Open

The intact nephron hypothesis: the concept and its implications for phosphate management in CKD-related mineral and bone disorder

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Mechanistic understanding of secondary hyperparathyroidism, vascular calcification, and regulation of phosphate metabolism in chronic kidney disease (CKD) has advanced significantly in the past five decades. In 1960, Bricker developed the 'intact nephron hypothesis', opening the door for hundreds of investigations. He emphasized that 'as the number of functioning nephrons decreases, each remaining nephron must perform a greater fraction of total renal excretion'. Phosphate *per se*, independent of Ca^{2+} and calcitriol, directly affects the development of parathyroid gland hyperplasia and secondary hyperparathyroidism. Vitamin D receptor, Ca^{2+} sensing receptor, and Klotho–fibroblast growth factor (FGF) receptor-1 complex are all significantly decreased in the parathyroid glands of patients with CKD. Duodenal instillation of phosphate rapidly decreases parathyroid hormone release without changes in calcium or calcitriol. The same procedure also rapidly increases renal phosphate excretion independently of FGF-23, suggesting the possibility of an 'intestinal phosphatonin'. These observations suggest a possible 'phosphate sensor' in the parathyroid glands and gastrointestinal tract, although as yet there is no proof for the existence of such a sensor. Evidence shows that phosphate has a key role in parathyroid hyperplasia by activating the transforming growth factor-α–epidermal growth factor receptor complex. Thus, control of serum phosphorus early in the course of CKD will significantly ameliorate the pathological manifestations observed during progressive deterioration of renal function.

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In advancing chronic kidney disease (CKD), there is a multitude of biochemical, physiological, and clinical alterations. In the nineteenth century, Bright[1] recognized the relationship between intrinsic kidney disease and the many abnormalities seen in the uremic state. The exact nature of events leading from the initial destruction of nephrons to the pathophysiological changes of end-stage renal function was not fully understood then. However, there is now significant evidence demonstrating that the many forms of CKD could give rise to the same pattern of derangements that relate principally to the extent of nephron destruction.

The physiopathology of 'chronic Bright's disease', an exposition of the 'intact nephron hypothesis' published in Bricker *et al.*,[2] provided a logical framework for understanding the changes in renal function that occur in animal models and in patients with CKD. Bricker *et al.* observed adaptive changes in renal function and in homeostasis that result from progressive CKD.

Bricker[3] emphasized that, as the number of functioning nephrons decreases, each remaining nephron must perform a greater fraction of total renal excretion. Bricker clearly explained the differences between substances such as creatinine, which are mainly handled by glomerular filtration, and sodium and phosphate, which are mainly handled by renal tubular reabsorption. For substances handled by glomerular filtration, a decrease in glomerular filtration induces a progressive increase in their plasma concentration early in the course of CKD. On the other hand, with substances such as sodium and phosphate that are mainly handled by tubular reabsorption, a decrease in renal tubular reabsorption of these substances will prevent a significant change in their blood concentrations, and their levels will remain normal in serum until advanced deterioration of renal function. Similarly, with substances such as potassium, which are mainly handled by renal tubular secretion, adaptive processes that will significantly increase the potassium secretion per nephron will maintain the levels of serum potassium within the normal range despite the fact that the patient may have severe renal impairment.

In the 1950s, if there was a consensus, 'it was that the processes of disease transformed the afflicted kidney into a disorganized and heterogeneous population of nephrons marred by a spectrum of functional disturbances which varied from one kidney to another, from one disease to

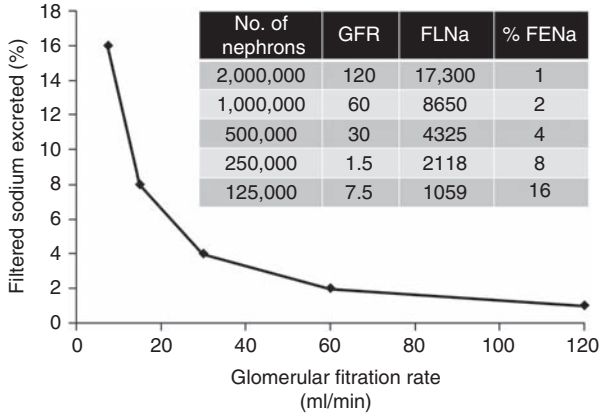

Figure 1 | Hypothetical treatment of sodium excretion in chronic kidney disease (see text for details). GFR, glomerular filtration rate; FENa, fractional excretion of sodium; FLNa, filtered load of sodium. Figure newly constructed from data in Bricker *et al.*[2] and Bricker.[3]

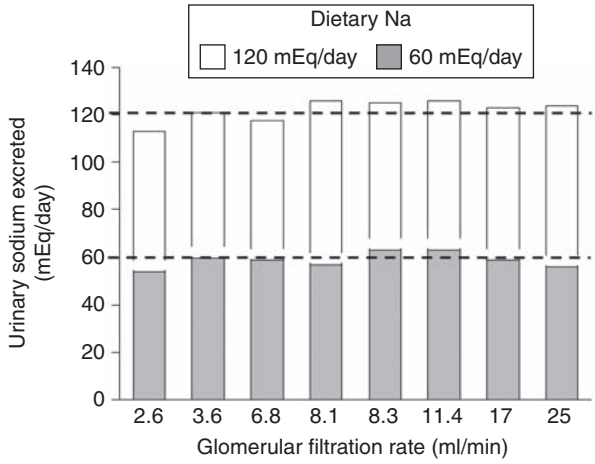

Figure 2 | Urinary sodium excretion in patients with advanced chronic kidney disease (stages 4–5). All patients with glomerular filtration rates ranging from 25 to 2.6 ml/min maintain perfect Na balance after ingestion of 60 or 120 mEq of Na/day. Reproduced by permission from Slatopolsky *et al.*[4] Copyright 1968 by the American Society for Clinical Investigation. Reproduced with permission from the American Society for Clinical Investigation in the format Journal via Copyright Clearance Center.

another, from one patient to another.'[3] Bricker[3] elaborated in great detail and challenged the idea that anatomical abnormalities cannot be followed by functional adaptations. He emphasized that 'the functional capacity of the residual nephrons of the diseased kidney is largely independent of the specific form of renal disease. The decrease in the number of nephrons is clearly responsible for many of the abnormalities that develop in the patient; the persistent nephrons permit the patient to survive.' One of the best examples is related to sodium excretion. A normal person with a glomerular filtration rate (GFR) of 120 ml/min will filter 17,000 to 18,000 mEq/day of Na. This person will excrete $\sim$1% of the filtered load of sodium provided the patient has a normal salt intake (8–12 g/day).

If the patient with CKD continues with the same diet but loses 50% of his/her renal function, the renal tubule must adapt and excrete twice the amount of Na per nephron, or roughly 2%. As the disease progresses, every time the patient loses 50% of his renal function, the fractional excretion of sodium should double. This hypothesis is depicted in Figure 1. Studies by Slatopolsky *et al.*[4] validated this hypothesis, comparing patients with normal GFRs (120 ml/min) with those with GFRs as low as 2.0 ml/min (Figure 2). When patients with inulin clearances from 25 to 2.6 ml/min were fed diets containing 60 or 120 mEq/day of Na, all patients were in perfect balance, clearly demonstrating that the lower the GFR and consequently the lower the number of nephrons, the greater the fractional excretion of Na. In other words, as GFR decreases, the greater the amount of Na excreted per remaining nephron. This indicates that patients with very low GFRs ($<$5 ml/min) ingesting 120 mEq/day of Na must excrete $>$30% of the filtered load of Na to maintain sodium balance.

To further correlate the relationship between Na excretion and bicarbonate reabsorption, Slatopolsky *et al.*[5] performed bicarbonate titrations in patients with CKD stages 3–5. Normal subjects and patients with CKD with GFRs $>$30 ml/min fell into one group. When extracellular fluid volume expansion was minimized by salt deprivation and diuretics in advance of the titration study, after the infusion of sodium bicarbonate, no definitive maximal tubular reabsorption for bicarbonate was observed. In the same group of patients, exaggerated expansion of the extracellular fluid led to an alteration in the patterns of bicarbonate reabsorption. Results from patients with GFRs of $\leqslant$20 ml/min resembled increased expansion, although extracellular fluid volume expansion was not exaggerated. However, those patients had a significant increase in Na excretion per nephron. On the other hand, patients with severe CKD stage 5 and nephrotic syndrome characterized by a low excretion of Na per nephron had a maximal tubular reabsorption for bicarbonate similar to normal subjects. There was a significant correlation between fractional excretion of sodium and bicarbonate reabsorption ($P<0.0001$; ref. 5).

THE INTACT NEPHRON AND CKD-RELATED MINERAL AND BONE DISORDER

CKD is characterized by several alterations in mineral homeostasis. Secondary hyperparathyroidism is present even in the early stages of CKD and leads to the development of high bone turnover, pathological fractures, vascular calcification, and other systemic alterations.

The maintenance of phosphate balance requires the excretion into the urine each day of the same amount of phosphorus that enters the extracellular fluid.[3] Several decades ago we demonstrated that, in dogs with experimental renal disease and in patients with different degrees of CKD, tubular reabsorption of phosphate decreases in proportion to the severity of CKD. At a GFR of 120 ml/min, $\sim$10% of the filtered phosphate is excreted (that is, tubular reabsorption of

phosphate is 90%). As GFR diminishes, the degree of phosphate excretion per nephron increases, and at very low filtration rate levels tubular reabsorption of phosphate is 10–20%, or 80–90% of the filtered load of phosphate is excreted into the urine[6] (Figure 3).

In our initial studies in dogs with 5/6 nephrectomy,[7] we demonstrated that if we greatly restricted phosphate intake from 1200 to 100 mg/day there was no need for adaptation, and the surviving nephrons could reabsorb 90% of the filtered load of phosphate. Moreover, in the same studies we demonstrated that, if phosphate is restricted from the beginning of nephron reduction, secondary hyperparathyroidism can be prevented, suggesting a critical role for phosphate retention in the elevation of parathyroid hormone (PTH). Subsequently, we developed the concept of 'proportional reduction'. In other words, if GFR is reduced in a step-wise manner over a period of weeks or months by gradual ligation of the branches of the renal artery, and at the same time phosphate intake is reduced in proportion to the decrease in GFR, there is no need for adaptation. We clearly demonstrated this hypothesis in a paper published in 1972 (ref. 8). In a group of dogs, GFR was gradually reduced from 56 to 40 to 19, and finally to 12 ml/min. At the same time, phosphate intake was gradually and proportionally reduced from 1200 to 234 mg/day. There were no changes in serum phosphorus, calcium, magnesium, tubular reabsorption of phosphate, or PTH, clearly indicating that we could prevent the development of secondary hyperparathyroidism by preventing the adaptation of phosphate excretion.

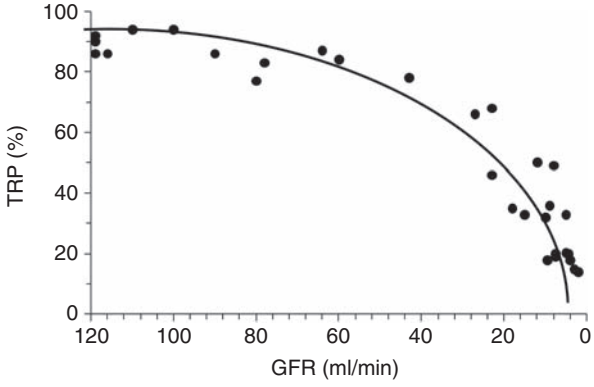

Figure 3 | Comparison of tubular reabsorption of phosphate (TRP) and glomerular filtration rate (GFR) in a group of normal subjects and 24 patients with different degrees of chronic kidney disease. The lower the GFR, the lower the TRP or the greater the excretion of phosphate per nephron. Reproduced by permission from Slatopolsky *et al.*[6] Copyright 1968 by the American Society for Clinical Investigation. Reproduced with permission from the American Society for Clinical Investigation in the format Journal via Copyright Clearance Center.

Subsequently, we learned the critical role of $1,25(OH)_2D_3$ (calcitriol) in the transcriptional repression of the *PTH* gene.[9,10] Because in advanced CKD the reduced renal mass may limit the production of calcitriol, studies were conducted[11] to clarify whether phosphate restriction improves secondary hyperparathyroidism in advanced CKD directly or indirectly. Dogs with experimental CKD were fed a low-calcium, low-phosphate diet for several weeks (Figure 4). It is well known that a low-phosphate diet can increase the levels of calcitriol, presumably by decreasing the levels of fibroblast growth factor (FGF)-23, which reduces the activity of 1α-hydroxylase. The results confirm a marked effect of phosphate restriction in secondary hyperparathyroidism. In humans and rats, calcium and phosphorus are well-known modulators of renal 1α-hydroxylase. However, in contrast to previous findings in patients with early CKD,[12] in the dogs with advanced CKD, significant reduction of phosphate intake did not increase the levels of calcitriol. In these studies, we observed a significant decrease in serum-ionized Ca and phosphate conditions known to stimulate 1α-hydroxylase, but plasma calcitriol did not increase. Finally, in 1996 (ref. 13) we demonstrated *in vitro* that phosphate *per se*, independent of ionized calcium or calcitriol, has a direct effect on PTH secretion. Parathyroid glands obtained from normal rats were incubated *in vitro* with culture media containing 0.2 or 2.8 mmol/l phosphorus. Again, the concentrations of ionized calcium and calcitriol were the same in both situations. The 2.8 mmol/l phosphorus concentration in the media greatly increased the secretion of PTH in comparison with the results obtained with 0.2 mmol/l

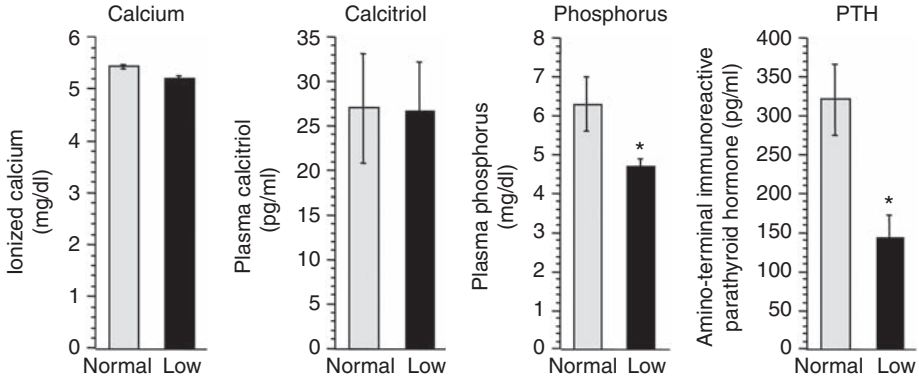

Figure 4 | Effects of a low-phosphate, low-calcium diet on serum calcium, calcitriol, phosphorus, and parathyroid hormone (PTH) in dogs with chronic kidney disease and established secondary hyperparathyroidism. *$p < 0.05$. Figure newly constructed from data in Lopez-Hilker *et al.*[11]

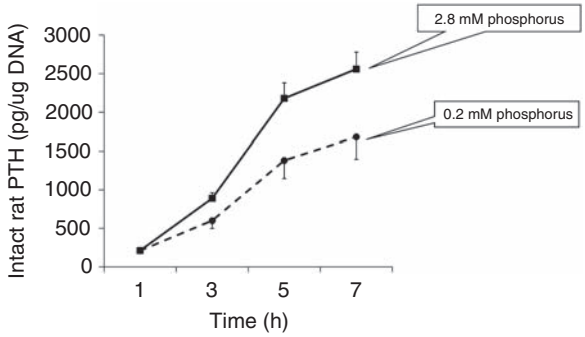

Figure 5 | The effects of phosphorus in the culture media on the secretion of parathyroid hormone (PTH) in a normal rat parathyroid gland *in vitro*. In the two experimental conditions, the concentrations of ionized calcium and $1,25(OH)_2D_3$ were identical. Error bars denote standard error. Adapted with permission from Slatopolsky *et al.*[13]

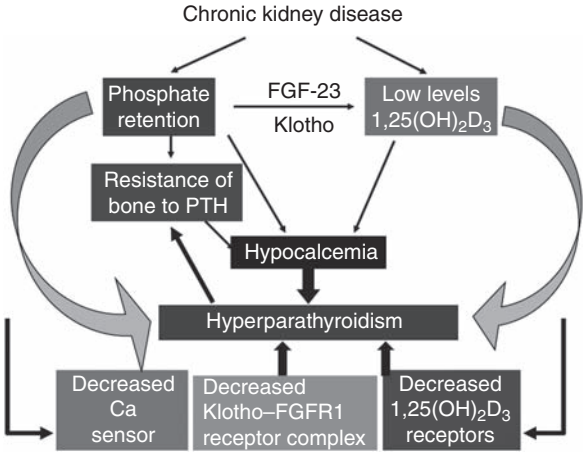

Figure 6 | Summary of the factors involved in the pathogenesis of secondary hyperparathyroidism. A decrease in ionizing calcium (ICa) is crucial in the development of secondary hyperparathyroidism. This change in ICa is secondary to phosphate retention and to low levels of $1,25(OH)_2D_3$. Phosphate retention increases fibroblast growth factor (FGF)-23, which, in conjunction with its cofactor, the Klotho protein, decreases the activity of the 1α-hydroxylase and increases the 24α-hydroxylase, thus decreasing the levels of circulating $1,25(OH)_2D_3$. In addition, phosphate retention, independent of change in ICa, posttranscriptionally increases the synthesis of parathyroid hormone (PTH). The $1,25(OH)_2D_3$, independent of calcium, suppresses the transcription of the *PTH* gene. Decreases in the vitamin D receptor, calcium sensor receptor, and Klotho–FGFR1 receptor complex in the parathyroid gland also aggravate the development of secondary hyperparathyroidism.

(Figure 5). In the same year, two other laboratories[14,15] clearly demonstrated a direct effect of phosphate on PTH secretion. The effect of phosphate on the secretion of PTH is posttranscriptional.[16,17] Figure 6 summarizes the mechanisms involved in the pathogenesis of secondary hyperparathyroidism.

I believe it is important to emphasize that the initial hypothesis regarding the mechanisms by which phosphate controls the regulation of PTH was made 50 years ago, and since then many factors have been discovered that also participate in the regulation of PTH. Regardless of these new developments, **the initial idea that phosphate *per se* controls PTH is correct**.

PHOSPHATE–PARATHYROID–INTESTINAL–RENAL AXIS

Several studies in experimental animal models have shown that a high phosphate intake can accelerate uremia-induced parathyroid hyperplasia and secondary hyperparathyroidism, whereas phosphate restriction prevents these abnormalities. The effect of phosphate is independent of serum calcium and $1,25(OH)_2D_3$ (refs 13, 18).

We conducted further studies in uremic rats with established parathyroid hyperplasia and secondary hyperparathyroidism to determine whether the change to a low-phosphate diet could modify the above abnormalities.[19] After 1 week of a low-phosphate diet, serum PTH returned to normal despite the persistence of parathyroid hyperplasia. Our studies did not show a change in the synthesis of PTH, but did show a suppression of PTH release. We also demonstrated an increase in intracellular PTH in the parathyroid gland in animals that were fed a high-phosphate diet and subsequently treated for 1 week with a low-phosphate diet. No apoptosis was observed in these glands. Because of our findings, we called our studies 'Hyperplasia of the parathyroid glands without secondary hyperparathyroidism.'[19]

Studies by Ritter *et al.*[20] in our laboratory demonstrated that after 2 weeks of CKD and a high-phosphate diet there was a significant loss of the calcium-sensing receptor. However, when the diet was switched to one that was lower in phosphate, serum PTH returned to normal in only 1 day but it took ~2 weeks for the calcium-sensing receptor to return to normal.

Further studies by Martin *et al.*[21] in our laboratory demonstrated that in rats with established secondary hyperparathyroidism there was a 60–80% decrease in serum phosphorus and PTH, with no change in serum calcium only 2 h after feeding the uremic rats a low-phosphate diet. Moreover, when a low-phosphate diet was given by gavage, serum PTH and phosphorus decreased significantly after only 15 min. On the other hand, when serum phosphate was acutely decreased by an infusion of glucose, no change in serum PTH was observed. All these results raise the question, 'Is there an acute regulation of PTH by dietary phosphate, and if so, is it mediated by a hormone possibly derived from the gastrointestinal tract?'

Recently, Berndt *et al.*[22] demonstrated that the instillation of phosphate in the duodenum of normal rats rapidly increases the renal excretion of phosphate. This was also seen in thyroparathyroidectomized rats, and there were no changes in the filtered load of phosphate or serum levels of FGF-23 or secreted frizzled-related protein. Moreover, these observations were also seen when the kidney was denervated, and the phosphaturia was not elicited when phosphate was instilled in other parts of the gastrointestinal tract, such as the stomach. Furthermore, when homogenates of duodenal

mucosa were infused intravenously there was a rapid increase in renal phosphate excretion. Berndt *et al.*[22] believe that there is a direct existence of an intestinal–renal axis specific for phosphate that is mediated by an unknown factor 'intestinal phosphatonin'. Moreover, they also propose the existence of an intestinal phosphate sensor.[23] Although this sensor has not yet been demonstrated, it is possible that the parathyroid gland also has a 'phosphate sensor'. Again, to date there is no scientific proof for the existence of such a sensor. This author, however, strongly believes in the existence of a 'phosphate sensor' in parathyroid glands. To date, all the important factors that control the release of PTH have a sensor in the parathyroid gland (that is, vitamin D receptor, calcium sensor receptor, Klotho–FGFR-1 receptor complex), and as phosphate also has a crucial role in the development of secondary hyperparathyroidism the existence of a phosphate sensor seems to be a reasonable possibility.

PHOSPHATE MODULATION OF PARATHYROID HYPERPLASIA

Parathyroid cell proliferation rather than hypertrophy is the main determinant of parathyroid enlargement and secondary hyperparathyroidism. The lack of an appropriate parathyroid cell line has impeded the identification of the mechanisms underlying the switch from a quiescent parathyroid cell to one that divides rapidly in response to hypocalcemia, hyperphosphatemia, or a decrease in $1,25(OH)_2D_3$ as part of the progression of CKD. Studies conducted by Dusso *et al.*[24] centered on phosphate control of parathyroid cell proliferation, as no change in the number of cells undergoing apoptosis has been observed in experimental conditions affecting parathyroid cells.[19] Dusso *et al.*[24] focused on dietary phosphate regulation of parathyroid expression of the cyclin/cyclin-dependent kinase inhibitor p21. Her studies demonstrate that, in early CKD, phosphate restriction may arrest uremia-induced parathyroid hyperplasia through a specific induction of the mRNA and protein levels of the cyclin-dependent kinase inhibitor p21. In addition, high phosphate intake induces transforming growth factor (TGF)-α, which seems to function as an autocrine signal to stimulate cell growth, thus worsening the parathyroid hyperplasia and the resultant secondary hyperparathyroidism. Growth signals from enhanced parathyroid TGF-α require activation of the epidermal growth factor receptor (EGFR): a 170-kDa membrane glycoprotein with intrinsic tyrosine kinase activity.[25] TGF-α-activated EGFR translocates to the nucleus, where it functions as a transcription factor. TGF-α/EGFR-driven growth involves TGF-α binding to the EGFR, which in turn induces EGFR dimerization and tyrosine phosphorylation of the EGFR by an intrinsic EGFR tyrosine kinase. This step is critical for the onset of downstream growth signal. Studies conducted by Cozzolino *et al.*[26] clearly demonstrate that a high phosphate dietary intake increases not only TGF-α but also the EGFR in the parathyroid gland of rats with CKD. The TGF-α–EGFR complex produces growth acceleration of benign and malignant tissue. Moreover, in the same studies,[26] the use of erlotinib, a tyrosine kinase inhibitor, counteracted the potent mitogenic signals triggered by kidney disease and phosphate retention. Low-calcium diets also increase the TGFα–EGFR complex. This study[26] demonstrates that the enhancement of parathyroid TGF-α and EGFR expression and growth signal occurs early after the onset of renal disease and is aggravated by a high-phosphate or a low-calcium diet.

Further studies by Dusso *et al.*[27] identify a tumor necrosis factor-α-converting enzyme (TACE) as the cause of the initial increase in parathyroid TGF-α, and this starts the vicious cycle for progression of parathyroid hyperplasia. TACE, also known as ADAM17, is a metalloproteinase essential for TGF-α signaling and several other EGFR ligands. The location of TACE at the cell membrane is mandatory for its function. Preliminary results[27] in uremic rats have shown that high phosphate intake also enhances parathyroid TACE content in early and established secondary hyperparathyroidism. However, high dietary phosphate may not affect parathyroid TACE expression directly, but may induce its translocation to the cell surface, causing the release of TGF-α required to initiate EGFR activity during parathyroid cell growth and TACE stabilization.

CONCLUSIONS

The intact nephron hypothesis has fueled 50 years of research and discovery in CKD. The adverse events of hyperphosphatemia in CKD, secondary hyperparathyroidism, calcium and vitamin D derangements, vascular calcification, and metabolic bone disorder are the results of the endocrine trade-offs required by the adaptation of nephrons attempting to preserve phosphate homeostasis. Restricting phosphate intake or, when that is not possible, restricting phosphate absorption by use of phosphate binders as GFR declines reduces the need for nephron adaptation and forestalls endocrine sequelae such as hyperparathyroidism. PTH is regulated at the levels of mRNA stability and protein exocytosis by extracellular phosphate concentration. Rapid (15-min) regulation of PTH by phosphate may require the release of an intestinal hormone. The increase in fractional excretion of phosphate after the instillation of phosphate in the intestine is rapid (15–20 min) and does not require PTH or FGF-23. Potentially, an 'intestinal phosphatonin' may be the mediator of this action. Finally, in the parathyroid gland, enhanced TGF-α and EGFR coexpression drives parathyroid hyperplasia in early CKD. Parathyroid TACE activity, itself controlled by phosphate and vitamin D, is crucial in transducing these growth signals and will be an important therapeutic target. Clearly, phosphate has direct effects at multiple organ sites suggesting a phosphate sensor. However, further studies are necessary to scientifically prove the existence of a phosphate sensor in the parathyroid glands and gastrointestinal tract.

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