Minireview

Intestinal phosphate absorption: The paracellular pathway predominates?

Matthew Saurette[1,2] and R Todd Alexander[1,2,3]

[1]Department of Physiology, University of Alberta, Edmonton, Alberta T6G 2R7, Canada; [2]The Women's & Children's Health Research Institute, Edmonton, Alberta T6G 1C9, Canada; [3]Department of Pediatrics, University of Alberta, Edmonton, Alberta T6G 2R7, Canada
Corresponding author: R Todd Alexander. Email: todd2@ualberta.ca

Impact statement

This review summarizes the work on transcellular intestinal phosphate absorption, arguing why this pathway is not the predominant pathway in humans consuming a "Western" diet. We then highlight the recent evidence which is strongly consistent with paracellular intestinal phosphate absorption mediating the bulk of intestinal phosphate absorption in humans.

Abstract

Hyperphosphatemia is nearly universal in patients with advanced chronic kidney disease and end stage renal disease. Given the considerable negative sequelae associated with hyperphosphatemia, i.e. increased cardiovascular disease, hastening of renal failure and death, reducing serum phosphate is a goal of therapy. In the absence of sufficient renal function, intestinal phosphate absorption is the remaining target to reduce plasma phosphate levels. Much work has been done with respect to understanding transcellular phosphate absorption. Both animal studies using inducible or intestinal NaPi-2b knockout mice and specific NaPi-2b inhibitors revealed this transporter as the primary mechanism mediating transcellular phosphate absorption in the intestine. However, this has not translated into effective phosphate lowering therapies in patients with kidney disease. More recently, it was observed that inhibition of the epithelial sodium hydrogen exchanger, sodium–hydrogen exchanger isoform 3 (NHE3), or its genetic deletion, decreases intestinal phosphate absorption. The mechanism mediating this effect is through increased transepithelial resistance and reduced paracellular phosphate permeability. Thus, NHE3 inhibition reduces paracellular phosphate permeability in the intestine. The transepithelial potential difference across intestinal epithelium is lumen negative and phosphate commonly exists as a divalent anion. Further, consumption of the typical Western diet provides a large lumen to blood phosphate concentration gradient. Based on these observations we argue herein that the paracellular phosphate absorption route is the predominant pathway mediating intestinal phosphate absorption in humans.

Keywords: Phosphate, nephrology, intestine, transport, paracellular, gut

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Introduction

Urinary phosphate (Pi) excretion is the main mechanism maintaining serum Pi levels within the physiological range.[1] However, during the progression of chronic kidney disease (CKD), there is a loss of renal function leading to impaired urinary Pi excretion. Combined with bone resorption, decreased renal Pi excretion in the presence of persistent intestinal Pi absorption results in hyperphosphatemia, i.e. elevated serum Pi, a hallmark of CKD and end stage renal disease (ESRD).[2] Unfortunately, hyperphosphatemia is associated with a number of negative sequelae including vascular calcification, cardiovascular disease, secondary hyperparathyroidism, and left ventricular hypertrophy.[3–6] Further, hyperphosphatemia is an independent risk factor for CKD progression and mortality in non-dialysis dependent patients.[7] Given that hyperphosphatemia in patients with CKD/ESRD is associated with significant negative clinical sequelae, reducing serum Pi is a focus of clinical practise guidelines.[8] As serum Pi levels are normally maintained through the modulation of renal Pi excretion, with declining renal function it necessitates reducing intestinal Pi absorption to normalize serum Pi levels. Understanding the mechanisms mediating intestinal Pi absorption is therefore essential to specifically and effectively target intestinal Pi absorption clinically. In this review, we wish to briefly summarize how transcellular intestinal Pi absorption occurs, then argue why this

pathway contributes less to intestinal Pi absorption in humans eating a typical Western diet. We then review what is known about paracellular Pi absorption and argue why this is perhaps a better therapeutic target.

There are multiple transport systems for intestinal Pi absorption

There are at least two transport systems responsible for intestinal Pi absorption, which have been separated into a sodium-dependent and a sodium-independent pathway.[9–11] Further characteristics of these routes include a saturable transcellular, sodium-dependent pathway (Fig.1) or a paracellular, sodium-independent pathway (Fig.2) that does not appear to saturate.[12,13] The rate-limiting step of transcellular intestinal Pi absorption is movement across the apical membrane of the enterocyte.[14] The predominant apical intestinal Pi transporter participating in transcellular absorption is the sodium-dependent phosphate cotransporter 2b (NaPi-2b), a type II transporter belonging to the solute carrier family (SLC34).[14] Kinetic analyses of mammalian NaPi-2b expressed in *Xenopus laevis* oocytes found an apparent Km^{Pi} of ~10 μM.[15] Given the low Km^{Pi} (high-affinity), this transporter is likely important for Pi absorption during periods of fasting when the luminal Pi concentration is low.

NaPi-2b expression is strongly regulated. Low serum Pi increases 1,25 $(OH)_2D_3$ levels which in turn increases NaPi-2b protein expression and sodium-dependent Pi uptake into jejunal brush boarder membrane vesicles (BBMVs).[16] Conversely, when serum Pi is high, FGF23, the major phosphatonin, i.e. phosphate regulating hormone, is secreted from osteocytes and osteoblasts.[17] FGF-23 inhibits the synthesis of active 1,25 $(OH)_2D_3$ thereby indirectly decreasing transcellular intestinal Pi absorption.[18] PTH is secreted from the parathyroid gland in response to decreased serum Ca^{2+} and/or elevated serum Pi[19] and acts on the kidney to induce phosphaturia.[20] PTH also indirectly increases NaPi-2b expression by increasing synthesis of 1,25 $(OH)_2D_3$.[21] In addition to hormonal regulation, NaPi-2b expression is directly regulated by dietary Pi levels. Interestingly, NaPi-2b protein expression in vitamin-D receptor KO mice increases following administration of a low Pi diet indicating that transcellular Pi absorption can be modulated through dietary Pi, independently of 1,25 $(OH)_2D_3$.[16] These regulatory characteristics are consistent with a pathway that fine tunes plasma phosphate levels.

In addition to the type II transporter NaPi-2b, the type III transporters (SLC20 family) PiT-1 and PiT-2 are expressed in the duodenum and jejunum of rats with PiT-2 also being expressed in the ileum.[22–24] In contrast, in mice, the jejunum expresses PiT-1 while the ileum expresses both PiT-1 and PiT-2. Circulating 1,25$(OH)_2D_3$ upregulates gene expression of PiT-2, but not PiT-1, while dietary Pi deprivation increases the expression of both, although with varying response rates.[23,24] Despite being able to transport phosphate across the plasma membrane, the contribution of the PiTs to overall intestinal Pi absorption is unlikely to be significant based on studies from intestinal specific NaPi-2b$^{-/-}$ mice. These animals display increased fecal Pi and compensatory reductions in urine Pi allowing them to maintain normophosphatemia. Deletion of intestinal NaPi-2b virtually abolishes sodium-dependent Pi transport into intestinal BBMVs consistent with PiT-mediated intestinal Pi uptake in the mouse being negligible.

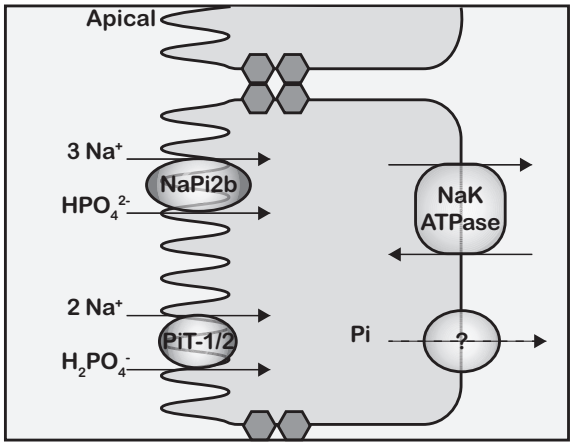

Figure 1. Transcellular intestinal phosphate (Pi) absorption. Transcellular, sodium-dependent, Pi absorption is secondarily active and utilizes the sodium concentration gradient established by the Na^+–K^+ ATPase. The apical transporter mediating the bulk of this is NaPi-2b; however, PiT-1 and PiT-2 may also play a minor role. Further the localization of each is species and intestinal segment specific. It is currently unclear how basolateral Pi efflux is mediated.

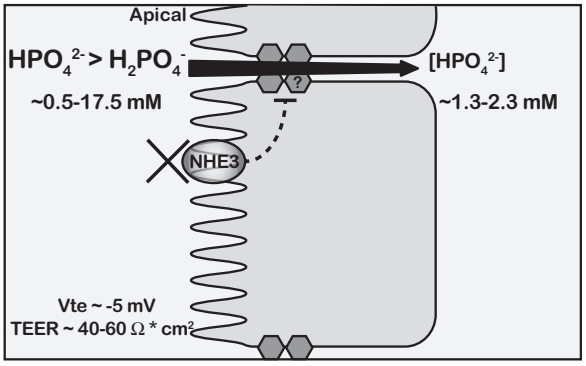

Figure 2. Paracellular intestinal phosphate (Pi) absorption. We argue intestinal Pi absorption occurs largely *via* the paracellular pathway, which is favored by the electrical (lumen negative) and chemical gradients. Inhibition of the NHE3 leads to an increased TEER and a reduction in the absolute permeability to phosphate. Values displayed are representative of rodents. TEER: transepithelial electrical resistance.

In addition to the sodium-dependent transcellular pathway, a sodium-independent transcellular pathway has also been proposed, although it is poorly characterized.[24,25] Candeal *et al.*[24] found that this sodium-independent transport preferentially moves $H_2PO_4^-$ over HPO_4^{2-} and has greater activity at low pH. The molecular identity of this pathway is unclear. Regardless, it can be assumed that the relative abundance of various transporters in different intestinal segments influences the relative contribution of the transcellular absorptive pathway to total Pi absorption, unfortunately complicating the situation is the fact that this property is species dependent.[26] In addition to transporter

abundance, the concentration of Pi in the intestinal lumen influences which route, i.e. either transcellular or paracellular, contributes a greater proportion to Pi absorption.

While transcellular Pi absorption predominates at low luminal Pi concentrations, given the low Km^{Pi} of NaPi-2b, the paracellular pathway likely contributes the bulk of Pi absorption once V_{max} has been reached. At concentrations >1 mM, phosphate absorption increases linearly with increasing luminal Pi concentration and does not saturate, a pattern consistent with passive paracellular transport.[27,28] This is because the paracellular movement of ions occurs passively, down their electrical and chemical concentration gradients, through tight junction (TJ) complexes between epithelial cells. TJ complexes are formed by the interaction of several transmembrane adhesion proteins including occludin, junctional adhesion molecules, and claudins. The claudin family has at least 24 members in mammals and plays an integral role in the barrier function of the TJ, whereby the first extracellular loop controls the resistance and charge selectivity of the pores they form.[29,30] Therefore, the unique combination of claudins in a TJ influences the resistance and permeability characteristics of an epithelium. The gastrointestinal tract is considered a "leaky" epithelium because it has a relatively low transepithelial electrical resistance (TEER). Moreover, the intestine displays a lumen negative transepithelial voltage[31] and hence there is a very large electrochemical gradient driving paracellular phosphate absorption across the intestine. Unfortunately, which claudin(s) contribute to Pi permeability is not known and overall intestinal paracellular Pi absorption has received less investigation relative to the transcellular pathway thus far.

Transcellular Pi absorption likely contributes less to total Pi absorption and is therefore less pathophysiologially relevant

Transcellular intestinal Pi absorption occurs primarily through the action of NaPi-2b. NaPi-2b is essential for Pi uptake into the developing embryo and thus global NaPi-2b deletion results in pups that are non-viable.[32] Consequently, Sabbagh *et al.*[33] developed a tamoxifen-inducible, conditional NaPi-2b$^{-/-}$ (CKO) mouse model.[32] Everted sac experiments using the major intestinal segments from these mice demonstrated that sodium-dependent active Pi transport was abolished with the deletion of NaPi-2b. In addition, sodium-independent Pi uptake into everted sacs was not different between wild-type and NaPi-2b CKO mice. This suggests that the sodium-dependent pathway is the predominant active transport pathway and that the sodium-independent transcellular pathway fails to compensate for the loss of NaPi-2b. Failure of the sodium-independent pathway to compensate for the loss of NaPi-2b is further supported by results from experiments utilizing an *in vivo* ileum loop model.[34] In brief, Pi absorption across mouse ileum, where movement is virtually entirely transcellular, was almost entirely (~90%) mediated by NaPi-2b. Similarly, intestinal-specific NaPi-2b$^{-/-}$ mice display markedly reduced[32] Pi uptake into ileal BBMVs.[35] However, despite these *ex vivo* experiments demonstrating reduced transcellular Pi transport, both the inducible, global NaPi-2b$^{-/-}$ and intestinal-specific NaPi2b$^{-/-}$ mice maintain normophosphatemia.[33,35,36] The intestinal sodium-independent pathway did not compensate in either model under the experimental conditions studied as both strains displayed increased fecal Pi excretion relative to wild-type mice. Interestingly in the experiments described, the mice consumed a chow containing primarily organic Pi, which is less bioavailable than inorganic Pi that is typically found in a "Western diet". The lower Pi bioavailability of these diets is associated with a lower luminal free Pi concentration and would favor absorption via the transcellular pathway. Assuming organic Pi in these diets is primarily from protein and not phytate, deletion of NaPi-2b would be expected to have a larger effect on total Pi absorption when the mice were fed an organic Pi diet. Further, NaPi-2b CKO mice do not have altered bone mineralization suggesting that compensatory renal reabsorption is sufficient to maintain normophosphatemia in these mice.

Both the NaPi-2b CKO and intestinal specific NaPi-2b KO mice display hypophosphaturia consistent with increased renal Pi reabsorption compensating for increased fecal Pi excretion. Changes to several phosphaturic hormones including PTH and FGF-23 could mediate reduced renal Pi excretion in these animals. Lower PTH levels would increase phosphate reabsorption from the proximal tubule by increasing the membrane abundance of NaPi-2a, NaPi-2c, and PiT-2.[37,38] This occurs by the binding of PTH to the PTH1R, which activates PKA and/or PKC, ultimately leading to the internalization and degradation of NaPi-2a.[39] Thus, lower PTH could increase renal Pi reabsorption in NaPi-2b CKO and intestinal-specific NaPi-2b$^{-/-}$ mice. However, in both models PTH levels were unaltered. In a similar fashion to PTH, FGF-23 decreases the abundance of NaPi-2a and NaPi-2c in the brush border membrane of the proximal tubule and decreases both their transcription and translation.[40,41] This appears to be the etiology of increased tubular reabsorption of Pi. Both the NaPi-2b CKO and intestinal-specific NaPi-2b$^{-/-}$ mice had significantly decreased FGF-23 levels relative to wild-type animals.[33,35,36] Consistent with these findings, NaPi-2b CKO and intestinal-specific KO mice have increased NaPi-2a.[33,35] Additionally, decreased intestinal Pi absorption, like dietary Pi deprivation, independently increases NaPi-2a and NaPi-2c protein expression.[42] Studies using thyroparathyroidectomized rats demonstrated that dietary Pi regulates renal Pi reabsorption independent of Pi regulating hormones.[21] This provides an additional potential explanation for how NaPi-2b CKO and intestinal-specific KO mice increase renal Pi reabsorption. Although clearly the predominant intestinal Pi transporter, these studies utilizing NaPi-2b CKO and intestinal-specific KO mice on an organic Pi diet reveal that the loss of NaPi-2b is compensated for by decreased renal Pi excretion. Thus, NaPi-2b inhibition was pursued as a phosphate lowering clinical target in persons with CKD/ESRD.

Interestingly, homozygous inactivating mutations in SLC34A2, the gene encoding NaPi-2b, are associated with pulmonary alveolar microlithiasis (PAM) in humans.[43]

NaPi-2b is highly expressed in the apical membrane of surfactant-producing type II alveolar cells where it exports phosphate produced by the metabolism of phospholipids, a component of surfactant.[44] Inactivating mutations in SLC34A2 produce a non-functional transporter and the buildup of phosphate in the alveolar space where it complexes with calcium. Consequently, patients suffering with PAM have microliths in their lungs. However, they do not have altered serum Pi indicating that Pi homeostasis is maintained despite the loss of NaPi-2b, perhaps due to renal compensation, increased Pi ingestion, or intestinal paracellular absorption of inorganic Pi?[45]

The strongest evidence suggesting that transcellular Pi absorption is not the predominant Pi absorption pathway in humans comes from studies employing NaPi-2b inhibitors. Rodent studies, including those detailed above, inferred that NaPi-2b is a good target to reduce hyperphosphatemia in patients with CKD.[33,46] Schiavi *et al.*[46] used an adenine-induced model of CKD to assess the contribution of NaPi-2b to hyperphosphatemia. They found that uremic NaPi-2b$^{-/-}$ mice had less of a rise in serum Pi and FGF-23 levels compared to wild-type mice after kidney damage was induced. Importantly, these animals appeared to have been fed a predominantly organic Pi diet. In contrast, when Ohi *et al.*[47] switched adenine-induced uremic mice from a standard organic mouse chow (0.9% Pi) to a casein-based synthetic chow (0.9% Pi), i.e. inorganic diet, the mice required the administration of 1% sevelamer carbonate in order to normalize serum Pi.[46] This is likely due to the increased Pi bioavailability/luminal free Pi concentration of the inorganic diet favoring paracellular Pi absorption. In agreement with the observed effects of NaPi-2b deletion in uremic mice, the NaPi-2b specific inhibitor, ASP3325, was found to decrease serum Pi both in healthy rats and those with adenine-induced kidney failure.[48] Again, these studies were likely performed on animals fed an organic Pi diet. Unfortunately, these results did not translate to humans. A clinical trial recently conducted on healthy human volunteers (phase 1) and hyperphosphatemic patients with ESRD undergoing hemodialysis (phase 2) examined the efficacy of single or multiple increasing doses of ASP3325.[49] Although generally well tolerated, ASP3325 did not affect urinary or fecal Pi excretion at any dose(s) in healthy subjects. Likewise, ASP3325 failed to reduce serum Pi, PTH, or FGF-23 in hyperphosphatemic ESRD patients. Given the specificity of ASP3325 for NaPi-2b, this suggests that NaPi-2b plays a minor role in intestinal Pi absorption in humans and that the transcellular pathway is likely less relevant to Pi absorption in humans.

In contrast to the negative ASP3325 clinical trial findings, nicotinamide decreases intestinal Pi absorption and reduces serum Pi in both rodents and humans.[50–53] Katai *et al.*[50] observed that sodium-dependent Pi uptake into BBMVs isolated from rat jejunum was significantly reduced in nicotinamide-treated rats. Further, *in vivo* experiments utilizing a rodent model of chronic renal failure found that nicotinamide reduced serum Pi by decreasing intestinal absorption of orally ingested radiolabeled Pi, perhaps by decreasing NaPi-2b expression.[52] To test the effect of nicotinamide on NaPi-2b co-transport, *Xenopus* oocytes were microinjected with RNA isolated from jejunum of either the wild-type or nicotinamide-treated rats.[50] When sodium-dependent and sodium-independent Pi co-transport was measured, V_{max} was reduced in the oocytes microinjected with RNA from nicotinamide-treated rats suggesting that nicotinamide reduces the transcription/translation of NaPi-2b. These studies are consistent with nicotinamide inhibiting intestinal Pi absorption, at least in part, through the negative transcriptional regulation of NaPi-2b. However, it is unlikely that this is the only mechanism as NaPi-2b inhibition with ASP3325 failed to induce a Pi lowering effect. Nicotinamide is known to have pleiotropic effects that may partly account for its efficacy.[54] Nicotinamide is the predominant substrate of nicotinamide adenine dinucleotide phosphoribosyl transferase, an enzyme found ubiquitously in mammalian cells, which catalyzes the rate-limiting step in the formation of NAD^+.[55,56] Therefore, ingested nicotinamide is readily converted to NAD^+ in enterocytes. Further, experiments assessing paracellular barrier function in Caco2 BBE cell monolayers found that treatment with extracellular NAD^+ increased expression of the TJ proteins occludin and ZO-1.[57] Interestingly, NAD^+ attenuated the hyperpermeability induced when these cells were exposed to an inflammatory milieu of proinflammatory cytokines, mimicking the systemic inflammation associated with CKD.[58] These results suggest that nicotinamide may modulate TJ properties indirectly by increasing NAD^+ biosynthesis and that nicotinamide has a range of effects in addition to NaPi-2b inhibition. Furthermore, no studies to our knowledge have assessed the effect of nicotinamide on paracellular Pi permeability. Resolving the mechanism by which nicotinamide decreases intestinal Pi absorption and assessing its effects on paracellular Pi permeability could provide insight into these seemingly different clinical results.

The intestinal electrochemical gradient favors paracellular Pi absorption

Intestinal paracellular Pi absorption depends on the concentration gradient across the intestinal epithelium, the electrical gradient, and the permselectivity of the TJs. The concentration of Pi in the intestinal lumen varies with dietary intake and bioavailability of the different Pi sources.[59] The concentration of Pi in the lumen of intestinal segments ranges between 4 and 10 mM in rats fed a normal Pi diet.[60] In humans, the concentration of Pi in aspirated jejunal fluid was between 0.5 and 17.5 mM depending on the dietary phosphate load.[28] Conversely, serum Pi is usually maintained between 0.75 and 1.45 mM in humans[61] and oscillates between 1.3 and 2.3 mM in rats.[62] Therefore, the transepithelial difference in Pi concentration favors absorption from intestinal lumen to blood in both rodents and humans. The Pi bioavailability of a diet is the proportion of total ingested Pi that is absorbed from the intestines into the circulation. Dietary Pi usually occurs as a mixture of inorganic Pi, i.e. food additives such as sodium or potassium phosphate and organic forms, i.e. protein and phytate. Absorption of Pi from protein-rich foods requires digestion by peptidases, including trypsin and other brush

border peptidases, and liberation of Pi moieties by intestinal alkaline phosphatase.[63] Therefore, the bioavailability of Pi from organic sources (~40–60%) is lower than inorganic sources (>90%) that do not require digestion.[64] Ultimately, a diet with lower Pi bioavailability does not have as large of a concentration gradient across the intestinal epithelium. Unfortunately, Western diets typically contain additives and preservatives, where inorganic Pi is the main component.[65] This greatly increases the luminal Pi concentration and consequently the driving force for intestinal Pi absorption.

In addition to the concentration gradient, the electrical driving force influences paracellular ion transport. A transmural potential difference of 5 mV (lumen negative) was measured throughout the small intestine of both man and rodents.[31] Being an anion, the electrical driving force therefore favors Pi absorption. The magnitude of the driving force, however, depends on the charge of the Pi species. The relative amount of the Pi species, HPO_4^{2-} or $H_2PO_4^-$, varies depending on the pH of the intestinal lumen, which ranges from ~6 in the duodenum to 7.4 in the terminal ileum of humans.[66] Thus, luminal Pi predominately exists as HPO_4^{2-} magnifying the electrical driving force.

There has been virtually no work examining the permselectivity of the intestinal epithelium for Pi. We recently measured Pi permeability across murine jejunum by inducing a diffusion potential for Pi versus Cl^- and found that this segment was approximately fivefold more permeable to Pi than Cl^-.[34] Together, both the driving forces for and permeability of the small intestine greatly favors paracellular Pi absorption from the intestine.

Inhibition or deletion of intestinal NHE3 decreases paracellular phosphate flux and increases fecal Pi

The sodium–hydrogen exchanger isoform 3 (NHE3) is an ion counter-transporter found in the kidneys and throughout the small intestine and colon.[67–69] Plasma membrane NHEs mediate the electroneutral exchange of one extracellular sodium ion for one intracellular proton. NHE3 has been implicated in a variety of functions including extracellular fluid volume homeostasis and blood pressure regulation.[70] In the small intestine, NHE 1–3 and 7 are expressed; however, NHE3 is the predominant NHE contributing to sodium absorption.[71,72] Consistent with its role in salt and water reabsorption, $NHE3^{-/-}$ mice have increased fluid in their intestines, low blood pressure, metabolic acidosis, and increased sodium in their feces.[71,73] In addition to reduced transcellular intestinal sodium absorption, $NHE3^{-/-}$ mice have increased TEER across the intestine when measured in Ussing chambers and decreased urine Pi.[74,75] A similar increase in TEER occurs when NHE3 is inhibited with 5-(N-ethyl-N-isopropyl)amiloride or the specific NHE3 inhibitor S-3226.[74–76] The increase in TEER with the loss or inhibition of NHE3 indicates the transporter likely influences the permeability of the intestinal epithelium. Further, decreased urine Pi in $NHE3^{-/-}$ mice is consistent with NHE3 participating somehow in intestinal Pi absorption. However, interpretation of these studies with global $NHE3^{-/-}$ mice is difficult because NHE3 is also expressed in the proximal tubule and thick ascending limb of the nephron. Constitutive, tubule-specific $NHE3^{-/-}$ mice (NHE3loxloxCre) have a 30% reduction in NaPi-2a abundance and severely reduced NaPi-2c abundance.[77] This is in contrast to global $NHE3^{-/-}$ mice, which have a 350% increase in NaPi-2a expression, likely compensating for reduced intestinal Pi absorption.[77] This complicates the interpretation of the global $NHE3^{-/-}$ model because the loss of NHE3 in the nephron may alter urinary Pi through its effects on NaPi-2a/c levels, potentially by influencing NHREF-1, a protein that tethers both NHE3 and NaPi-2a/c to the cytoskeleton. To avoid this confounding situation, intestinal-specific $NHE3^{-/-}$ mice were generated; however, most pups die within a few days after birth.[70] Although an intestinal epithelial-specific $NHE3^{-/-}$ model was recently generated,[78] there has not been an assessment of the effect on paracellular Pi flux. However, Pi flux across human $NHE3^{-/-}$ ileum monolayers is reduced suggesting that, at least in human intestinal monolayers, the loss of NHE3 reduces Pi absorption.[34]

Given its physiological role and potential impact on intestinal Pi absorption, NHE3-specific inhibitors were evaluated for their ability to decrease sodium and Pi absorption. Two small molecule inhibitors of intestinal NHE3 have been investigated: SAR218034 (SAR) and tenapanor hydrochloride. When administered orally, both SAR and tenapanor are minimally absorbed from the intestine as evinced by the low plasma concentrations of the drugs observed post-administration (~1 nmol).[79,80] In healthy rats, inhibition of NHE3 by either SAR or tenapanor increases fecal Pi and decreases urine Pi.[34,79,80] Tenapanor-treated 5/6th NPX rats, an animal model for CKD, also display a similar reduction in urinary Pi excretion and increased stool Pi.[81] This is not the result of non-specific inhibition of transcellular Pi transporters by tenapanor as this drug does not inhibit transcellular Pi transporters when expressed in cell culture, nor does it reduce Pi flux across human intestinal monolayers when NHE3 is deleted.[34,81] That tenapanor reduces intestinal Pi absorption via a direct effect on NHE3 is further supported by a lack of effect of tenapanor on Pi absorption in mouse ileum monolayers, a location where Pi transport is mediated entirely by NaPi-2b.[33,34] Finally, although the studies to date have focused on the effect of tenapanor on the small intestine, the colon expresses NHE3 and demonstrates significant Pi permeability.[82] It is therefore likely that it also reduces phosphate absorption from this segment as well.

The mechanism by which tenapanor reduces intestinal Pi absorption was recently investigated by King and colleagues. In a rat intestinal loop model, when rats were given an oral bolus containing radioactive Pi, increasing luminal Pi caused a proportionate increase in urinary Pi excretion. Pi excretion increased linearly with luminal Pi concentration and did not saturate at high concentrations. Tenapanor attenuated urinary Pi excretion at all luminal concentrations proportionately. This pattern is consistent with paracellular transport and is in agreement with pioneering physiologic studies that demonstrate the same linear concentration dependence of Pi absorption in both rabbits and humans.[10,11] To determine whether tenapanor reduced paracellular Pi absorption by either reducing water and sodium flux or altering paracellular permeability, an enteropooling study was conducted. In

this study, cecal Pi concentration was measured at defined time points post ingestion of a high Pi (1.2%) meal. Because cecal Pi concentration and mass increased with tenapanor treatment, rather than remaining constant as would be predicted if there is decreased diffusional driving force due to luminal water retention, it was concluded that the reduced intestinal Pi absorption is due to decreased paracellular Pi permeability. Consistent with the enteropooling study, bi-ionic dilution potential experiments on mouse jejunum and human intestinal monolayers found that tenapanor decreases paracellular Pi permeability and increases TEER in both human duodenum monolayers and mouse jejunum.

Exactly how tenapanor reduces paracellular Pi permeability and increases TEER is not clear. The increase in TEER may be due to decreased cytosolic pH (pHi). Consistent with this, when the TEER of human ileum monolayers was measured in Ussing chambers, treatment with tenapanor or acidification of the apical medium produced a rapid increase in TEER. The rapid rate at which tenapanor increased TEER leads to the hypothesis that reduced pHi caused a pH-sensitive conformational change in a TJ protein. Importantly, dynamic gating of TJs is a proposed mechanism regulating barrier function of an epithelium. More specifically, the vitamin D-regulated claudin-2 channel is dynamically gated, alternating between an open and closed state. This was demonstrated using an elegant trans-TJ patch clamp technique that measures conductance across a single claudin channel.[83] Further *in silico* studies based on these patch clamp results and taking into account the structure of the TJ strands found that the local gating kinetics defined the global epithelial barrier function.[84] As King and colleagues suggest, potentially the pHi decrease caused by NHE3 inhibition alters the gating kinetics of claudins ultimately decreasing intestinal Pi permeability. Unfortunately, which claudins mediate paracellular Pi flux remain elusive warranting further investigation.

Inhibition of paracellular Pi absorption reduces serum Pi in ESRD patients and increases fecal Pi in healthy volunteers

Consistent with the effect of NHE3 inhibition on rodents and enteroid monolayers, tenapanor reduces intestinal phosphate absorption in human volunteers. In a phase 1, double-blind, randomized, placebo-controlled study in healthy Japanese volunteers, the safety and pharmacokinetic/dynamics was assessed for a single (180 mg) or repeated doses (15, 30, 60, or 90 mg $\times$ 2 daily) of tenapanor.[85] In these healthy human volunteers, repeated doses (15–90 mg) caused increases in stool sodium and Pi content and decreases in urinary sodium and Pi content. Thus, inhibition of paracellular Pi absorption by tenapanor translates into humans. Serum Pi was not evaluated in the phase 1 trial.[85] However, a phase 2b trial was conducted to assess if tenapanor led to clinically relevant reductions in serum Pi in persons with ESRD on hemodialysis.[86] Tenapanor treatment led to a dose-dependent reduction in serum Pi levels, with the largest reductions seen in the 10–30 mg twice daily dosing groups. This suggests that inhibition of paracellular Pi transport with tenapanor provides clinically relevant decreases in serum Pi and is, therefore, a potential therapy for treating hyperphosphatemia. In ESRD patients, intact FGF-23 levels are increased almost 1000-fold over healthy individuals and increase with worsening kidney function.[87] Increased levels of FGF-23 are associated with cardiovascular disease including left ventricular hypertrophy[88] and mortality.[89] Given that increases in intact FGF-23 accompanying CKD and hyperphosphatemia have deleterious effects, a secondary analysis of the phase 2 placebo-controlled, randomized clinical trial was carried out to assess the effect of tenapanor on FGF-23.[90] Following washout of Pi binders and four weeks of treatment with tenapanor, serum Pi and intact FGF-23 levels were significantly reduced. Further, the magnitude of reduction in FGF-23 was correlated to the magnitude of reduction of serum Pi levels. Inhibition of intestinal paracellular Pi transport therefore not only reduces serum Pi, but also has downstream effects on serum FGF-23. The exact mechanism for how tenapanor decreases serum FGF-23 is unclear and it is also currently unknown if this tenapanor-mediated reduction in serum FGF-23 leads to clinically relevant improvements in cardiovascular health. Currently a phase 3 trial is ongoing to evaluate the safety and efficacy of tenapanor to treat hyperphosphatemia in patients with ESRD on dialysis.[91] The study consists of a 4-week binder washout period, a 26-week treatment period, and a randomized withdrawal period where patients will either remain on tenapanor or a placebo. The primary outcome measure is a placebo-adjusted decrease in serum Pi of at least 1.2 mg/dl during the controlled randomized withdrawal period in the population administered tenapanor. The effect of tenapanor on serum Pi from baseline and on serum FGF-23 will be assessed. If results from this phase 3 trial support that tenapanor decreases serum Pi and is effective at reducing hyperphosphatemia in ESRD patients, this will provide additional evidence that the paracellular pathway is physiologically more relevant than the transcellular pathway to human intestinal phosphate absorption, at least those with ESRD.

Conclusion

Hyperphosphatemia is associated with declining renal function and significant negative clinical sequelae including vascular calcification, cardiovascular disease, renal osteodystrophy, and mortality. Unfortunately, current therapies aimed at reducing serum Pi, i.e. dietary Pi restriction and oral Pi binders can lead to protein malnutrition, high pill burden, and can cause compensatory increases in transcellular Pi absorption.[92–95] In this review, we summarized the various intestinal Pi transport pathways arguing that the paracellular pathway plays a more prominent role in intestinal Pi absorption in humans and is therefore a better target to reduce serum Pi in patients with CKD. Several lines of evidence support this conclusion. Although NaPi-2b is the major transcellular Pi transporter, and its inhibition was successful in rodents, the specific inhibitors of NaPi-2b fail to decrease serum Pi levels in humans. In contrast, specific inhibitors of NHE3 increase stool Pi and decrease urinary Pi indicating decreased intestinal Pi

absorption in both rodents and humans. Importantly, the method of action of these inhibitors is by increasing TEER and decreasing paracellular Pi permeability and thus absorption. The efficacy of these inhibitors over NaPi-2b inhibitors implies the paracellular pathway is more physiologically relevant in humans. These inhibitors, offer a new approach and some promise to reduce serum Pi, i.e. attenuating paracellular Pi permeability, in patients suffering with CKD and hyperphosphatemia.

Authors' contributions: MS wrote the initial manuscript and RTA edited it for important scientific content.

DECLARATION OF CONFLICTING INTERESTS

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