Phosphate homeostasis and the renal-gastrointestinal axis

Joanne Marks,1 Edward S. Debnam,1 and Robert J. Unwin1,2
1London Epithelial Group, Department of Neuroscience, Physiology, and Pharmacology, and 2Centre for Nephrology, University College London Medical School, London, United Kingdom

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Phosphate Homeostasis

Body phosphate homeostasis is determined by modulation of intestinal uptake of dietary phosphate, renal phosphate reabsorption and excretion, and the exchange of phosphate between extracellular and bone storage pools. Until recently, the accepted view has been that phosphate balance is achieved mainly by control of phosphate reabsorption in the proximal tubule and that intestinal absorption of dietary phosphate plays only a limited role. However, studies have shown that instillation of phosphate into the small intestine, specifically the duodenum, can result in acute changes in serum phosphate concentration (46, 109) and that release of an “enteric phosphatonin” in response to a phosphate load rapidly promotes renal phosphate excretion (12). Moreover, studies using sodium-phosphate cotransporter NaPi-IIb knockout (NaPi-IIb−/−) mice have shown that absence of this intestinal brush-border membrane (BBM) transporter triggers compensatory renal mechanisms to maintain phosphate homeostasis (109). Thus these findings suggest that intestinal phosphate absorption plays a more significant part in phosphate homeostasis than was previously recognized.

Disturbances in phosphate homeostasis may signify and cause important clinical disorders. Hypophosphatemia may be due to malnutrition, malabsorption, or inherited disorders affecting renal phosphate reabsorption such as hypophosphatemic rickets, X-linked hypophosphatemia, and tumor-induced osteomalacia (4, 16). Prolonged phosphate deficiency is associated with bone demineralization, leading to skeletal defects such as rickets in children and osteomalacia in adults, and can also increase the risk of nephrolithiasis (97, 103), rhabdomyolysis, hemolysis, respiratory failure from muscle weakness, and reduced myocardial contractility (4). Hyperphosphatemia is a common and serious complication of chronic renal failure (CRF) (54), contributing to secondary hyperparathyroidism, and to the increased cardiovascular morbidity and mortality. Treatments to maintain a normal serum phosphate concentration (and calcium-phosphate solubility product) are important for long-term survival in CRF (70); however, targeting the kidney to prevent hyperphosphatemia is made more difficult by the progressive decline in renal function, resulting in a reduced ability of the kidneys to excrete excess phosphate and respond to phosphaturic agents or hormones like parathyroid hormone (PTH). Therefore, more attention has been devoted to developing gut-related therapies to control serum phosphate levels. The mainstays of this approach are dietary phosphate restriction and/or the use of dietary phosphate binders (most of which are anion exchangers), but these treatments may worsen malnutrition in CRF and might even contribute to accelerated vascular calcification. A more effective strategy against hyperphosphatemia would be to directly target phosphate absorption by inhibiting intestinal transport of phosphate;
however, in contrast to the kidney, less is known about the mechanisms involved in enterocyte phosphate uptake or how it is regulated. In addition to this lack of information, there are important constraints to the experimental approaches that are used to study intestinal phosphate transport, as well as marked differences between the rat and mouse models commonly used to investigate phosphate absorption. This review aims to summarize our current understanding of the mechanisms and regulation of intestinal phosphate transport, as well as its relationship to renal phosphate handling.

**Sodium-Dependent Phosphate Cotransporters**

Classically, phosphate balance is thought to depend on the function of sodium-dependent phosphate transporters that are members of the solute carrier family SLC34 (85), which include three type II transporters, NaPi-IIa (SLC34A1), NaPi-IIc (SLC34A3), and NaPi-IIb (SLC34A2). Our current understanding of phosphate movement across the proximal tubule cell and enterocyte is that expression of these transporters at the BBM provides the rate-limiting step for the transcellular uptake of phosphate (85, 88). The basic mechanism of phosphate transport across the proximal tubular and small intestinal BBM is very similar, although tissue-specific transport proteins are involved. Typically, these have been recognized to be NaPi-IIa and NaPi-IIb in the kidney and gut, respectively. However, evidence is now emerging that members of the SLC20 family, or type III transporters, specifically PiT1 and PiT2, may also play an important role in phosphate transport. These proteins were originally identified as receptors for retroviruses, but they have now been shown to mediate sodium-dependent phosphate transport (26). Early studies proposed that PiT transporters were located at the basolateral membrane (BLM) of proximal tubule cells and enterocytes and that they mediated the entry of phosphate from the interstitial fluid (7, 26). However, it is now clear that PiT2 is present at the renal BBM (22, 134) and PiT1 at the intestinal BBM (46), and that the abundance of these proteins is controlled by dietary phosphate load (22, 46, 134). In contrast, very little is known about the processes involved in the movement of phosphate across the BLM into the blood.

The type II and type III families of transporters have distinct characteristics: they differ in their preferences for phosphate, their regulation, and whether they are inhibited by phosphonoformic acid (PFA) (summarized in Figs. 1 and 2). Type II transporters have a preference for divalent \( \text{HPO}_4^{2-} / \text{H}_2\text{PO}_4^- \) phosphate and are inhibited by PFA; NaPi-IIa and NaPi-IIb are electrogenic and transport phosphate with a stoichiometry of 3:1 \( \text{Na}^+ / \text{H}_2\text{PO}_4^- \):\( \text{HPO}_4^{2-} / \text{H}_2\text{PO}_4^- \), whereas, NaPi-IIc is electroneutral with a 2:1 \( \text{Na}^+ / \text{H}_2\text{PO}_4^- \) stoichiometry. In contrast, type III transporters prefer monovalent phosphate \( \text{H}_2\text{PO}_4^- \); they are all electrogenic and are not inhibited by PFA (at least at concentrations \(<1 \text{ mM} \) and they have a reported stoichiometry of 2:1 \( \text{Na}^+ : \text{H}_2\text{PO}_4^- \) (reviewed in detail in Ref. 137).

Fig. 1. Factors controlling type II and type III phosphate transporters in the rat and mouse proximal tubule. Arrows represent increased (↑), decreased (↓), or no change (↔) in protein expression or transport activity of each transporter by the different regulatory factors; phosphonoformic acid (PFA) inhibits type II transporters, but it has no effect on type III transporters.
Renal Phosphate Transport

Since the main focus of this review is intestinal phosphate transport, the reader is referred to several recent and comprehensive reviews of renal phosphate transport (15, 42, 104). The following section (summarized in Fig. 1) briefly discusses our current understanding of phosphate handling by the kidney, with particular emphasis on the likely involvement of type III transporters.

Renal Type II Transporters

The type II transporters, NaPi-IIa and NaPi-IIc, are expressed at the proximal tubule BBM, and they are collectively responsible for the reabsorption of ~80% of filtered phosphate. NaPi-IIa has been localized throughout the proximal tubule (S1–S3 segments), with highest protein levels found in the S1 segment (27, 29). Generation of NaPi-IIa knockout mice (NaPi-IIa\(^{-/-}\)) has established that this transporter is responsi-
ble for ~70% of renal phosphate absorption (8). PTH, FGF-23, and dietary phosphate are considered to be the major regulators of NaPi-IIa protein levels. BBM expression of NaPi-IIa is reduced within minutes in response to PTH (6, 59), and within 2 h in response to altered dietary phosphate load (117, 134). This adaptation occurs by activation of molecular motifs within the protein that are responsible for its endocytosis or exocytosis, together with alterations in protein-protein interactions that stabilize the protein at the BBM (15, 86, 87).

NaPi-IIc protein is highly expressed in rodents during weaning, with levels diminishing with age (112); in contrast to NaPi-IIa, this protein is only present in the S1 segment of the proximal tubule (93, 112). Recent reevaluation of the role of this transporter in renal phosphate handling in adult rodents has shown that expression of NaPi-IIc protein is regulated by dietary phosphate (117, 133), dietary magnesium (130), metabolic acidosis (93), and FGF-23 (114); however, there are conflicting reports on the role of PTH in NaPi-IIc regulation (84, 104, 130). Importantly, changes in NaPi-IIc protein levels in response to dietary phosphate occur over a longer time course than NaPi-IIa (117, 134), and the cellular mechanisms of internalization of the transporter are different (117, 133). Studies using NaPi-IIc−/− mice have shown that protein levels of NaPi-IIc are increased and that this protein may account for ~30% of renal phosphate reabsorption (129). Somewhat surprisingly, studies using NaPi-IIc knockout mice suggest that this transporter is involved in the calcium/vitamin D axis and that in rodents, at least, it plays only a minor role in phosphate homeostasis (116). Interestingly, genetic studies in humans have recently identified NaPi-IIc mutations as the cause of hereditary hypophosphatemic rickets with hypercalciuria (HHRH) (9, 56). From these studies, and the finding that heterozygous mutations in NaPi-IIa do not result in changes in phosphate excretion (66, 138), it has been proposed that NaPi-IIc plays a more significant role in phosphate homeostasis in humans (139). However, there has been a recent report of a recessively inherited form of the renal Fanconi syndrome (with hypophosphatemia and phosphaturia, but without hypercalciuria) due to a homozygous loss-of-function mutation in NaPi-IIa (77). While this may suggest a more significant, or even dominant, role for NaPi-IIa in human renal phosphate handling, the puzzle is the associated and more generalized proximal tubular dysfunction and renal impairment, which have been attributed to the cytosolic retention and putative toxicity of the mutant NaPi-IIa protein.

Renal Type III Transporters

Interestingly, even though double knockout mice for NaPi-IIa and NaPi-IIc display severe hypophosphatemia, and significantly increased phosphaturia, there is still residual renal phosphate reabsorption (115). Thus other phosphate transporters are able to maintain some phosphate reabsorption in the absence of type II transporters. In this context, PiT1 and PiT2 mRNAs have been detected in the kidney. Reports have shown that renal PiT1 and PiT2 gene expression is increased in metabolic acidosis (93) and that PiT2 mRNA is decreased during dietary potassium deficiency (22). In contrast, to NaPi-IIc, renal PiT2 mRNA is expressed at the same level during weaning and maturation (69).

To date, PiT2, but not PiT1, protein has been localized in the kidney. Immunohistochemistry has shown PiT2 to be present at the BBM and that its abundance is regulated by dietary phosphate (134). Localization of the transporter in rats depends on dietary phosphate load: under normal phosphate conditions, the protein is restricted to the S1 segment, whereas dietary phosphate restriction induces expression of PiT2 protein in all segments of the proximal tubule (22). In the kidney, the type III transporters adapt to dietary phosphate composition more slowly than the type IIa transporters, with changes in PiT2 protein detectable only after 8 h (134). In addition, dietary potassium deficiency (22) and FGF-23 (131) decrease and metabolic acidosis increases (136) renal PiT2 protein levels. However, although changes in gene expression and protein level suggest that PiT2 may play a role in renal phosphate reabsorption, functional evidence is still lacking. Elegant studies measuring the pH dependence of phosphate uptake into BBM vesicles suggest that the involvement of PiT2 in phosphate uptake can vary with pH (136) and that it could contribute from ~3 to ~40% of total phosphate uptake at pHs of 7.5 and 6.0, respectively. However, proximal tubular fluid pH, even under conditions of metabolic acidosis, does not fall below pH 6.6 (35, 48), making it unlikely that PiT2 would make more than a modest contribution to renal phosphate reabsorption in vivo. However, until a specific inhibitor of PT-mediated transport is identified, the functional role and contribution of type III transporters to renal phosphate handling under different conditions will remain uncertain.

Intestinal Phosphate Transport

Much less is known about intestinal phosphate transport compared with renal phosphate reabsorption (summarized in Fig. 2). Early studies in the rat revealed that the duodenum and jejunum were responsible for the bulk of intestinal phosphate absorption (30, 140) and that transport could be resolved into sodium-dependent and -independent components (19, 21, 68). Kinetic analysis of phosphate transport across rat jejunal BBM revealed a process with a K_m of 0.1 mM (14, 73, 74). The type II phosphate transporter NaPi-IIb was later identified and proposed to be exclusively responsible for the sodium-dependent component of transport (51). Genetic studies of NaPi-IIb expressed in Xenopus laevis oocytes revealed this protein to be a high-affinity phosphate transporter with a K_m of ~10 μM (43, 135). It has been shown subsequently that NaPi-IIb is regulated by a number of factors, including 1,25-dihydroxyvitamin D_3 [1,25(OH)_2D_3] and dietary phosphate load (49, 85). In contrast, the sodium-independent component of phosphate transport seems to be unregulated (31, 57, 58).

Immunohistochemical studies in mice have localized NaPi-IIb protein to the BBM along the entire villus length (148). Other work using autoradiography has shown that phosphate uptake is restricted to enterocytes in the mid- to upper regions of duodenal and jejunal villi (79), a pattern of distribution that has also been found for active uptake of other substrates such as glucose (33), iron (94), and 1,25(OH)_2D_3-stimulated calcium transport (17). This localized distribution probably reflects the time required for fully functional expression of phosphate transport proteins and the development of the electrochemical driving force for uptake across the BBM, both of which occur during enterocyte migration along the villus.
Role of NaPi-IIb in Intestinal Phosphate Transport

An important finding from studies reevaluating intestinal phosphate transport in rodents is that NaPi-IIb protein is expressed in the ileum of mice (105, 126), a region that was not considered previously to be a major site of phosphate absorption. It is now clear that the profile of phosphate absorption along the rat and mouse small intestine displays important differences (Fig. 2) (80, 105). Studies using the in situ intestinal loop technique have shown that phosphate is absorbed along the entire mouse small intestine, the highest rate occurring in the ileum, which is paralleled by the highest levels of NaPi-IIb mRNA and protein. In contrast, maximal absorption across the rat small intestine is seen in the duodenum and jejunum, with very little phosphate absorption occurring in the ileum (80). In vitro studies using everted sacs (109) and BBM vesicles (46, 105) have confirmed these species differences in the localization of phosphate absorption; significantly, the pattern seen in rats is similar to that reported in humans (19, 141). However, it is important to note that segmental studies may not reflect regional contributions to overall phosphate transport in the intact unanesthetized animal, because transit times through individual intestinal segments must also be taken into account. One study of compartmental modeling in the rat that took into consideration transit times demonstrated that the upper, mid-, and lower small intestine contribute equally to overall phosphate absorption (143). Since NaPi-IIb expression is highest in the rat proximal small intestine (46), it is reasonable to speculate that there is an as yet unidentified transport process responsible for ileal phosphate absorption.

Despite the importance of NaPi-IIb in intestinal phosphate transport, there remains a lack of consensus on the relative contributions of sodium-dependent and -independent phosphate transport. A recent in vivo study showed no apparent requirement of phosphate uptake for sodium (143); in contrast, other studies concluded that sodium-dependent mechanisms are largely responsible for phosphate uptake in the rat jejunum and mouse ileum (38, 109). The involvement of NaPi-IIb protein in the sodium-dependent component has been confirmed using PFA (38) and nicotineamide (109), both of which are type II transporter inhibitors, and by finding that sodium-dependent phosphate transport is absent in NaPi-IIb−/− mice (109). However, it is important to note that studies comparing phosphate absorption in NaPi-IIb−/− and wild-type mice showed that even when the protein is maximally induced in wild-type animals by feeding them a low-phosphate diet, it only contributes ~50% to overall phosphate absorption following an acute phosphate load (109). Interestingly, inactivating mutations of NaPi-IIb in humans do not seem to result in a significant reduction in serum phosphate levels, as might be expected if this protein plays a dominant role in intestinal phosphate absorption (28). However, there is growing evidence that when intestinal NaPi-IIb protein is downregulated, there is an increase in renal NaPi-IIa protein expression (109); conversely, upregulation of NaPi-IIa results in a decrease in NaPi-IIb protein levels (106). At present, the signals for these compensatory changes in renal and intestinal phosphate handling that can maintain normophosphatemia have not been identified.

Thus studies of intestinal phosphate absorption have provided important insights into the mechanisms involved, but they have also highlighted the difficulty in making generalizations from data obtained using different experimental methods and different animal species, often of varying maturity. Our interpretation is that active sodium-dependent phosphate transport is NaPi-IIb mediated and that it occurs maximally in the rat jejenum and mouse ileum. However, this transporter might only dominate under fasting or low dietary phosphate conditions, and sodium-independent transport may prove to have a more important role in overall phosphate absorption along the small intestine.

Intestinal Type III Transporters

As in the kidney, there has also been interest in the potential role of type III transporters in intestinal phosphate transport. A recent study revealed that PiT1 mRNA is present throughout the small intestine, with the highest levels found in the ileum; in contrast, mRNA levels of PiT2 are low in all intestinal segments. Gene expression of PiT1 is unaffected by changes in dietary phosphate (46), but mRNA levels of PiT2 may be increased by 1,25(OH)2D3 (57).

Interestingly, gene expression of NaPi-IIb and PiT1 mRNAs also occurs in rat distal colon, a region that is not usually considered to be a site of active phosphate absorption (Wagner CA, personal communication). If confirmed, this observation could have implications for the effectiveness of phosphate binders in CRF. The high levels of short-chain fatty acids normally present in the colonic lumen would be expected to displace phosphate from the anion exchanger binding sites and make it available for absorption (144), potentially negating any upstream benefit of binders on phosphate balance.

PiT1 protein has been localized to the BBM of enterocytes in the rat duodenum and jejunum, with the highest levels found in the jejunum. Although gene expression of PiT1 is highest in the rat ileum, its protein is not found there, at least under normal dietary phosphate conditions (46). PiT2 protein has recently been detected in the mouse ileum, although a regional profile for this transporter has not been reported (106). In contrast to PiT2 in the kidney, protein levels of PiT1 (46) in the small intestine seem not to be regulated by changes in dietary phosphate concentration. The precise role of PiT transporters in intestinal phosphate absorption remains to be determined; however, in light of the recent finding that sodium-dependent phosphate transport is not present in NaPi-IIb−/− mice (109), it seems unlikely that PiT-mediated transport constitutes a major alternative pathway for sodium-dependent phosphate absorption.

Regulation of Intestinal Phosphate Transport

The phosphate requirement in young animals is elevated because of its importance to skeletal development during rapid growth. As in the kidney, there is an age-related decline in intestinal phosphate absorption (18, 145) that has been correlated with decreased gene and protein expression of NaPi-IIb (5, 61, 145). Our own preliminary data indicate an age-dependent decrease in intestinal gene expression for PiT2 (Nadaraja S, personal communication), but not PiT1.

Dietary phosphate and 1,25(OH)2D3 are thought to be the most important physiological regulators of intestinal phosphate absorption (49, 85), although EGF (146, 147), glucocorticoids (5, 20), estrogens (148), metabolic acidosis (44, 126) and, more
recently, phosphatoninins (78, 83) also affect intestinal phosphate absorption. Unlike the control of NaPi-IIa in the kidney, the current view is that PTH does not regulate NaPi-Iib expression directly (68, 88) but that it can affect intestinal transport of phosphate indirectly by its stimulatory effect on 1,25(OH)₂D₃ synthesis.

Early studies suggested that increased intestinal phosphate transport induced by a low-phosphate diet depended on stimulation of renal 25-OH-vitamin-D₃-1α-hydroxylase (1αOHase), leading to raised circulating levels of 1,25(OH)₂D₃ (32, 50) and enhanced NaPi-Iib protein expression. However, the finding that upregulation of NaPi-Iib in response to a low-phosphate diet occurs in vitamin D receptor null mice, and in 1αOHase-deficient mice (24, 113), suggests that diet-related alterations in intestinal phosphate absorption can occur independently of changes in 1,25(OH)₂D₃. More recent studies have reevaluated the regional response of the small intestine to changes in dietary phosphate and 1,25(OH)₂D₃ status. Adaptation of rats to a low-phosphate diet for 7 days results in increased sodium-dependent phosphate transport and NaPi-Iib protein levels in the jejunum, but not in the duodenum (46). A similar regional response is seen in rats treated for 24 h with 1,25(OH)₂D₃ (80). In contrast, rats adapted to a low-phosphate diet and then acutely exposed to a high-phosphate diet showed an unexpected increase in duodenal, but not jejunal, phosphate uptake and NaPi-Iib protein levels (46). These findings highlight the importance of NaPi-Iib in both acute and chronic adaptation of intestinal phosphate transport, and they also suggest that different regulatory mechanisms may be region specific.

A group of proteins collectively known as phosphatoninins, due to their phosphaturic effect, have emerged as novel candidates in the regulation of phosphate homeostasis. The phosphatoninins of current interest are FGF-23, so far the most prominent one (121), FGF-7 (25), secreted frizzled-related protein 4 (sFRP-4) (10), and matrix extracellular phosphoglycoprotein (MEPE) (108). All have been shown to induce hypophosphatemia through a reduction in NaPi-IIa protein expression and to cause increased renal phosphate excretion (11); however, little is known about their effects on intestinal phosphate transport. Detailed information on the secretion and general actions of phosphatoninins can be found in several recent reviews (11, 60, 118, 142).

FGF-23 is the most studied phosphatonin to date, and it is now widely accepted as a key regulator of phosphate homeostasis: gain-of-function mutations in FGF-23 are the cause of autosomal dominant hypophosphatemic rickets. FGF-23 has been shown to reduce expression of NaPi-IIa and NaPi-IIc at the proximal tubule BBM (45, 114) and to influence circulating 1,25(OH)₂D₃ concentrations: transgenic mice overexpressing FGF-23, or normal mice treated with FGF-23, have reduced serum 1,25(OH)₂D₃ levels (13, 120, 122), and FGF-23 null mice have increased serum 1,25(OH)₂D₃ concentrations (121). These changes in 1,25(OH)₂D₃ occur through an effect of FGF-23 on renal 1αOHase activity (123) and result in reduced intestinal sodium-dependent phosphate transport due, at least in part, to decreased expression of NaPi-Iib protein (55, 83, 110).

It is now known that FGF-23-induced changes in renal phosphate transport and 1,25(OH)₂D₃ synthesis are dependent on its permissive interaction with the protein Klotho (65, 91, 92). Interestingly, Klotho exists in membrane-bound and circulating forms (39, 81), and it is emerging that these probably have distinct modes of action. Membrane-bound Klotho interacts with FGFR1c at the BLM of proximal tubule cells to form high-affinity receptors for FGF-23 (39, 45, 47, 127). Activation of this complex results in reduced NaPi-IIa and NaPi-IIc protein expression, and decreased 1,25(OH)₂D₃ synthesis, as described above. In contrast, secreted Klotho has been shown to increase cell surface expression of some renal cation channels, for example, TRPV5 and ROMK, via its intrinsic enzyme activity (reviewed in Ref. 64). Recent data suggest that the secreted form of Klotho can also influence renal phosphate handling. Hu et al. (52) have shown in an elegant study that Klotho mRNA and protein are present in the proximal tubule and, using free-flow tubule micropuncture studies, that Klotho protein is secreted into proximal tubular fluid. The secreted protein, through its glucuronidase activity and independent of any interaction with FGF-23, directly inactivates surface membrane-expressed NaPi-IIa, resulting in phosphaturia (52). Intriguingly, a form of Klotho known as β-Klotho has been found in the small intestine; it interacts with mouse FGF-15 (human FGF-19) to inhibit sodium-dependent bile acid transport (125), but whether this or another isofrom could have a similar enzymatic effect on NaPi-IIb is unknown.

MEPE is the only other phosphatonin known to influence intestinal phosphate absorption. However, unlike FGF-23, the inhibitory action of MEPE on intestinal phosphate absorption occurs independently of changes in circulating levels of 1,25(OH)₂D₃, and its effect on phosphate transport is confined to the jejunum (78). Although the major cellular sources of MEPE are osteoblasts, osteocytes, and odontoblasts (107), MEPE mRNA and immunoreactivity have been detected in the normal kidney, with highest levels of the protein present in the BBM of proximal convoluted tubules (95). This has led to the suggestion that MEPE could be an autocrine or paracrine factor secreted into the tubular lumen, where it may bind to apical cell surface receptors. Our unpublished data indicate that MEPE mRNA is also expressed in the small intestine, with highest levels present in the duodenum (Fig. 3). Taken together, these findings suggest that MEPE might be a local regulator of phosphate transport in both the kidney and small intestine. The potential for FGF-7 and sFRP-4 to influ-
ence intestinal phosphate transport is unknown. Based on current evidence, it seems likely that phosphatoninns have complex interrelated actions on both the kidney and small intestine and that they can act on these targets to control phosphate balance and prevent hyperphosphatemia.

Gut-Renal Interactions in Control of Serum Phosphate Concentration

It has recently been proposed that the small intestinal mucosa secretes a substance with phosphatonin-like actions in the kidney. Thus intraduodenal infusion of 1.2 M sodium phosphate, but not sodium chloride, increased phosphate excretion within 20 min (12). The response was not due to altered serum levels of phosphate, PTH, FGF-23, sFRP-4, an increase in glomerular filtration rate, or the result of a neural reflex. The authors concluded that an intestinal phosphate sensor triggers the release of a phosphaturic factor from the duodenal mucosa. This observation also raises the question of whether the phosphaturic factor could locally affect intestinal phosphate transport following changes in dietary phosphate load. We have shown gene expression of MEPE in the small intestine, particularly in the duodenum (Fig. 3) and so MEPE might be the “intestinal phosphatonin” proposed by Berndt et al. (11). Since postprandial-elevated levels of serum phosphate have been linked to increased cardiovascular risk, particularly in patients with CRF (2, 34, 124), the actions of this phosphaturic factor could have implications for control of intestinal phosphate transport in the response to altered dietary phosphate and for the management of hyperphosphatemia. As already mentioned, acute feeding of a high-phosphate diet to rats rapidly increases serum phosphate levels as a result of increased duodenal NaPi-IIb protein expression (46). This adaptation is associated with a decrease in renal NaPi-IIa protein expression, presumably to restore phosphate balance. Moreover, acute administration of a phosphate load to NaPi-IIb−/− mice leads to a blunting of the expected increase in serum phosphate level (109). Thus the existence of an intestinal phosphatonin raises the possibility of targeting NaPi-IIa activity to prevent hyperphosphatemia in susceptible individuals. Another intriguing phenomenon that may have relevance to a potential link between the gastrointestinal tract and kidney in phosphate handling is the phosphaturia (and hypophosphatemia) commonly observed following partial hepatectomy (90). FGF-23, FGF-7 and sFRP4, and PTH, do not seem to be responsible for this effect; however, MEPE and its biologically active proteolytic breakdown products known as acidic serine-aspartate-rich-motif peptides (ASARM), which are also phosphaturic, might be, since the liver is a rich source of the protease cathepsin B that can produce ASARM from MEPE (89).

Intestinal Phosphate Transport in CRF

Patients with CRF present with hyperphosphatemia, 1,25(OH)2D3 deficiency, secondary hyperparathyroidism, and increased serum FGF-23 concentrations. It has been suggested that FGF-23 contributes to the maintenance of normal serum phosphate levels during declining renal function; however, at GFR values below 30 ml/min, the regulatory influence of FGF-23 fails, resulting in hyperphosphatemia (40, 119). The apparent renal resistance to FGF-23 in CRF may depend on a number of factors: it could be the result of decreased levels of NaPi-IIa protein per se, or a result of reduced levels of the Klotho-FGFR1 receptor complex, a phenomenon that occurs in the parathyroid glands of uremic patients (63). While levels of renal Klotho are known to decline in the kidneys of patients with CRF (62, 63), it has not been established whether the expression of FGFR1 receptors also decreases. However, it has been shown that the C-terminal fragment of FGF-23 can bind to the Klotho-FGFR1 signaling complex and competitively inhibit FGF-23 bioactivity (47). Whether this finding has any relevance to FGF-23 resistance in CRF has not been confirmed; moreover, both increased intact (119) and C-terminal (67) FGF-23 have been reported in CRF, but the relative ratios of each have not been determined. The mechanisms involved in the failure of FGF-23 to maintain phosphate balance in CRF and its role in the development of secondary hyperparathyroidism are areas of current and intense investigation.

Experimental 5/6 nephrectomy has proved to be a good model for clinical CRF (53, 96, 111) and has been widely used to investigate the effects of CRF on phosphate balance. Phosphate excretion is enhanced in CRF animals, the result of a decrease in renal NaPi-IIa protein expression (36), whereas intestinal phosphate transport and NaPi-Iib expression are unaffected in this model (73, 79). Attempts to reduce intestinal phosphate absorption using phosphate binders (3, 54) and a low-phosphate diet are standard therapies in the management of hyperphosphatemia in CRF. Studies in CRF animals show that dietary phosphate restriction decreases urinary phosphate excretion via increased NaPi-IIa expression (36), but it has no effect on intestinal phosphate handling (79). However, since renal function is impaired in CRF, renal adaptation to a low-phosphate diet has a limited impact on serum phosphate concentration.

The differential effect of CRF on renal and intestinal phosphate absorption indicates that specific mechanisms regulate phosphate transport in these tissues. PTH is considered to be a major physiological regulator of renal phosphate reabsorption, with high circulating PTH levels stimulating rapid endocytosis of NaPi-IIa from the proximal tubule BBM (6, 132). In contrast, PTH does not directly influence intestinal expression of NaPi-IIb (68, 88), which may explain, at least in part, the failure to observe changes in intestinal phosphate absorption in CRF. However, increased serum levels of FGF-23 and the deficiency of 1,25(OH)2D3 in CRF would be expected to inhibit intestinal phosphate transport; yet, the appropriate decrease in serum FGF-23 and increase in 1,25(OH)2D3 in response to dietary phosphate restriction is maintained in CRF rats (111), findings that are at variance with the unaltered intestinal phosphate absorption in CRF. At present, the mechanisms responsible for these differences in phosphate handling in normal and CRF animals remain unclear, but it seems that the 1,25(OH)2D3-vitamin D receptor axis does not influence intestinal phosphate absorption in CRF.

Phosphate binders are widely used to control hyperphosphatemia in clinical CRF; however, none of the currently available binders is ideal. Aluminum-containing binders are highly effective but are not used because of the risk of aluminum toxicity (3, 72). Calcium-containing phosphate binders in the form of calcium carbonate and calcium acetate are inexpensive and effective in reducing intestinal phosphate absorption and in lowering circulating phosphate levels (72); but, they can cause hypercalcemia, especially when given with...
vitamin D analogs (as they often are), which may promote vascular calcification (3, 70). Aluminum- and calcium-free phosphate binders are now available, but they are expensive and less effective at binding phosphate than the more traditional binders (72). A novel binder that has shown some promise in clinical trials is lanthanum carbonate (3, 71). It has been shown to control hyperphosphatemia without adding to body calcium load (41). Adverse effects are primarily gastrointestinal in nature but are no more troublesome than conventional treatments (41); in clinical trials, no toxic effects have been reported over 4 years of follow-up (102). However, another potential problem with widespread use of phosphate binders in an aging population of patients with CRF is the commonly associated constipation and the increased risk of bowel perforation with underlying diverticular disease (1).

An alternative approach to the use of binders to limit hyperphosphatemia in CRF is to target renal and intestinal transport of phosphate directly. Several potential inhibitors have been investigated. PFA is a competitive inhibitor of renal and intestinal phosphate transport in vitro (76); however, there are conflicting reports on its ability to reduce serum phosphate concentration in CRF rats (23, 75). Administration of nicotinamide to rats with CRF also decreases phosphate uptake across the jejunal BBM (58) and prevents hyperphosphatemia (37); measurements of serum urea and creatinine suggest that nicotinamide can also prevent or slow a further decline in renal function (37). When given to hemodialysis patients, oral nicotinamide reduced hyperphosphatemia (128), although treatment was associated with significant gastrointestinal side effects that may limit its clinical usefulness. Phosphophloretin, a phosphorylated form of the plant chalcone phloretin, has been shown to be a competitive inhibitor of renal (100) and intestinal sodium-dependent phosphate transport in rabbits (98), rats (98), and humans (99) and to reduce serum phosphate levels in normal (98) and uremic rats (101). Finally, JTP-59557, a triazole derivative, is a noncompetitive inhibitor of phosphate transport across the rat small intestine in vivo, which is probably due to a action on NaPi-IIb (82). However, the effect of this compound on phosphate absorption in uremic rats is unknown.

It is now becoming clear that in the face of declining renal function, the small intestine may be an important therapeutic target in the management of hyperphosphatemia. However, to develop effective therapies it is important that we understand more about the mechanisms that control intestinal phosphate transport. Over the past few years, our understanding of this process has increased significantly; specifically, generation of a NaPi-IIb/− mouse has clearly demonstrated a role for this transporter in intestinal phosphate absorption. However, whether NaPi-IIb only functions during conditions of fasting or low dietary phosphate needs to be confirmed. The finding that PiT1 is expressed at the enterocyte BBM means that the role of this transporter in phosphate transport has to be more carefully examined, particularly in the context of its relative contribution to sodium-dependent uptake. Interesting regional-specific adaptations of intestinal phosphate transport to acute and chronic dietary phosphate loads are also beginning to emerge. New findings about the function of these different regions and the evidence suggesting that acute changes in duodenal phosphate load can influence renal phosphate handling have significantly increased our understanding of the regulatory role of the small intestine in phosphate homeostasis.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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F295

Review

INTESTINAL PHOSPHATE ABSORPTION

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