Physiology of Calcium and Phosphate Homeostases

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I. ABSTRACT

Calcium and phosphate homeostases are controlled by bidirectional calcium and phosphate fluxes, occurring at the levels of intestine, bone, and kidney. The latter organ plays a central role in regulating the extracellular concentration of either ion. Sensitive and efficient regulatory mechanisms, involving extracellular calcium sensing, are triggered by changes in calcium demand or supply. Similarly, the renal handling of phosphate can adjust its capacity to meet the need for phosphate of the organism. Not only calciotropic peptides or steroid hormones are capable of modifying the different calcium and phosphate fluxes to various extents, but also a variety of local factors are implicated in the regulation of calcium and phosphate homeostasis, in order to protect the organism against a deficiency or an overload. Finally, by directly influencing renal tubular calcium and phosphate transports, or by releasing calcium from intracellular stores, calcium itself plays the role of an effector on homeostatic mechanisms.

II. INTRODUCTION

Calcium and phosphate play prominent roles in the regulation of cell function. In addition, both are tightly connected to the process of bone mineralization, in which they participate in the formation of hydroxyapatite crystal deposited in specific regions with the collagen fibril networks. The hydroxyapatite crystal ensures the structural rigidity of the skeleton in its function of standing body support, and in the protection and housing of bone marrow. The regulation of calcium and phosphate homeostases is aimed at maintaining extracellular calcium and phosphate

concentration and balance as constant as possible, to protect the organism against deficiency or overload of these ions. Extracellular calcium concentrations are maintained remarkably stable, because of the high sensitivity of a variety of cell systems or organs, including the central nervous system, muscle, and exo-/endocrine glands, to small variations of extracellular calcium concentrations. In contrast, to fulfill the requirements of adequate mineral supply for osteoid tissue mineralization, the level of extracellular inorganic phosphate is adjusted to meet the demand of the organism. The homeostasis of both ions and their balance are controlled by a series of hormones and factors tightly interrelated in complex regulatory systems. The production of some of these agents is regulated by the concentration of the solute they are controlling, through negative feedback mechanisms, justifying thereby the term of calciotropic hormones as far as calcium homeostasis control is concerned.

III. BODY DISTRIBUTION OF CALCIUM

Calcium is the fifth inorganic element in abundance in the body. For a 70-kg subject, the calcium mass represents about 1300 grams, 99% of which is in bone and teeth, mostly as hydroxyapatite [1]. A small portion of bone calcium (approximately 1%) is rapidly exchangeable with extracellular fluid, contributing to the regulation of the homeostasis of extracellular calcium concentration, by serving as buffer and storage [2]. During puberty, there is nearly a doubling of body mineral stores through an increase in the size of the skeleton, with minor changes in volumetric bone density, i.e. the amount of bone in bone [3, 4]. By the end of the second decade, most of the body mineral capital is accumulated, though a very few percents of bone consolidation have been suggested to occur during the third decade, particularly in males [5].

Approximately 1% of total body calcium is intracellular. At the intracellular level, calcium homeostasis is controlled by an influx following an electrical and chemical gradient, through selective calcium channels [6–8]. As compared with the 1 mmol/L extracellular calcium concentration, a 10 000-fold lower concentration in the cytosol is maintained by the constant extrusion of calcium, through a calcium–magnesium ATPase and sodium–calcium exchange mechanisms [9, 10]. Intracellular calcium homeostasis is also maintained by a dynamic equilibrium with intracellular mitochondrial and microsomal stores [11, 12]. The concentration of cytosolic-free calcium is around 0.1 μmol/L. This concentration is critical in controlling cell membrane permeability, a large variety of enzymatic reactions, endocrine and exocrine hormone secretions, and in regulating cardiac, skeletal and smooth muscles contraction (Table I). Calcium is also implicated in the control of cell replication and apoptosis [13, 14]. Regulation of intracellular enzymatic functions is achieved through the interaction with various calcium-binding proteins such as, for instance, calmodulin or troponin, or the actin–myosin system, for muscle contraction [2, 8].

About 0.1% of total body calcium is in the extracellular compartment. Extracellular calcium concentration plays a major role in the integrity and stability of cell membrane, in intercellular adhesion, in blood clotting, and it influences neuromuscular excitability. Plasma calcium is tightly regulated in a narrow range, particularly the ionized form, which amounts to approximately 50% of total plasma calcium, and which represents the physiologically active form, whereas 40% is bound to proteins, mostly albumin, and 10% is under the form of ultrafiltrable ion complexes. The binding equilibrium of calcium with albumin is determined by the pH. Indeed, acute acidosis is associated with a decreased binding and, thereby, a higher proportion of the ionized form. In opposite, acute alkalosis decreases ionized calcium by increasing calcium bound to serum albumin.

IV. DETERMINANTS OF EXTRACELLULAR CALCIUM CONCENTRATION

Extracellular calcium concentration is maintained in a dynamic equilibrium through fluxes occurring at the level of the intestine, bone, and kidney (Fig. 1). At steady state, when body ions balance is zero, as in nongrowing individuals, the amounts of solutes entering the extracellular space

TABLE I. Physiological Roles of Calcium and Phosphate

	Calcium	Phosphate
Structural constituent	Hydroxyapatite (99% body calcium) Exchangeable pool (mineral storage)	Hydroxyapatite (85% body phosphorus) Nucleic acids Carbohydrates Lipids
Function	Intracellular signal transduction Cell adhesion Cell proliferation and differentiation Membrane permeability (neuromuscular excitability, muscle contraction, neurotransmission) Cytoskeleton (cell motility) Exo-/endosecretion Coagulation	Energy storage and delivery Intracellular signal transduction Enzyme activity Acid–base homeostasis

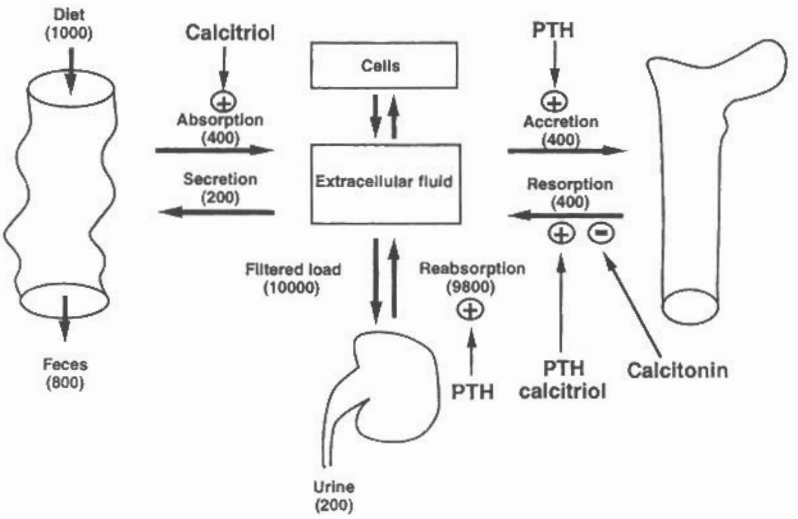

FIGURE 1 Main fluxes (mg/day) controlling calcium homeostasis.

are matched by the amounts leaving it. By controlling the calcium output, the kidney plays a central role in the maintenance of calcium homeostasis.

A. Intestinal Flux

Net intestinal absorption of calcium represents the difference between the amounts of solutes absorbed and secreted into the gut lumen. In humans, under normal conditions, intestinal absorption of calcium constitutes approximately 20% of ingested calcium. Net intestinal absorption of calcium depends on dietary intakes, on the capacity of the intestinal wall to transport calcium, on the bioavailability of calcium present in the intestinal lumen, and on the secretory flux. Under normal conditions, the secretory flux does not appear to vary markedly. However, it could be increased in pathological conditions such as coeliac disease. The intestinal calcium absorptive capacity is mainly controlled by calcitriol, which stimulates the transport through both genomic and nongenomic mechanisms [15–19]. The interaction of calcitriol with its specific nuclear receptor triggers the synthesis of a variety of proteins, including its receptor itself, calbindins, and the calcium–magnesium ATPase pump, which is located in the enterocyte basolateral membrane. Duodenum possesses the highest concentration of calcitriol receptors, and is the site of the intestine most sensitive to the vitamin metabolite in terms of calcium absorptive capacity. However, the short length of this segment and the rapid transit time suggest that it may not play a quantitative major role in the overall net intestinal calcium absorption. Thus, jejunum and ileum could quantitatively absorb more, despite a less efficient calcium transport capacity. Parathyroid hormone does not exert any direct effect on the intestinal cells [20, 21]. The importance of bioavailability of calcium at absorptive sites is illustrated by the impairment of calcium absorption induced by the formation of complexes with anions, such as phosphate, sulfate, phytate, or oxalate [22, 23]. For instance, the colonic mucosa is equipped with a powerful vitamin D-sensitive mechanism of calcium transport. However, the absorption is quantitatively little, since calcium is in the large intestine lumen under a form not available to the site of absorption [24]. At steady state, a 24-hour urinary excretion of calcium is mainly the reflection of daily net intestinal calcium absorption. The intestinal absorptive capacity can be evaluated by measuring calcium isotope absorption. A deconvolution analysis of calcium isotopes after oral and intravenous simultaneous administration of 2 different tracers, allows one to determine intestinal calcium transport. A simple evaluation is obtained by measuring the increase in urinary calcium after an oral calcium load [25, 26].

B. Bone Fluxes

On average, about 1% of total bone calcium exchanges every month, through a mechanism involving bidirectional fluxes. The main regulators of these fluxes are parathyroid hormone and calcitriol, and possibly calcitonin as an inhibitor of osteoclastic bone resorption under certain conditions [18, 19, 27, 28]. A large variety of substances, either circulating or produced locally, or present in the bone matrix, such as prostaglandins, thyroid hormones, glucocorticoids, sex hormones, growth factors, or interleukins,

components of the RANK-ligand/osteoprotegerin system, produced by the immune or hematopoietic systems, or bone cells, are capable of influencing bone remodeling, and thereby the bidirectional calcium fluxes [29–32]. In fasting urine, calcium excretion related to creatinine is a direct reflection of net bone [33–35]. Indeed, after an overnight fast, provided there is no calcium supplement taken in the previous evening and the patient does not have a postmicturitional urine residue, calcium appearing in the urine originates mostly from bone. The adjustment by creatinine corrects for urine concentration. Multiplying this ratio by serum creatinine provides urinary calcium excretion per glomerular filtration rate unit.

C. Soft Tissues

A minute amount of body calcium is intracellular. Thus, any calcium shift from or into the intracellular compartment does not significantly influence extracellular calcium homeostasis, except maybe in response to parathyroid hormone. The transient decrease in plasma calcium observed in the minutes following parathyroid hormone injection has been attributed to a parathyroid hormone-mediated acute transfer of calcium into cells [36, 37].

D. Renal Fluxes

Approximately 75% of plasma calcium is ultrafiltrable. After filtration, more than 95% of calcium is reabsorbed. Thus, the amount of calcium appearing in the urine represents the difference between the amounts filtered and reabsorbed. At steady state, this amount is mainly the reflection of net fluxes into the extracellular fluid of calcium originating from intestine and bone. The proximal tubule reabsorbs 60–70% of calcium, this reabsorption is tightly connected to that of sodium [38] (Fig. 2). Then, 20–30% of filtered calcium is reabsorbed along the loop ascending limb, and 10% at the level of the distal tubule. These two latter reabsorptive sites are influenced by parathyroid hormone. The renal tubular capacity to reabsorb calcium is the main determinant of extracellular calcium concentration. Any change of this capacity is able to induce variations of plasma calcium from 1.5–3.8 mmol/L [33, 34]. This concept has been established by the study of the relationship between urinary calcium excretion and plasma calcium in patients suffering from a lack or an excess of parathyroid hormone. Another example of the central control of extracellular calcium by the renal tubule is given by the syndrome of familial hypocalciuric hypercalcemia, the features of which include an increase in renal tubular reabsorption of calcium independent of parathyroid hormone [39–41]. Various situations or pharmacological agents can modulate the renal tubular reabsorption of calcium. Alkalosis stimulates renal tubular reabsorption of calcium, whereas acidosis decreases it. Thiazides and lithium salts increase the reabsorption of calcium, through mechanisms which are independent of parathyroid hormone [39, 40, 42]. Phosphate deficiency, pharmacological doses of calcitonin and loop diuretics are associated with an increase in calcium clearance [38]. Even large variations of the glomerular filtration rate do not cause major changes in calcemia, since the renal tubule can easily maintain the

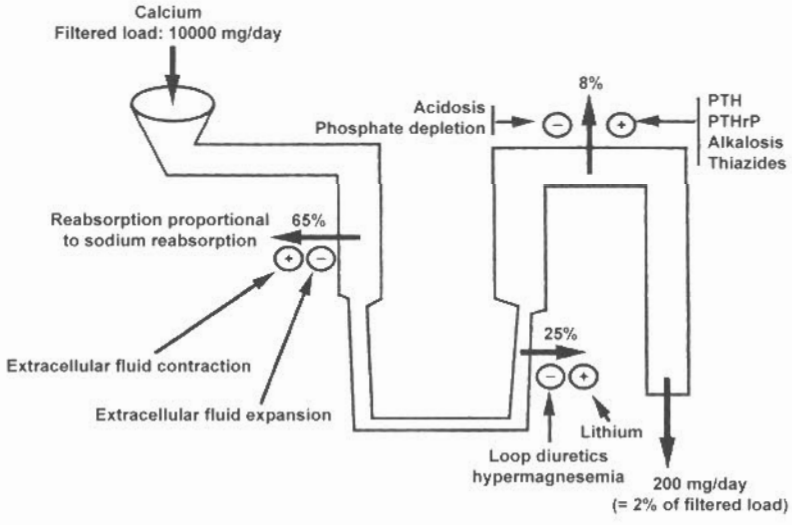

FIGURE 2 Central role of the kidney in controlling calcium homeostasis.

calcium excretion by modulating its reabsorptive capacity. However, the capacity of the kidney to excrete calcium can be overwhelmed under certain circumstances, particularly in case of large calcium loads resulting from extensive bone destruction and/or very high net calcium intestinal absorption, for instance as a consequence of vitamin D excess, which can lead to an increase in extracellular calcium levels.

V. RELATIVE IMPORTANCE OF THE VARIOUS CALCIUM FLUXES IN CONTROLLING EXTRACELLULAR CALCIUM HOMEOSTASIS

An important role in the regulation of calcium fluxes and balance between the various body compartments is played by the above-mentioned systemic hormones. Other hormones such as insulin, growth hormone, insulin-like growth factors, parathyroid hormone-related protein [28, 43–45], glucocorticoids, and sex hormones, as well as locally produced and acting interleukins [46], transforming growth factors and colony-stimulating factors, which are capable of directly influencing calcium metabolism, could also modify the target cell sensitivity to parathyroid hormone and/or calcitriol [29–31, 47–49]. However, their secretion does not appear to be directly controlled by variations in extracellular calcium and/or calcium demand.

Any disturbance of the above-described fluxes can result in an alteration of extracellular calcium homeostasis. For instance, an excess of vitamin D is associated with an increase in intestinal absorption of calcium and of bone resorption, and leads to hypercalcemia when the renal excretion capacity is overwhelmed [50, 51]. On the other hand, when renal tubular reabsorption of calcium is stimulated, such as by parathyroid hormone or by parathyroid hormone-related protein, plasma calcium levels can rise, despite very minute changes in calcium influx into the extracellular fluid compartment [33, 34]. Then, despite an increase in renal tubular reabsorption of calcium, urinary excretion is elevated as a consequence of a higher filtered load. The relative and quantitative contribution of calcium mobilization from bone, and of renal tubular reabsorption of calcium, to hypercalcemia induced by parathyroid hormone-related protein, can be estimated in studying the model of thyroparathyroidectomized rats chronically infused with parathyroid hormone-related protein [52].

Thyroparathyroidectomy prevents the contraregulation to variations in extracellular calcium concentrations by endogenous hormones. The elevation of plasma calcium is determined by both increased bone resorption and enhanced renal tubular reabsorption of calcium. However, the complete inhibition of bone resorption by a bisphosphonate, at a dose which fully normalized fasting urinary calcium excretion, taken as a reflection of net bone resorption, is associated with an approximately 30% decrease, but not a correction of plasma calcium. Thus, the residual hypercalcemia can be attributed to a renal tubular reabsorption effect, which accounted for more than two-thirds of the elevated plasma calcium (Fig. 3A). Indeed, it is well established that bisphosphonates are devoid of any direct effect on the renal handling of calcium [53]. To influence the renal tubular reabsorption, the administration of an agent (the free radical scavenger WR-2721), known to impair the tubular reabsorption of calcium through a parathyroid hormone-independent mechanism [54, 55], is able to acutely increase calcium excretion and to further reduce plasma calcium (Fig. 3B). The predominance of stimulated renal tubular reabsorption of calcium or of increased bone resorption in determining an altered extracellular calcium homeostasis can be demonstrated in a variety of clinical disorders associated with hypercalcemia [50] (Table II, Fig. 4).

VI. HOMEOSTATIC RESPONSES TO HYPOCALCEMIA

To maintain the extracellular calcium concentration as constant as possible, the response to acute variations implies changes in the various fluxes, without a necessarily major alteration in total body calcium stores. In contrast, when the organism is chronically submitted to calcium deficiency, its capacity to absorb calcium from the gut or to retain it in the kidney cannot match the need; then, to maintain extracellular calcium concentration, the skeletal mineral is mobilized, leading to a progressive decrease in bone mineral mass [1]. This mechanism might be implicated in the pathogenesis of senile osteoporosis. To recognize changes in extracellular calcium concentration, parathyroid cells have a sensitive calcium sensor, capable of transmitting the information to the parathyroid hormone-synthesizing and -releasing machinery.

A. Extracellular Calcium-sensing Receptor

A central role in the regulation of calcium homeostasis is played by parathyroid hormone produced by the parathyroid glands, which recognize alterations in plasma calcium [2, 28, 56]. Any change in plasma calcium is detected by a cell membrane-associated calcium sensor/receptor, which can also be activated by other divalent cations [2, 41, 57].

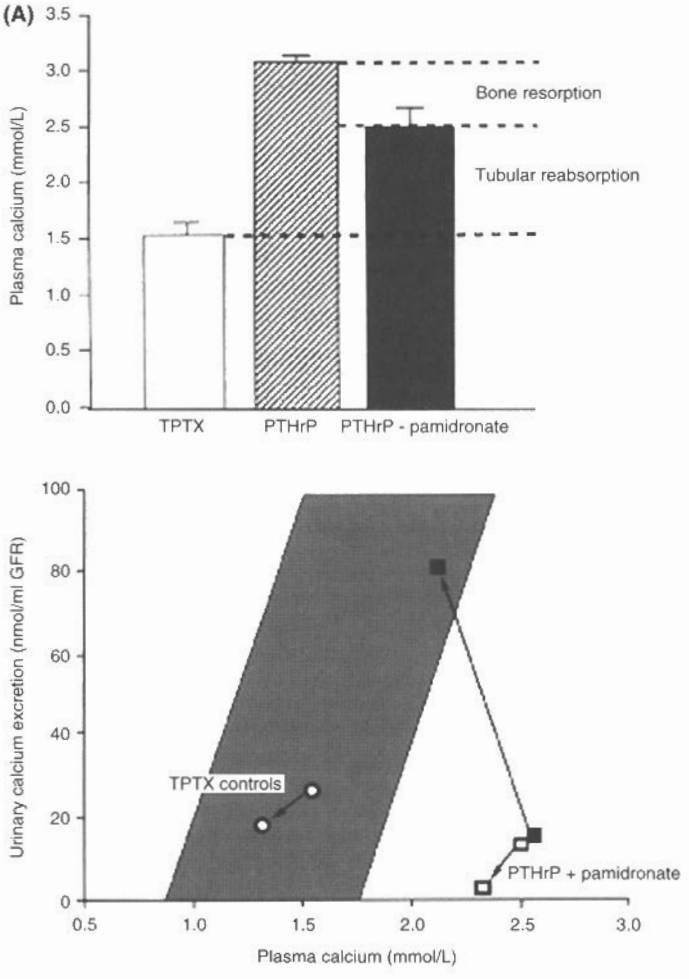

FIGURE 3 Relative contribution of net bone resorption and renal tubular reabsorption to parathyroid hormone-related protein-induced hypercalcemia in thyroparathyroidectomized rats. (A) Pair-fed animals were chronically infused by subcutaneous osmotic minipumps with synthetic parathyroid hormone-related protein (1–34) during 7 days. The bisphosphonate pamidronate was given subcutaneously at a dose which normalized bone resorption, as reflected by the correction of fasting urinary calcium excretion (adapted from Rizzoli et al., *J Bone Miner Res* **4**: 759–765, 1989). (B) The free radical scavenger WR-2721 was acutely administered to parathyroid hormone-related protein-infused and pamidronate-treated rats. The arrows connect the points before and after WR-2721 acute administration. WR-2721 acutely increased the clearance of calcium.

This 1078-amino acid polypeptide belongs to the receptor family of 7 transmembrane domains cell membrane-associated and guanine nucleotide-binding protein-coupled. This structure is present in parathyroid cells, in C cells of the thyroid, in renal epithelial cells, and in the central nervous system. A raise in extracellular calcium concentration stimulates protein kinase C and leads to an increase in cytosolic free calcium, thereby inhibiting parathyroid hormone synthesis and release. Conversely, a decrease in plasma calcium triggers the exocytosis of PTH within seconds, whereas it takes hours to increase PTH synthesis, and days to stimulate parathyroid cell proliferation [2, 28, 56]. Various mutations have been reported, which account for hyper- or hyposecretion of parathyroid hormone

TABLE II. Hypercalcemic Disorders

	Increased bone resorption	Increased renal tubular reabsorption of calcium
Endocrine disorders		
Primary hyperparathyroidism	+	+
Hyperthyroidism	++	–
Malignancy	+ or ++	+ or –
Granulomatous disorders (with calcitriol production)	++	–
Disuse	++	–
Drug-induced		
Vitamin D poisoning	++	–
Milk-Alkali syndrome	–	+
Thiazide diuretics	–	+
Lithium salts	–	+
Benign familial hypocalciuric hypercalcemia	–	+

in relation to variations in extracellular calcium concentration [58]. In familial hypocalciuric benign hypercalcemia, circulating levels of parathyroid hormone are insufficiently suppressed for the degree of calcemia, and the renal tubular reabsorption of calcium is increased through a parathyroid hormone-independent mechanism [39, 40, 59]. This disorder appears to be due to mutations associated with hypofunction of the cell membrane calcium-sensing mechanism [58, 60]. Thus, higher plasma calcium levels are necessary to inhibit PTH secretion. A similar syndrome can be reproduced in transgenic mice by the incorporation of transgenes displaying the same mutations [61]. Interestingly, the same biochemical pattern can be encountered in patients treated with lithium salts [62, 63]. Conversely, in certain cases of familial

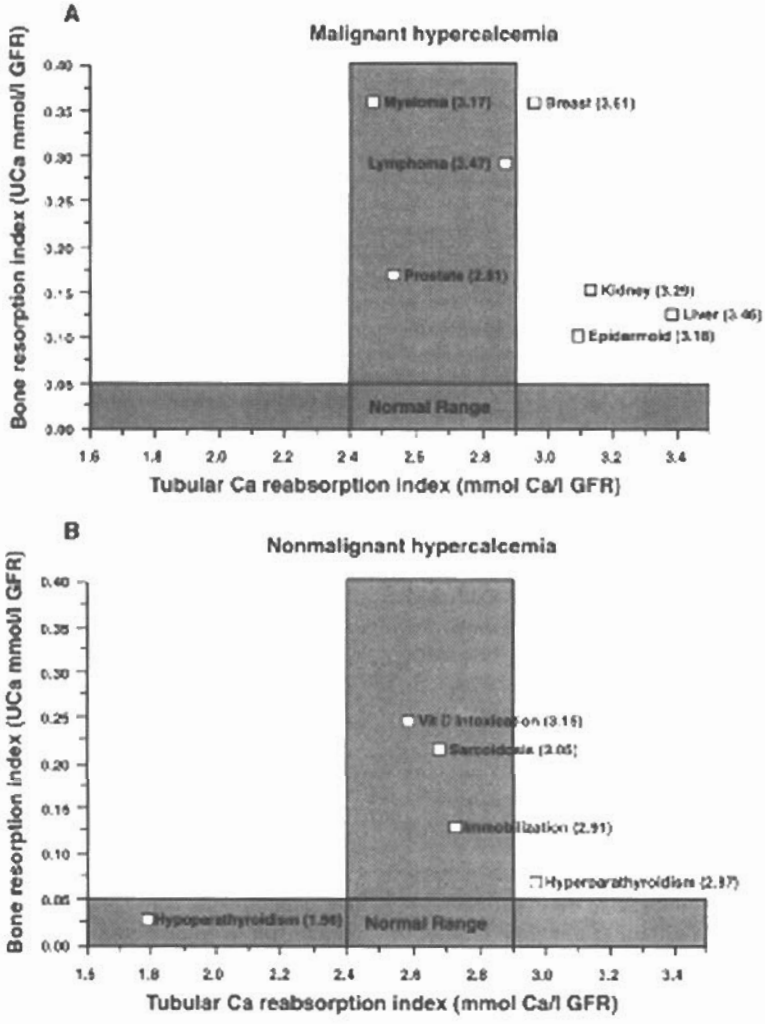

FIGURE 4 Relationship between bone resorption, as evaluated by the bone resorption index (BRI) and tubular reabsorption of calcium index (TRCaI) in rehydrated patients with malignant (A) or non malignant (B) hypercalcemia. In parentheses the mean plasma calcium concentration is given. This figure is taken from Buchs et al. (*Bone* **12**: 47–56, 1991), with the permission of the publisher.

hypoparathyroidism, a mutation-induced hyperactive calcium-sensing receptor has been found [59]. Under these conditions, parathyroid hormone production is not stimulated despite reduced calcium levels. Calcium-sensing receptor activity can be modulated by calcimimetics, which enhance its sensitivity to extracellular calcium [58]. Under these conditions, PTH secretion is inhibited. Conversely, calcilytics are negative modulators of the calcium-sensing receptor, leading thereby to a stimulation of PTH secretion. There are other structures recognizing changes in extracellular calcium concentration [64–67]. In osteoblasts, they may be distinct from those present in parathyroid cells [68–70].

B. Effectors

The perturbation of plasma calcium is corrected by the stimulation of the tubular reabsorption of calcium, and the mobilization of calcium from bone mineral (Fig. 5). Parathyroid hormone itself, as well as a decrease in calcium and/or phosphate concentrations, directly stimulates the synthesis of calcitriol at the renal level. The latter hormone contributes to an increase in plasma calcium through a mobilization of calcium from bone and a stimulation of intestinal calcium absorption. Thus, calcitriol plays a central role in the intestinal adaptation to low calcium intake. This adaptative mechanism is blunted in the elderly, as a consequence of a decreased synthesis of, and a lower response to, calcitriol [71, 72]. It appears therefore that the integrated control of extracellular calcium homeostasis is governed by the requirement of maintaining extracellular calcium concentration in a very narrow range. This system is quite efficient in its capacity to respond to a calcium need. Conversely, prevention of overwhelming by calcium is ensured by the reversal of these systemic mechanisms. Although calcitonin is able to inhibit bone resorption and to increase the renal clearance of calcium, when given in pharmacological doses, it is improbable that this hormone significantly participates in the physiological defense against hypercalcemia.

During body growth, or in the third trimester of pregnancy, when calcification of the fetus bone takes place, the need for calcium results in an increase in intestinal calcium absorption. Elevated levels of calcitriol are responsible for stimulating intestinal calcium absorption [16, 18, 19]. On the other hand, with, for instance, estrogen deprivation at menopause, bone bidirectional calcium fluxes are increased, with resorption overcoming accretion, resulting in a net negative skeletal balance. Under these conditions, a reduced intestinal calcium absorption and/or a higher urinary calcium excretion can be viewed as a homeostatic mechanism attempting to prevent the extracellular calcium compartment from being overloaded. A similar reaction can occur with prolonged immobilization and the consequent net calcium loss.

VII. CALCIUM AND BONE GROWTH

Several observational studies have suggested that increasing the calcium intake would promote a greater bone mass gain, and thereby a higher peak bone mass [5]. Furthermore, several prospective randomized, double-blind, placebo-controlled intervention trials indicate that calcium supplementation can increase bone mass gain,

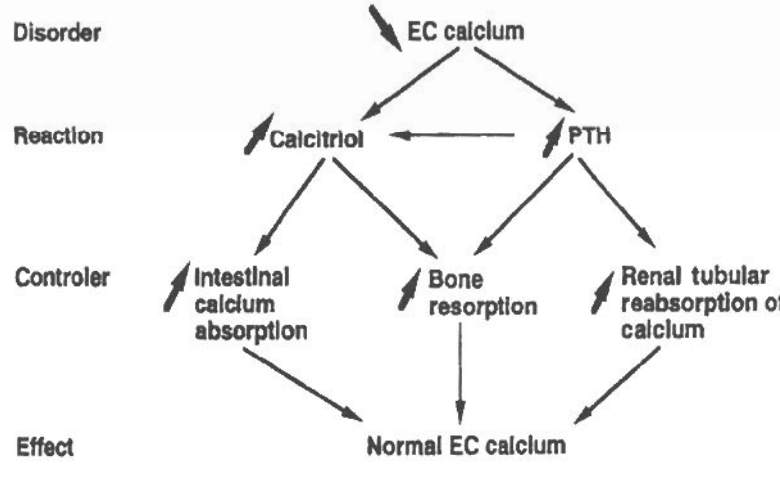

FIGURE 5 Homeostatic response to a decrease in extracellular (EC) calcium concentration.

although the magnitude of the calcium effects appears to vary according to the skeletal sites examined, the stage of pubertal maturation at the time of the intervention, and the spontaneous calcium intake [73–78]. Furthermore, these effects could be modulated by an interaction with vitamin D receptor genotype [79]. The positive effects of calcium supplementation have essentially been ascribed to a reduction in bone remodeling. Indeed, in one study the plasma level of osteocalcin, a biochemical marker of bone remodeling in adults, was significantly reduced in the calcium-supplemented children [73]. This interpretation is also in keeping with the currently favored mechanism proposed to account for the inhibitory effect of calcium on age-related bone loss. In the elderly, the calcium effect on bone remodeling is usually ascribed to an inhibition of parathyroid hormone secretion, as the plasma level tends to increase with aging [80–85].

In a double-blind, placebo-controlled study on the effects of calcium supplementation in prepubertal girls, changes in scanned bone area and in standing height suggest that calcium supplementation could affect bone modeling in addition to bone remodeling [76, 77]. Indeed, milk calcium-enriched foods enhanced the gain of both mean scanned bone area of several skeletal sites, and statural height in the group of spontaneously low-calcium consumers, to the level achieved by the spontaneously high-calcium consumers. Morphometric analysis of the changes observed in the lumbar spine and in femoral diaphysis suggests that calcium could enhance both the longitudinal and the cross-sectional growth of the bones [76, 77]. Effects of calcium on the secretion or action of growth factors, including the IGFs-IGF-binding proteins system, could be implicated [86, 87]. Bone mineral density was measured 7.5 years after the end of calcium supplementation. In these young adult girls, it appeared that menarche occurred earlier in the calcium-supplemented group, and that persistent effects of calcium were mostly detectable in those subjects with an earlier puberty [77]. Finally, because of a higher response to calcium supplements preferentially observed in a certain vitamin D receptor genotype [79], it remains to be determined whether the interaction between vitamin D receptor genotype and the influence of calcium supplementation on bone mass accrual affects modeling and/or remodeling.

VIII. BODY DISTRIBUTION OF PHOSPHORUS

Phosphorus is the sixth element in abundance in the body, mostly under the form of phosphate. Its mass represents about 1% of body weight, i.e. 700 g for an individual of 70 kg [1]. More than 80% of phosphate is in bone, the remaining in soft tissues, in the cellular and extracellular compartments. Inside the cells, phosphate can be either under an inorganic form, or an organic one, as a constituent of carbohydrates, lipids, or nucleic acids (Table I). It plays a crucial role in the energy storage and delivery, in the regulation of a large variety of different enzymes activity, and in intracellular signal transduction mechanisms. It also contributes to ensure intracellular and plasma membrane stability, and is implicated in the modulation of hemoglobin affinity for oxygen. Phosphate enters the cells through an active transport system, the energy for which is provided by the extra/intracellular sodium gradient [88, 89]. Extracellular phosphate represents 0.1% of body phosphate. In plasma, one-third of phosphate is inorganic, of which 90% is ultrafiltrable. Two-thirds are parts of circulating phospholipids. At physiological pH, the divalent form of inorganic phosphate predominates. This anion contributes to the maintenance of extracellular acid–base homeostasis. Inorganic phosphate concentration varies in relation to age and body growth rate. In adults, its concentration ranges between 0.8 and 1.4 mmol/L, whereas it is 1.4–2.7 and 1.3–2.0 in the neonate and the adolescent, respectively. There is a nycthemeral rhythm in inorganic phosphate concentration, with highest values encountered in the late afternoon.

IX. DETERMINANTS OF EXTRACELLULAR PHOSPHATE CONCENTRATION

The levels of extracellular phosphate are determined by the balance between dynamic fluxes from and into extracellular compartment, which occur in intestine, bone, soft tissue, and kidney (Fig. 6).

A. Intestinal Fluxes

A balanced normal diet provides sufficient amounts of phosphate in most circumstances, so that phosphate deficiency from dietary origin usually does not occur. In the intestinal lumen, phosphate can be complexed with various cations, including aluminum or calcium, which thereby prevents its absorption. This property is applied in the prevention of phosphate overload in the frame of advanced renal failure and phosphate retention. The jejunum exhibits the highest absorptive capacity. Two mechanisms are involved. The first one, which predominates in the proximal small intestine, is an active and saturable transport system, activated by calcitriol [17, 21]. The second one is a passive

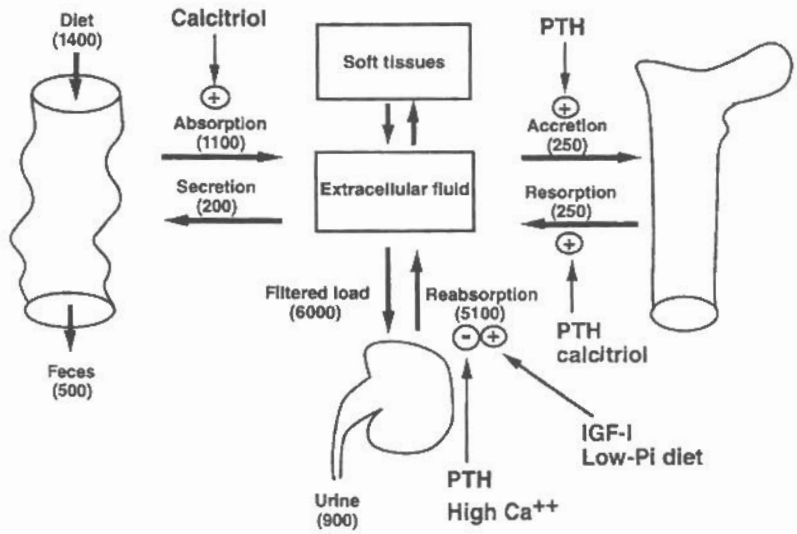

FIGURE 6 Main fluxes (mg/day) controlling phosphate homeostasis.

and nonsaturable transport, which plays quantitatively the most important role. Indeed, an increase in phosphate intake is accompanied by a commensurate increment of net intestinal phosphate absorption. The saturable component becomes negligible at high phosphate intake. Under normal conditions, 60–70% of dietary phosphate is absorbed. At steady state, daily urinary phosphate excretion is a direct reflection of phosphate absorbed in the intestine, and thus of the phosphate intake.

B. Bone Fluxes

The processes of bone formation and resorption implicate bidirectional fluxes of phosphate from and into bone mineral. Osteoclastic bone resorption releases phosphate into the extracellular fluid. The deposition of phosphate into newly formed osteoid tissue is dependent on its extracellular concentration. In addition, the plasma membrane of osteoblastic cells, as well as extracellular matrix vesicles found in epiphyseal cartilage and in woven bone, are endowed with a saturable, carrier-mediated phosphate transport system, modulated by various hormones and growth factors [90–92]. These extracellular matrix vesicles could play a role in the initiation of the calcification process. The plasma membrane-associated receptor for gibbon ape leukemia virus [93], which functions as a sodium-dependent phosphate transporter, distinct from the renal type I and II sodium-dependent phosphate transporters, has been found in osteoblastic cells and is regulated by IGF-I [94]. Thus, this type III phosphate transporter could participate in the control of the flux of phosphate deposition into bone.

C. Renal Fluxes

Approximately 70% of the filtered inorganic phosphate is reabsorbed in the proximal tubule through saturable sodium co-transport mechanisms [95], whereas about 20% of the filtered load is excreted in the urine (Fig. 7). An important step in the transfer of phosphate from the lumen to peritubular capillaries takes place in the epithelial cell brush-border membrane. At least two different sodium-dependent phosphate transporters have been identified in the renal tubule, which share little homology in their amino acid sequence [88, 89]. The type I transporter appears to be constitutive, nonselective, and unregulated. The type II transporter is controlled by dietary phosphate intake or by parathyroid hormone/parathyroid hormone-related protein. Short-term exposure to a low-phosphate diet stimulates the insertion of type II phosphate transporters into the apical membrane, whereas parathyroid hormone decreases it. Prolonged low dietary phosphate intakes increase the expression of type II transporter. Its presence in the brush border membrane is markedly depressed in X-linked hypophosphatemic rachitic mice [88, 89]. However, this defect has been mapped to another gene than that coding for the type II phosphate transporter. The gene mutated in the case of X-linked hypophosphatemia codes for an endopeptidase, for which the substrate is, however, still not clearly elucidated [96–99].

The maximal tubular reabsorption capacity (TmPi/GFR) is the main determinant of plasma phosphate concentration [95]. Since the tubular reabsorption is a saturable process, fractional reabsorption for a given TmPi/GFR varies according to the filtered load. Therefore, fractional

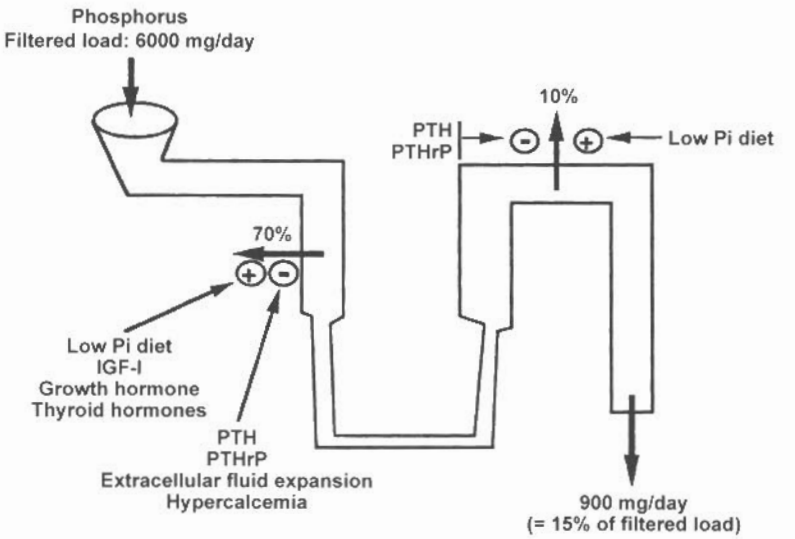

FIGURE 7 Central role of the kidney in controlling phosphate homeostasis.

excretion or reabsorption cannot be taken as a reliable reflection of the tubular reabsorptive capacity. This capacity is controlled by the phosphate supply and the need for phosphate of the organism [100]. Not only is the low dietary phosphate-mediated stimulation of phosphate transport independent of parathyroid hormone, but it prevents the phosphaturic response to the hormone [101]. During growth, a state of need for phosphate, or during phosphate deficiency, TmPi/GFR is increased [95].

In proximal tubule kidney cells, phosphate transport is activated by IGF-I, whereas it is decreased by parathyroid hormone, or its tumoral analog parathyroid hormone-related protein [102–104]. Other factors could influence the sodium-dependent phosphate transport [47, 48]; however, their contribution to alterations observed in physiological or pathophysiological situations remains to be established. IGF-I, which plays a prominent role in the longitudinal growth of bone, also increases the renal synthesis of calcitriol [105], which in turn stimulates the intestinal absorption of phosphate [17, 21] (Table III).

TABLE III. Factors Affecting Phosphate Renal Tubular Reabsorption

Factors that decrease Pi reabsorption	Factors that increase Pi reabsorption
Phosphate loading	Phosphate depletion
Parathyroid hormone/PTHrP/cAMP	Parathyroidectomy
Volume expansion	Volume contraction
Hypercalcemia	Hypocalcemia
Carbonic anhydrase inhibitors	Growth hormone
Fibroblast growth factor 23	Insulin-like growth factor-I
Frizzled receptor protein 4	

Thus, by acting indirectly on the intestine through calcitriol, and directly on the kidney, IGF-I contributes to make positive the phosphate balance, favoring thereby the mineralization of newly formed bone (Fig. 8). Parathyroid hormone, or parathyroid hormone-related protein, as well as a state of phosphate overload, or elevated extracellular calcium concentration reduce TmPi/GFR. Calcitriol appears to be devoid of any direct and significant effect on the renal tubular reabsorption of phosphate under physiological conditions.

Factor(s) other than PTH or PTHrP are known to increase phosphate excretion, through influencing renal tubular reabsorption of phosphate. Although the molecules implicated are not fully elucidated, the name "phosphatonin" has been suggested [106, 107]. Evidence for the existence of such factor(s) has been obtained from pathological conditions such as tumor-associated osteomalacia, X-linked hypophosphatemia, or autosomal dominant hypophosphatemic rickets. Genomic and proteomic procedures allowed one to identify fibroblast

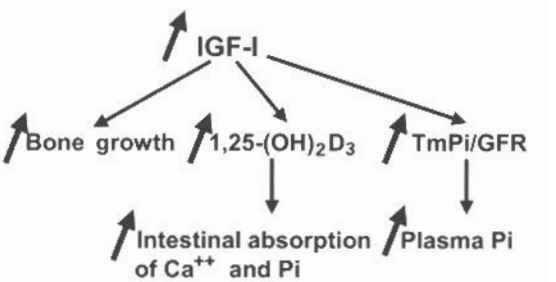

FIGURE 8 Effects of IGF-I on bone and on mineral homeostasis.

growth factor 23 (FGF23), frizzled receptor protein 4 (FRP4), and matrix extracellular phosphoglycoprotein (MEPE) has exhibited phosphaturic activity [107–110] (Fig. 9).

D. Soft Tissues

In constrast to calcium, phosphate fluxes from and into the intracellular compartment can alter extracellular phosphate concentration. During fasting, phosphate mobilization from soft tissues, such as the liver, can contribute to an increase in plasma phosphate. Inversely, during the post-absorptive phase, extracellular phosphate is transferred into soft tissues, as a result of incorporation into carbohydrates or lipids under the influence of insulin [111, 112]. Hyperphosphatemia, in the frame of a tumor lysis syndrome following the initiation of cytotoxic therapy of hematologic malignancies, or hypophosphatemia occurring during the treatment of diabetic ketoacidosis with insulin, are examples of the role played by soft tissues in phosphate homeostasis under pathological conditions [113, 114].

X. HOMEOSTATIC RESPONSES TO CHANGES IN PHOSPHATE SUPPLY OR DEMAND

Phosphate deficiency stimulates calcitriol production, which increases the transfer of phosphate from the intestine and mobilization from bone mineral (Fig. 10). The renal synthesis and release of calcitriol are related to the phosphate supply and the demand of the organism. On the other hand, low extracellular phosphate concentration is associated with higher calcium concentration through a physicochemical process. In turn, the inhibition of parathyroid hormone release decreases urinary phosphate excretion.

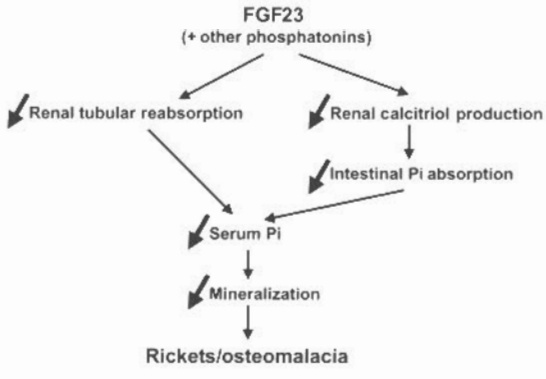

FIGURE 9 Effects of "phosphatonins" on Pi homeostasis.

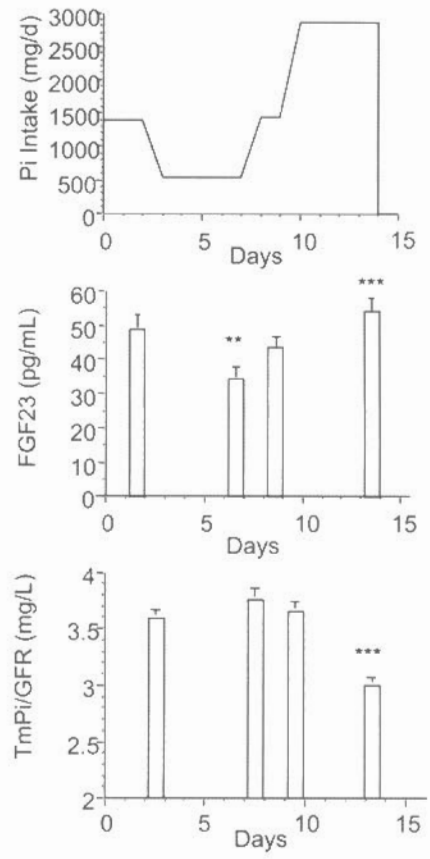

FIGURE 10 Renal Pi handling and serum FGF23 levels in relation to dietary Pi intakes (from Ferrari et al., *J Clin Endocrinol Metab* 2005, with permission of the publisher).

The most powerful mechanism for phosphate retention is certainly the system, the molecular nature of which is still not elucidated, which allows the kidney to adapt very tightly its capacity of reabsorbing phosphate to dietary supplies and to the need of the organism in phosphate, in a parathyroid hormone-independent manner [88, 89, 95, 96, 100, 101, 115]. FGF23 could contribute to this dietary phosphate-induced modulation of renal tubular transport. Indeed, increasing dietary phosphate intakes was associated with higher FGF23 levels in young healthy males undergoing acute changes in phosphate supply [116] (Fig. 11). The adaptation to low phosphate intakes in terms of calcitriol production and changes in renal tubular phosphate reabsorption is missing in X-linked hypophosphatemic rickets [115]. This indicates that the homeostatic response to meet the phosphate supply and need is altered in this disorder, leading to a renal phosphate leak and inadequate calcitriol levels for the degree of hypophosphatemia, despite the need for phosphate for bone growth and mineralization. Various mutations in the gene to which X-linked hypophosphatemic rickets has been mapped, have been reported, both in humans and in mice [97–99].

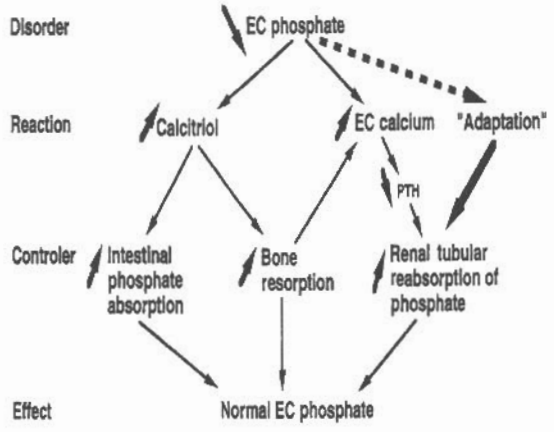

FIGURE 11 Homeostatic response to a decrease in extracellular (EC) phosphate concentration.

The role played by this *PHEX* (phosphate-regulating gene with homology to endopeptidase on the X chromosome) gene product, which is a zinc-binding endopeptidase, in the regulation of phosphate homeostasis, is still not elucidated.

As mentioned above, this phosphate homeostatic mechanism could play a role in the increase of renal tubular reabsorption of phosphate observed during growth, possibly in association with IGF-I, but also in disorders characterized by an increased demand, as for instance in extensive osteoblastic metastases [117]. An inhibition of the same system could also be implicated to avoid phosphate overload in the frame of a decreased nephron mass, as for instance in progressive renal failure [118, 119]. There is also evidence that elevated extracellular phosphate concentration could also be able to directly increase parathyroid hormone production [120]. This mechanism is apparently less efficacious than the one triggered by hypocalcemia. Because of the phosphaturic effects of parathyroid hormone, such a regulatory system certainly represents an attempt to maintain the homeostasis of phosphate economy.

XI. CONCLUSIONS

The physiology of calcium and phosphate homeostases is regulated by coordinated bidirectional calcium and phosphate fluxes, occurring at the levels of intestine, bone, and kidney. These fluxes are influenced by calciotropic peptides or steroid hormones, and by a variety of locally produced factors. By directly modifying renal tubular calcium and phosphate transport, or by releasing calcium from intracellular stores, calcium itself functions as a regulator. In the control of extracellular concentration of either ion, the kidney tubule reabsorptive capacity plays a central role. Sensitive and efficient regulatory mechanisms involving local calcium sensing are triggered by changes in calcium demand or supply. Similarly, the renal handling of phosphate can adjust its capacity to meet the need of the organism. The regulation of calcium and phosphate homeostases aims at fighting against a deficiency or an overload. In response to a variation of any regulatory flux, a series of homeostatic responses and adjustments is triggered, leading to a new steady state, in which the initially altered variable has been corrected.

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