile acids can increase the activity of the epithelial sodium channel (ENaC) to promote absorption. This detailed study by Wiemuth *et al.*[7] screened the taurine conjugates of 6 bile acids commonly found in rat bile and demonstrated that 4 of these influence ENaC activity. This occurred to differing extents, with the cholesterol moiety of the bile acid, rather than the amino acid it is conjugated to, considered crucial for ENaC activation. In contrast, at high concentrations, bile acids reduce absorption via calcium-dependent inhibition of the apical Na^+/H^+ exchangers (NHEs) and SLC26 Cl^-/HCO_3^- exchanger (CBE) to induce diarrhea. However, they likely increase secretion due to activation of the basolateral potassium calcium-activated channel subfamily N Member 4 (KCNN4) and via alterations in proteins levels of aquaporins (AQP), specifically AQP3, 7, and 8.[6] In the small intestine, bile deficiency induced by biliary duct ligation

decreases protein levels of the sodium-glucose–linked transporter 1, resulting in reduced glucose absorption; both transport function and SGLT1 levels can be normalized by administration of a conjugated bile acid mixture.[8] These changes to intestinal glucose absorption are one of the ways in which bile acids appear to play their unexpected role in glucose homeostasis, although exactly which bile acids are involved has not been elucidated. Intriguingly, the study by Hashimoto *et al.*[3] also suggests that intestinal magnesium absorption may be regulated by LCA, although the underlying cellular mechanisms were not investigated. Therefore, although it is accepted that bile acids play a part in the pathogenesis of several diseases,[6] and as such can be therapeutically exploited, there is a growing understanding of how these molecules may also be involved in normal intestinal transport physiology. Importantly, future studies should systematically compare the effect of individual bile acids to elucidate their exact role in normal gastrointestinal physiology.

The physiological and pathophysiological impact of bile acids on intestinal function is inherently dependent on the gut microbiota. It plays a central role in the metabolism of bile acids, through deconjugation and dehydroxylation reactions, to generate unconjugated free bile acids and secondary bile acids, respectively. Importantly, the diversity of the gut microbiome significantly influences bile acid pool size and its composition, and numerous disease states, including CKD, can cause dysbiosis. Phosphate is one of the major nutrients used by gut bacteria for survival and reproduction, so changes in dietary phosphate levels may therefore affect the composition of the microbiome. Indeed, dietary phosphate restriction in patients with CKD has been shown to diminish symbiotic bacteria, whereas probiotic treatment of dialysis patients reduces serum phosphate levels, presumably through alterations to the bacterial population. In addition, Rahbar Saadat *et al.* propose that long-term ingestion of phosphate binders may also alter the gut microbiome and provide benefits beyond reduced phosphate absorption (reviewed in Rahbar Saadat *et al.*[9]). Exactly how alterations to the gut microbiome in CKD impact intestinal phosphate handling has not been studied in enough detail to draw any definitive conclusions, but perhaps the findings of Hashimoto *et al.*[3] shed some light on the potential link between the gut microbiome, bile acids, and phosphate homeostasis.

In conclusion, although numerous regulators of transcellular calcium and phosphate absorption have been identified, the factors that control paracellular calcium and phosphate absorption require more investigation. Understanding the cellular processes and how they are regulated will help develop receptor-selective drugs for the treatment and prevention of diseases associated with calcium and phosphate homeostatic dysregulation.

Disclosure

The author declared no competing interests.

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