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# New insights into phosphate homeostasis

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**Extracellular phosphate is crucial for many bodily functions, including skeletal development, energy metabolism, cell signalling and the regulation of protein synthesis. Body phosphate homeostasis is determined by the regulation of intestinal uptake of dietary phosphate, renal phosphate reabsorption and the exchange of phosphate between extracellular and bone storage pools. On a day-to-day basis, phosphate balance is achieved largely through the control of phosphate reabsorption in the proximal tubule: in the steady state, renal phosphate excretion reflects dietary intake, and daily urinary phosphate excretion correlates with its absorption from the diet.**

Disturbances in phosphate homeostasis can have important clinical consequences. Hyperphosphataemia is a common and serious complication of chronic renal failure (CRF),[1] leading to secondary hyperparathyroidism, and increased cardiovascular morbidity and mortality. Maintaining a normal plasma phosphate concentration (and calcium-phosphate solubility product) is critical to long-term survival in CRF.[2] However, targeting the kidney to prevent hyperphosphataemia in CRF is made difficult by the progressive decline in renal function. For this reason, attention has been focused on developing gut-related therapies to reduce plasma phosphate levels in patients with CRF. The mainstays of this approach are dietary restriction and/or the use of dietary phosphate binders. However, these treatments can lead to, on the one hand, malnutrition, and on the other, can contribute to accelerated vascular calcification. A more effective strategy would be to inhibit intestinal phosphate transport directly. Hypophosphataemia is less common clinically, but can occur as a consequence of malnutrition, malabsorption or inherited disorders affecting renal phosphate reabsorption, such as hypophosphataemic rickets, X-linked hypophosphataemia and tumour-induced osteomalacia (TIO).

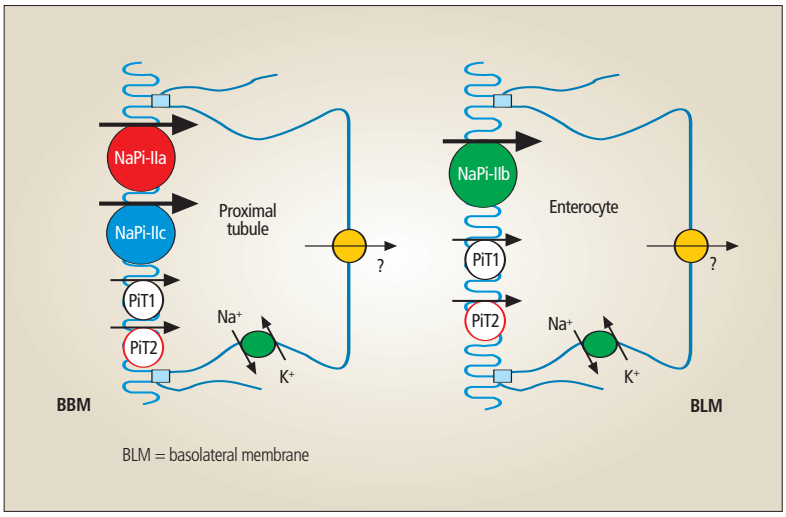


*Figure 1. Currently identified proteins responsible for phosphate transport across renal and intestinal epithelia. Arrow size represents the relative contribution of each transporter to phosphate transport across the brush border membrane (BBM). All transporters shown at the BBM are sodium dependent. The transporter responsible for efflux of phosphate from these cells has not yet been identified*

## Phosphate transporters

Normal phosphate balance utilises three sodium-dependent phosphate transporters (NaPi-IIa, NaPi-IIb and NaPi-IIc) that are differentially expressed at the brush border membrane (BBM) of renal and intestinal epithelial cells (see Figure 1). These transporters are members of the solute carrier family SLC34.[3]

In the kidney, the isoform NaPi-IIa is localised throughout the proximal tubule (S1–S3 segments), with highest expression in the S1 segment.[4,5] Numerous studies over the last 15 years have established that this protein is the major transporter involved in renal phosphate reabsorption, and that NaPi-IIa possesses molecular motifs responsible for its rapid regulation by endocytosis and exocytosis.[6,7] Parathyroid hormone (PTH) and dietary phosphate are the major regulators of NaPi-IIa; however, NaPi-IIa has also been shown to be regulated by other circulating factors, including vitamin D, growth factors, glucocorticoids and thyroid hormone.[6] More recently, a group of proteins known as phosphatonins has been identified, which, like PTH, can cause phosphaturia by inhibiting NaPi-IIa.[8]

Another renal isoform, NaPi-IIc, is expressed only at the BBM of the S1 segment of the proximal tubule.[9,10] Originally, NaPi-IIc was thought to play a role in phosphate reabsorption only during early growth and development, since its expression level seemed to depend on the age of the animal.10 However, recent re-evaluation of this transporter in renal phosphate handling has shown that in adult rodents it is regulated by

PTH,[11] dietary phosphate,[12,13] dietary magnesium,[14] metabolic acidosis,[9] and the phosphatonin FGF-23.[15] Importantly, changes in NaPi-IIc protein levels occur over a much longer time period than NaPi-IIa, and the mechanisms of internalisation are also different.[11–13] Interestingly, recent gene analysis studies have identified NaPi-IIc as the genetic cause of hereditary hypophosphataemic rickets with hypercalciuria (HHRH).[16,17] From these studies, and the finding that heterozygous mutations in NaPi-IIa do not result in changes in phosphate excretion,[18,19] it has been proposed that NaPi-IIc plays a more important role in phosphate homeostasis in man. In contrast, studies using NaPi-IIc knockout mice suggest that this transporter is more involved in the calcium/vitamin D axis, and that in rodents it plays only a minor role in phosphate homeostasis.[20] These findings indicate that the relative contribution of NaPi-IIa and NaPi-IIc to renal phosphate reabsorption, and thus phosphate homeostasis, is species specific.[21]

In the small intestine, the isoform NaPi-IIb is believed to be the rate-limiting step for phosphate absorption.[3,22] Dietary phosphate and 1,25 (OH)2D3 (1,25-dihydroxyvitamin D3) and are thought to be the most important physiological stimuli of intestinal phosphate absorption,[3,23] although epidermal growth factor,[24,25] glucocorticoids,[26,27] oestrogens[28] and systemic metabolic acidosis[29,30] can also affect intestinal phosphate absorption. Interestingly, the profile of phosphate absorption along the rat and mouse small intestine display striking differences.[31,32] The profile in the rat is much closer to that reported in man, making the rat a more appropriate animal model in which to study intestinal handling of phosphate. Indeed, there is growing recognition of the role of the gut in phosphate balance, especially when this is disturbed, as in renal failure; however, compared with the kidney, relatively little is known about the mechanisms and control of intestinal phosphate absorption.

Recent findings suggest that PiT1 and PiT2 proteins, members of the SLC20 family, are also involved in phosphate homeostasis. These proteins were originally identified as receptors for retroviruses, but have subsequently been shown to mediate sodium-dependent phosphate transport. In this context, they were originally considered to be located at the basolateral membrane (BLM) of proximal tubule cells and enterocytes, where they were thought to mediate the influx of phosphate from blood, so as to maintain cellular phosphate levels.[33,34] However, studies have shown that both PiT1 and PiT2 are expressed at the BBM of renal and intestinal epithelia,[35,36] and that changes in dietary phosphate load can affect the level of PiT2 protein in these tissues.[35,37] The finding that renal PiT2 (and PiT1) mRNA is also increased in metabolic acidosis[9] provides further evidence for the involvement of this class of transporter in renal phosphate handling. These findings highlight the need to investigate the role of these transporters in normal and abnormal phosphate homeostasis.

## Phosphatonins

Recent attention has focused on the novel circulating factors known as phosphatonins, and their role in phosphate balance. Phosphatonins are bone-derived phosphaturic proteins that rapidly reduce plasma phosphate concentration. They were first identified in plasma and bone tumours from patients with TIO, a disorder causing hypophosphataemia and renal phosphate wasting.[38] Phosphatonins have since been detected in diseases that are phenotypically similar to TIO such as X-linked hypophosphataemic rickets. The phosphatonins of current interest are fibroblast growth factor 23 (FGF-23),[39] secreted frizzled related protein 4 (sFRP-4),[40] and matrix extracellular phosphoglycoprotein (MEPE).[41] FGF-23 is the most studied phosphatonin to date, and has been shown to require the associated co-receptor Klotho for its cellular actions.[42] FGF-7 is the most recent phosphaturic growth factor to be isolated.[43,44]

**There is growing recognition of the role of the gut in phosphate balance**

Studies have established that the hypophosphataemic action of FGF-23, sFRP-4 and MEPE is due, at least in part, to reduced NaPi-IIa protein expression.[8] Recent evidence indicates that the small intestine is also a target for phosphatonin action. The hypophosphataemic action of FGF-23 involves both reduced expression of NaPi-IIa and suppression of renal 1,25 (OH)2D3 synthesis, which itself reduces NaPi-IIb protein expression and intestinal phosphate absorption.[45] Interestingly, studies in our laboratory suggest that other phosphatonins may more directly influence intestinal phosphate uptake, as the inhibitory action of MEPE on intestinal phosphate absorption is independent of circulating 1,25 (OH)2D3 levels.[46] Phosphatonins are, therefore, likely to have interrelated actions on both the kidney and small intestine, and are a potential means of manipulating phosphate balance to avoid hyperphosphataemia.

## Other novel regulators of phosphate homeostasis

A recent study showed that intraduodenal infusion of sodium phosphate, but not sodium chloride, promoted renal phosphate excretion within

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20 **[Units?]**.[47] The response was not due to altered plasma levels of phosphate, PTH, FGF-23, sFRP-4, increased GFR, or a result of any neural reflex. The authors concluded that raised levels of intestinal phosphate can trigger the release of a phosphaturic factor from the intestinal mucosa. This finding raises the question whether this phosphaturic factor can also affect intestinal phosphate transport, as well as wider issues of the role of gut-renal crosstalk in renal phosphate handling following changes in dietary intake of phosphate.

## The Ca2+-sensing receptor and phosphate transport

The Ca2+-sensing receptor (CaSR) was first localised to chief cells in the parathyroid gland, where it controls PTH secretion by detecting low levels of extracellular calcium and transducing this signal into increased PTH release. The receptor is also sensitive to other divalent (for example, Mg2+) and trivalent cations. CaSR has also been found in both renal and intestinal epithelia,[48,49] and recent studies have provided evidence that the CaSR might also play a more direct role in the regulation of renal phosphate handling. Short-term intravenous infusion of the calcimimetic R-568 decreased, whereas intravenous administration of the calcilytic NPS 2143 increased, phosphate excretion.[50,51] The fact that changes in dietary phosphate alter expression of both NaPi-IIa and the renal CaSR has led to the suggestion that co-regulation of CaSR and NaPi-IIa could be involved in the 'fine tuning' of phosphate reabsorption along the proximal tubule.[52] Moreover, 1,25 (OH)2D3 has been shown to regulate the renal CaSR,[53] and CaSR activation can blunt PTH-induced inhibition of renal phosphate absorption.[54] It has also been shown recently that the upregulation of NaPi-IIc protein, seen in response to a high magnesium diet, occurs directly through stimulation of the renal CaSR.[14]

## *Key points*

- **A potential effective strategy for treating CRF-induced hyperphosphataemia would be targeted inhibition of the intestinal phosphate transporter, NaPi-IIb by phosphatonins.**
- **In rodents and humans, the relative contribution of NaPi-IIa and NaPi-IIc to renal phosphate reabsorption and, therefore, phosphate homeostasis may differ.**
- **Reinvestigation of the mechanisms of phosphate transport by renal and intestinal epithelia, particularly the role of NaPi-IIc and the PiT transporters, is required to fully understand the contribution of these proteins to phosphate homeostasis.**

Despite the apparent association between CaSR and renal phosphate transport, little is known about the role of the intestinal CaSR. It may function as an amino acid 'taste receptor',[55] but its potential role in phosphate homeostasis is unknown. It is just possible that the actions of orally administered calcimimetics could involve a more direct effect on phosphate balance via an action on the intestinal CaSR, although this needs to be investigated.

Finally, vascular smooth muscle cells also express the phosphate transporters already described, as well as the CaSR. Increased cellular uptake of phosphate has been implicated in the process of vascular calcification, and so direct targeting of phosphate transport may have even wider benefits for CRF ■

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