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Three feedback loops precisely regulating serum phosphate concentration

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Parathyroid hormone (PTH) and vitamin D were considered the major factors regulating phosphate homeostasis. Now, with the identification of fibroblast growth factor 23 (FGF23), a phosphaturic molecule inhibiting calcitriol and PTH, they need to be integrated into three feedback loops involving parathyroid, bone, and kidney. PTH and calcitriol are required for the appropriate synthesis of FGF23 by bone cells. PTH also regulates klotho expression in the kidney and thereby the phosphaturic action of FGF23.

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Parathyroid hormone (PTH) and vitamin D have been recognized as the main regulators of phosphate homeostasis.[1] PTH accomplishes it via two opposite effects: it reduces serum phosphate by decreasing its renal reabsorption, and increases it either by directly stimulating bone turnover and phosphate release or by indirectly stimulating intestinal phosphate absorption through its stimulatory effect on renal 1α-hydroxylase activity and $1,25(OH)_2D_3$ (calcitriol) production. PTH binds to PTH/PTHrP receptor type 1 (PTHR1) in renal and bone cells and activates several intracellular second messengers, including Gsα-dependent cyclic adenosine monophosphate (cAMP), inositol triphosphate, phospholipase C, free calcium, and diacylglycerol.[1] Some of them, namely, cAMP and phospholipase C, lead to the internalization of the sodium–phosphate cotransporters NPT2a and NPT2c in proximal tubular cells, and to a fall in renal phosphate reabsorption. In bone cells, PTHR1s are found mostly in osteoblasts, and not in osteoclasts, which confers to PTH its anabolic action, increasing bone formation. However, most importantly physiologically, PTH stimulates osteoblast RANKL (receptor activator of nuclear factor-κB ligand) production, which in turn increases osteoclast number, bone resorption, and subsequently phosphate release.[1]

Active vitamin D, or calcitriol, results from a cascade of metabolic steps beginning with cutaneous ultraviolet-dependent generation of cholecalciferol from 7-dehydrocholesterol and ergosterol, followed by liver 25-hydroxylation of cholecalciferol, and lastly by renal 1α-hydroxylation of 25(OH)D. Vitamin D metabolites are transported by the vitamin D-binding protein (DBP). They bind to the same receptor (VDR) with distinct affinities, thousands of times greater for calcitriol than for 25(OH)D. The complex vitamin D/VDR forms nuclear heterodimers with RXR that interact with the vitamin D-responsive element on target genes to produce its physiological effects. Calcitriol increases renal NPT2a expression and phosphate reabsorption, as well as intestinal NPT2b expression and phosphate absorption. Its positive effect on intestinal and renal phosphate absorptions is counterbalanced by the stimulation of fibroblast growth factor 23 (FGF23) production. Calcitriol also modulates both bone resorption, through its differentiating effects on monocyte-macrophage-osteoclast cells, and bone formation, through its multiple actions on osteoblasts. At the parathyroid gland, vitamin D suppresses synthesis of PTH by directly repressing its gene, and, indirectly, by increasing calcium-sensing receptor (CaR) expression and the sensitivity of parathyroid cells to extracellular calcium. Thus, vitamin D can increase or decrease serum phosphate concentration depending on its balanced effect on parathyroid glands, bone, and intestine.

FGF23 is a circulating 32-kDa peptide secreted by osteocytes, osteoblasts, and osteoclasts, in response to hyperphosphatemia and vitamin D.[2] FGF23 reduces serum phosphate by two mechanisms: it decreases renal phosphate reabsorption by lowering NPT2a and NPT2c expression, and it diminishes calcitriol synthesis by inhibiting 1α-hydroxylase and by stimulating its catabolizing enzyme 24,25-hydroxylase. The reduced calcitriol decreases intestinal NPT2b expression and phosphate absorption. FGF23 binds to and activates a composite receptor formed by the conjunction of FGF receptor 1 (FGFR1), FGFR3, and/or FGFR4 with klotho. Klotho is a 130-kDa transmembrane protein identified in 1997 in a genetically created mouse strain, which displays hyperphosphatemia, hypercalcemia, high calcitriol, premature ageing, and shortened lifespan.[3] Klotho is expressed mainly in distal tubular cells, which contrasted, at the beginning, with the phosphaturic effect of FGF23 in proximal tubules; however, klotho expression is also present in proximal tubular cells. Klotho can also be shed from the cellular membrane by metalloproteases, and released into the circulation in a soluble form of smaller molecular weight with not yet fully understood physiological functions.[4]

FGF23 and klotho may regulate PTH, as evidenced by the expression of all players involved in FGF23 signaling in parathyroid glands, including FGFR1, FGFR3, and klotho. FGF23 directly decreases PTH mRNA expression and PTH secretion *in vitro* as well as in *in vivo* experiments. It may also indirectly repress the *PTH* gene through the stimulation of parathyroid 1α-hydroxylase activity and local production

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of calcitriol. Both effects depend on klotho, as suggested by the observations that reduced expression of klotho and FGFR1 might explain the resistance of parathyroid cells to FGF23 in chronic kidney disease,[5] as well as in one case of hyperphosphatemic familial tumoral calcinosis due to an inactivating *klotho* mutation.[6] Inversely, and independently of FGF23, klotho stimulates PTH secretion through the stabilization of Na/K-ATPase at the cell membrane.[7] However, to date, it is unknown whether klotho, FGF23, and FGFR1 interact with the CaR, the main regulator of PTH secretion.

Overexpression of FGF23 and injection of FGF23 into mice cause hypophosphatemia and reduce calcitriol; inversely, mice lacking FGF23 have hyperphosphatemia and high calcitriol. Similarly, the phenotype of klotho-deficient mice is quite similar to that of FGF23 knockout animals, depicting hyperphosphatemia, high calcitriol, and reduced lifespan, suggesting that these abnormalities occur because FGF23 cannot act on the organs where klotho is normally expressed, namely, the kidney and parathyroid gland.[3] Intriguingly, most of the phenotype of FGF23 and klotho knockout mice can be rescued by a phosphate-restricted diet, and by disruption of vitamin D signaling pathways by the silencing of either the VDR or the 25-hydroxyvitamin D-1α-hydroxylase gene. These findings demonstrate that the presence or the absence of klotho determines the tissue specificity and the effects of FGF23.

López *et al.*[8] (this issue) asked whether PTH could regulate skeletal FGF23 production. They hypothesized that if FGF23 reduces PTH, this should lead to hypocalcemia and hyperphosphatemia. Such hypocalcemia would be magnified by low calcitriol and hyperphosphatemia. Therefore, there should be a mechanism protecting against the hypocalcemia in case of persistent high FGF23, and this mechanism should block FGF23 production. The decreased synthesis of calcitriol induced by low PTH actually inhibits FGF23 production, but, on the other hand, the increase in phosphate that follows low PTH levels would tend to stimulate FGF23 synthesis. PTH might be necessary for FGF23 secretion, and thus low PTH would result in decreased FGF23. This would also help to understand the contradictory action of FGF23 on plasma phosphorus. FGF23 directly decreases plasma phosphate, while, indirectly through decreasing PTH, it facilitates an increase of serum phosphate.

The authors found paradoxically low FGF23 levels in parathyroidectomized (PTX) animals in spite of hyperphosphatemia, suggesting that phosphate alone was unable to stimulate FGF23 synthesis, and that other factors such as PTH and/or calcitriol were required. This assumption should be taken with caution, since, despite low PTH and calcitriol, high FGF23 is seen in PTH-null mice and in patients with hypoparathyroidism. Similarly, after total parathyroidectomy, the peak of serum phosphate always precedes FGF23 increase by several days, illustrating that phosphate is the primary stimulus for FGF23 production. Low PTH was probably not solely responsible for the low FGF23 in PTX animals. As the authors discuss, calcitriol was also decreased, which is probably more important than stimulation of FGF23 by PTH. Indeed, vitamin D regulates FGF23 independently of phosphate, as suggested by the reduced FGF23 levels in animals with *VDR* gene knockout selectively in chondrocyte cells. However, it remains to be investigated what would be the effect of supraphysiological doses of PTH on serum FGF23 levels in vitamin D-deprived animals, either by *VDR* or *CYP27B1* knockout or by binephrectomy. It is also unknown how parathyroidectomy would modify the number and activity of bone cells producing FGF23.

The administration of physiological and supraphysiological doses of PTH in

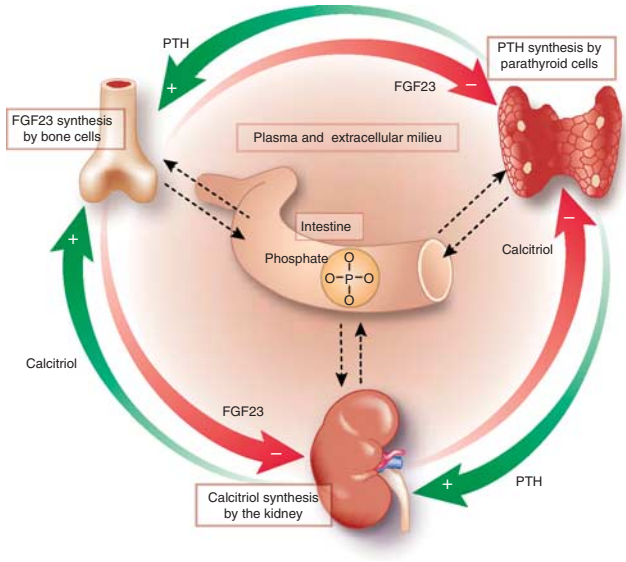

Figure 1 | Regulatory mechanisms of phosphate homeostasis. From conception and throughout life, vertebrates need phosphate for energy, nucleic acids, cellular membrane constitution, skeletal formation, and a multitude of other vital functions. The only source of phosphate is external, from daily dietary food, which has to travel in the gut and become available for intestinal absorption, either actively through the cells or passively through the paracellular pathway. Once absorbed, inorganic phosphate enters into the extracellular compartment, particularly into the circulating plasma, where its concentration is tightly regulated by the three organs depicted here, parathyroid, bone, and kidney, interacting with one another through three feedback loops. Parathyroid glands produce parathyroid hormone (PTH), which, on the kidney, stimulates phosphate excretion and calcitriol synthesis; then, in turn, low phosphate and calcitriol directly inhibit PTH production. On the bone, PTH stimulates fibroblast growth factor 23 (FGF23) production and phosphate release following an increase in bone remodeling. FGF23 inhibits PTH secretion, but phosphate will tend to stimulate PTH production. FGF23, at the kidney level, stimulates urinary phosphate excretion and inhibits calcitriol production, tending to reduce serum phosphate levels by these two mechanisms. On the other hand, the renal PTH-stimulated calcitriol production stimulates FGF23 production by bone cells. These three counterregulatory loops maintain tightly controlled intestinal absorption and serum phosphate concentration. Certainly, there is now evidence that there should be an intestinal phosphate sensor that alerts one of these organs (the kidney), or probably more than one (dashed lines), in case of phosphate overload or phosphate deficiency.

PTX animals increased FGF23 and calcitriol and normalized serum phosphate levels. Several observations had already suggested that PTH could stimulate skeletal FGF23 production. Administration of (1-34)PTH to healthy individuals increased serum FGF23. Moreover, constitutively activated PTHR1 in Jansen's disease, which can be considered as a primary hyperparathyroidism with high PTH and hypophosphatemia, showed elevated FGF23.[9] Similarly, high FGF23 is often seen in secondary hyperparathyroidism and can predict its severity and its resistance to vitamin D therapy in chronic kidney disease patients.[10] In the same patients, serum FGF23 significantly declined after parathyroidectomy. López *et al.*[8] confirm and extend these previous findings, demonstrating that PTH, either directly or indirectly via calcitriol, is necessary for skeletal FGF23 production.

Probably, one of the most interesting findings of this study is the lack of effect of FGF23 on urinary and serum phosphate in the absence of PTH. PTX animals receiving physiological doses of calcitriol had high FGF23 but did not normalize serum phosphate, implying that, at least in the kidney, there was a key factor regulated by PTH that was required for the phosphaturic action of FGF23. This factor was certainly klotho, as the authors found a reduction of klotho expression in the kidneys of PTX animals. Importantly, renal klotho expression was restored to normal levels after PTH supplementation, allowing FGF23 to exert its phosphaturic action and the normalization of serum phosphate levels.

In summary, the study by López *et al.*[8] shows that PTH is necessary in maintaining normal circulating FGF23 levels, that changes in calcitriol levels unquestionably play a role in the stimulatory effect of PTH on FGF23, and that PTH regulates klotho expression in the kidney and thereby the phosphaturic effect of FGF23. It confirms the existence of three feedback loops, involving parathyroid glands, bone, and kidney, regulating serum phosphate concentration (Figure 1) and opens a number of areas for clinical and experimental research in the field of phosphate homeostasis.

DISCLOSURE
Pablo Ureña Torres has received fees from Abbott, Amgen, Genzyme, Shire, and Fresenius for clinical research studies and consulting and for speaking at promotional meetings.

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Calcium sensing in podocytes

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Besides its primary function in maintaining systemic calcium homeostasis, the calcium-sensing receptor (CaSR) is expressed by many cell types, with different, sometimes opposite, regulatory functions. Novel work from Oh and collaborators shows that activation of CaSR in podocytes has prosurvival effects and protects the cell from puromycin aminonucleoside damage. Given that the cellular consequences of CaSR activation are largely context-dependent, further studies will be required to elucidate its precise role in podocyte physiology and pathophysiology.

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Since 1883, when Ringer discovered that a trace amount of Ca^{2+} from tap water was sufficient to induce contraction of a frog's heart, the role of Ca^{2+} in regulating physiological functions has been continually investigated. We now know that appropriate concentrations of extracellular and intracellular Ca^{2+} are vital to the survival of all organisms from the simplest unicellular organism to mammals and that highly regulated processes are required to provide constant and appropriate quantities of the ion to cells and tissues. Therefore, sophisticated mechanisms enable cells to detect minor changes in extracellular Ca^{2+} content to counteract and modify their behavior accordingly.

In major organisms, Ca^{2+} has to be controlled both systemically and locally, and in mammals, the control of systemic calcium homeostasis is mediated by the calcium-sensing receptor (CaSR).

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