Primary Disorders of Phosphate Metabolism

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Phosphorus plays an important role in growth, development, bone formation, acid-base regulation, and cellular metabolism. Inorganic phosphorus exists primarily as the critical structural ion, phosphate (PO4), which serves as a constituent of hydroxyapatite, the mineral basis of the vertebrate skeleton, and at the molecular level, providing the molecular backbone of DNA. Its chemical properties allow its use as a biological energy store as adenosine triphosphate. Additionally, phosphorus influences a variety of enzymatic reactions (e.g. glycolysis) and protein functions (e.g. the oxygen-carrying capacity of hemoglobin by regulation of 2,3-diphosphoglycerate synthesis). Finally, phosphorus is an important signaling moiety, as phosphorylation and dephosphorylation of protein structures serves as an activation signal. Indeed, phosphorus is one of the most abundant components of all tissues, and disturbances in its homeostasis can affect almost any organ system. Most phosphorus within the body is in bone (600-700 g), while the remainder is largely distributed in soft tissue (100-200 g). The plasma contains 11-12 mg/dL of total phosphorus (in both organic and inorganic states) in adults. Inorganic phosphorus (Pi) primarily exists as phosphate (PO4), and is the commonly measured fraction, found in plasma at concentrations averaging 4 mg/dl in older children and adults. Plasma Pi concentrations values in children are higher, often up to 8 mg/dl in infants, and gradually declining throughout childhood to adult values. The organic phosphorus component is primarily found in phospholipids and is not routinely assessed, and comprises approximately two-thirds of the total plasma phosphorus (1). Thus the term “plasma phosphorus” generally is used when referring to plasma inorganic Pi concentrations, and because plasma inorganic Pi is nearly all in the form of the PO4 ion, the terms phosphorus and phosphate are often interchangeably used in the clinical chemistry laboratory.

The critical role that phosphorus plays in cell physiology has resulted in the development of elaborate mechanisms designed to maintain phosphate balance. These adaptive changes are manifest by a constellation of measurable responses, the severity of which is modified by the difference between metabolic Pi need and exogenous Pi supply. Such regulation maintains the plasma and extracellular fluid phosphorus within a relatively narrow range and depends primarily upon gastrointestinal absorption and renal excretion as mechanisms to effect homeostasis. Although investigators have recognized a variety of hormones which influence

these various processes, in concert with associated changes in other metabolic pathways, the sensory system, the messenger and the mechanisms underlying discriminant regulation of Pi balance remain incompletely understood.

While long-term changes in Pi balance depend on these variables, short-term changes in Pi concentrations can occur due to redistribution between the extracellular fluid and either bone or cell constituents. Such redistribution results secondary to various mechanisms including: elevated levels of insulin and/or glucose; increased concentrations of circulating catecholamines; respiratory alkalosis; enhanced cell production or anabolism; and rapid bone remineralization.

REGULATION OF PHOSPHORUS HOMEOSTASIS

The majority of ingested phosphorus is absorbed in the small intestine; hormonal regulation of this process has undergone relatively limited study, and is thought be limited, at least in comparison to well-established hormonal mechanisms regulating Pi homeostasis in the kidney. Indeed the kidney has long been considered the dominant site of regulation of Pi balance, as renal tubular reclamation of filtered Pi occurs in response to complex regulatory mechanisms. Although the fate of Pi has generally been considered a matter of renal elimination, incorporation into organic forms in proliferating cells, or deposition into the mineral phase of bone as hydroxyapatite, the role of intestinal phosphate transport warrants further study. Indeed it appears that presentation of Pi to the intestine can affect systemic phosphate handling before changes in serum Pi concentration are evident. Moreover, in the setting of severe phosphorus deprivation, the phosphate contained in bone mineral provides a source of phosphorus for the metabolic needs of the organism. The specific roles that the intestine and kidney play in this complex process are discussed below.

GASTROINTESTINAL ABSORPTION OF PHOSPHORUS

Studies of Pi absorption in the intestine have yielded variable results, in part due to confounding influences of nutritional status, the effects of anesthesia on gut transit, species differences, and potential effects of studying whole organisms as opposed to isolated bowel segments. The small intestine is the dominant site of Pi absorption; in mice Pi is absorbed along the entire length of small bowel, but at the highest rate in the ileum. In rats, duodenum and jejunem provide the primary sites of Pi absorption, whereas very little occurs in ileum. This is felt to be more consistent with the pattern of Pi absorption in humans, however studies are subject to the confounding issues noted above. In normal adults net Pi absorption is a linear function of dietary Pi intake. For a dietary Pi range of 4 to 30 mg/kg/day, the net Pi absorption averages 60 to 65% of the intake (2). Intestinal Pi absorption occurs via two routes (Figure 1), a cellularly mediated active transport mechanism and diffusional flux, largely through a paracellular shunt pathway (3).

Controversy exists as to what proportion of intestinal Pi absorption is absorbed via sodium-dependent mechanisms and what proportion is Na-independent. In this regard, the major Na+-dependent phosphate cotransporter identified in intestinal brush border membranes is

NaPi-IIb, a member of the SLC34 solute carrier family, also referred to as type II sodium-phosphate cotransporters (4). A major role for NaPi-IIb transporters is evident in intestine, but NaPi-IIb is also expressed in lung, colon, testis/epididymus, liver, and in mammary and salivary glands. Like NaPi-IIa, predominantly a renal tubular protein, NaPi-IIb is electrogenic, maintains a 3:1 stoichiometry of Na: Pi, and has a high affinity for Pi binding (5-9). Depending upon species and bowel segment, NaPi-IIb transporters can be regulated by 1,25 dihydroxyvitamin D (↑), FGF23 (↓), low Pi diet (↑), and acute phosphate loading (↑). Energy for the electrochemical uphill process is provided by the sodium gradient, which is maintained by sodium-potassium ATPase. The phosphate incorporated into intestinal cells by this mechanism is ferried from the apical pole to the basolateral pole likely through restricted channels such as the microtubules. Exit of Pi from the enterocyte across the basolateral membrane and into the circulation is a poorly understood process. More recently members of the SLC20 solute carrier family, the type III sodium-phosphate cotransporters Pit1 and Pit2, have been found to be variably expressed in the intestine (10). Their role is not clear; in NaPi-IIb-/- mice there is no residual sodium-dependent Pi transport activity, suggesting that the type III transporters are of limited significance in the intestine. Finally it does not appear that Na-independent Pi transport is a regulated phenomenon. Given the variable nature and segment-specific regulation of NaPi-IIb, the ultimate impact on overall phosphate homeostasis appears to be of a lesser magnitude at the intestine than at the kidney.

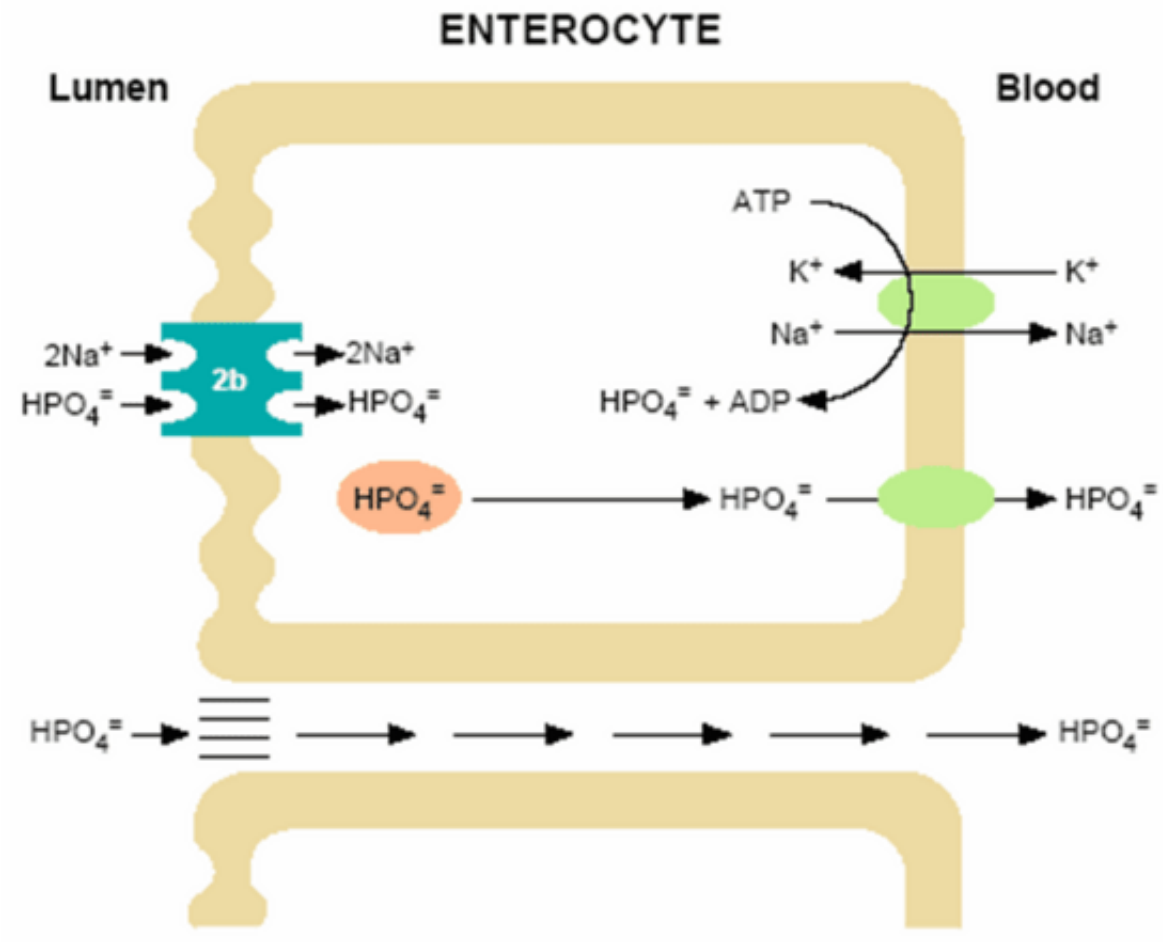


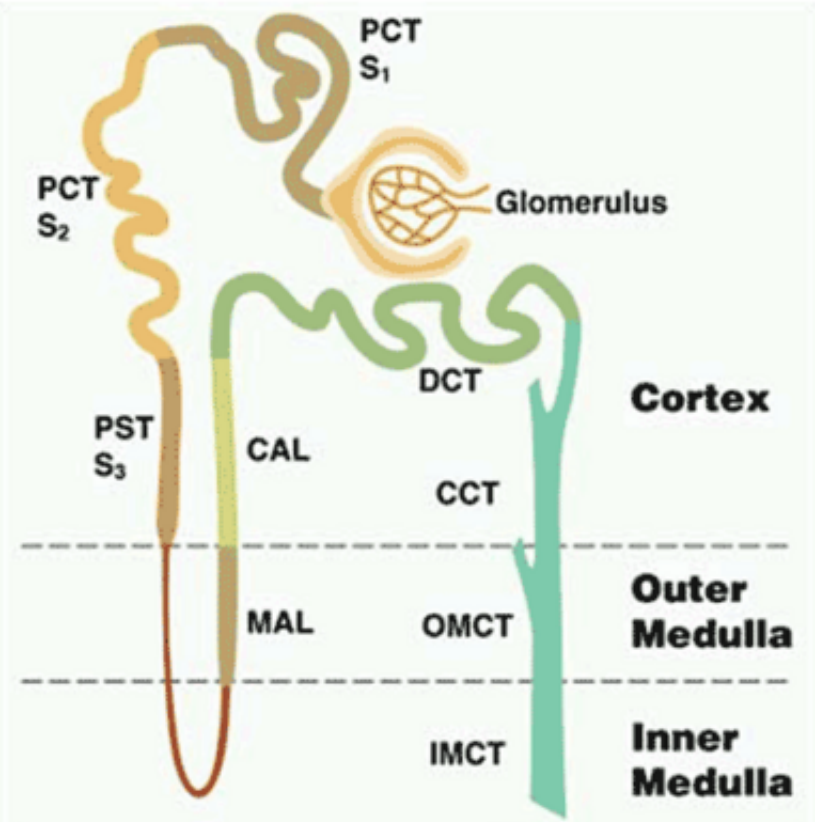
Figure 1.Model of inorganic phosphate (HPO4=) transport in the intestine. At the luminal surface of the enterocyte the brush border membrane harbors sodium-dependent phosphate transporters of the NaPi-IIb type. NaPi-IIb transporters are electrogenic, have high affinity for Pi, and a stoichiometry of 3 Na ions: 1 phosphate. Energy for this transport process is provided by an inward downhill sodium gradient, maintained by transport of Na+ from the cell via a Na+/K+ ATPase cotransporter at the basolateral membrane. The HPO4= incorporated into the enterocytes by this mechanism is transferred to the circulation by poorly understood mechanisms. The bulk of HPO4= absorption occurs via a sodium-independent process(es) such diffusional aborption across the intercellular spaces in the intestine.

As most diets contain an abundance of Pi, the quantity absorbed nearly always exceeds the need. Factors which may adversely influence the non-regulable, sodium-independent process are the formation of nonabsorbable calcium, aluminum or magnesium phosphate salts in the intestine and age, which reduces Pi absorption by as much as 50%.

RENAL EXCRETION OF PHOSPHORUS

The kidney responds rapidly to changes in serum Pi levels or to dietary Pi intake. The balance between the rates of glomerular filtration and tubular reabsorption (11) determines net renal handling of Pi. Pi concentration in the glomerular ultrafiltrate is approximately 90% of that in plasma, as not all of the plasma Pi is ultrafilterable (12). Since the product of the serum Pi concentration and the glomerular filtration rate (GFR) approximates the filtered load of Pi, a change in the GFR may influence Pi homeostasis if uncompensated by commensurate changes in tubular reabsorption.

The major site of phosphate reabsorption is the proximal convoluted tubule, at which 60% to 70% of reabsorption occurs (Figure 2). Along the proximal convoluted tubule the transport is heterogeneous, with greatest activity in the S1 segment. Further, increasing, but not conclusive, data supports the existence of a Pi reabsorptive mechanism in the distal tubule. Currently, however, conclusive proof for tubular secretion of Pi in humans is lacking (13).



	Phosphate Reabsorption	PTH Sensitive Adenylate Cyclase	PTH Inhibited Phosphate Reabsorption	Calcitonin Sensitive Adenylate Cyclase	Calcitonin Inhibited Phosphate Reabsorption
PCT	60–70%	++	++	–	++
PST	15–20%	++	++	–	++
DCT	5–10%	++	++	++	–
CAL	0%	++	–	++	–
MAL	0%	–	–	++	–

Figure 2.Distribution of Pi reabsorption and hormone-dependent adenylate cyclase activity throughout the renal tubule. The renal tubule consist of a proximal convoluted tubule (PCT), composed of an S1, S2 and S3 segment, a proximal straight tubule (PST), also known as the S3 segment, the loop of Henle, the medullary ascending limb (MAL), the cortical ascending limb (CAL), the distal convoluted tubule (DCT) and three segments of the collecting tubule: the cortical collecting tubule (CCT); the outer medullary collecting tubule (OMCT); and the inner medullary collecting tubule (IMCT). Pi reabsorption occurs primarily in the PCT but is present is the PST and DCT, sites at which parathyroid hormone (PTH) dependent adenylate cyclase is localized. In contrast, calcitonin alters Pi transport at sites devoid of calcitonin dependent adenylate cyclase, suggesting that Pi reabsorption in response to this stimulus occurs by a distinctly different mechanism.

At all three sites of Pi reabsorption, the proximal convoluted tubule, proximal straight tubule and distal tubule, PTH has been shown to decrease Pi reabsorption either by a cAMP-dependent process, or in some cases a cAMP-independent signaling mechanism. In contrast, calcitonin-sensitive adenylate cyclase maps to the medullary and cortical thick ascending limbs and the distal tubule (Figure 2) (14). Althought calcitonin has been shown to inhibit Pi reabsorption in proximal convoluted and straight tubules by a cAMP-independent mechanism, the physiologic importance of this action is likely limited. It appears that the major regulators of renal tubular phosphate retention are PTH and the novel member of the fibroblast growth factor family, FGF23 (see below).

Mechanism of Phosphate Transport

Investigations of the cellular events involved in Pi movement from the renal tubule luminal fluid to the peritubular capillary blood indicate that Pi reabsorption occurs principally by a unidirectional process that proceeds transcellularly. Entry of Pi into the tubular cell across the luminal membrane proceeds by way of a saturable active transport system that is sodium-dependent (analogous to the sodium-dependent co-transport in the intestine) (Figure 3). The rate of Pi transport is dependent on the abundance of transporters functioning in the membrane, and the magnitude of the Na+ gradient maintained across the luminal membrane. This gradient depends on the Na+/ATPase or sodium pump on the basolateral membrane. The rate limiting step in transcellular transport is likely the Na+-dependent entry of Pi across the luminal membrane, a process with a low Km for luminal phosphate (~0.43M) which permits highly efficient transport.

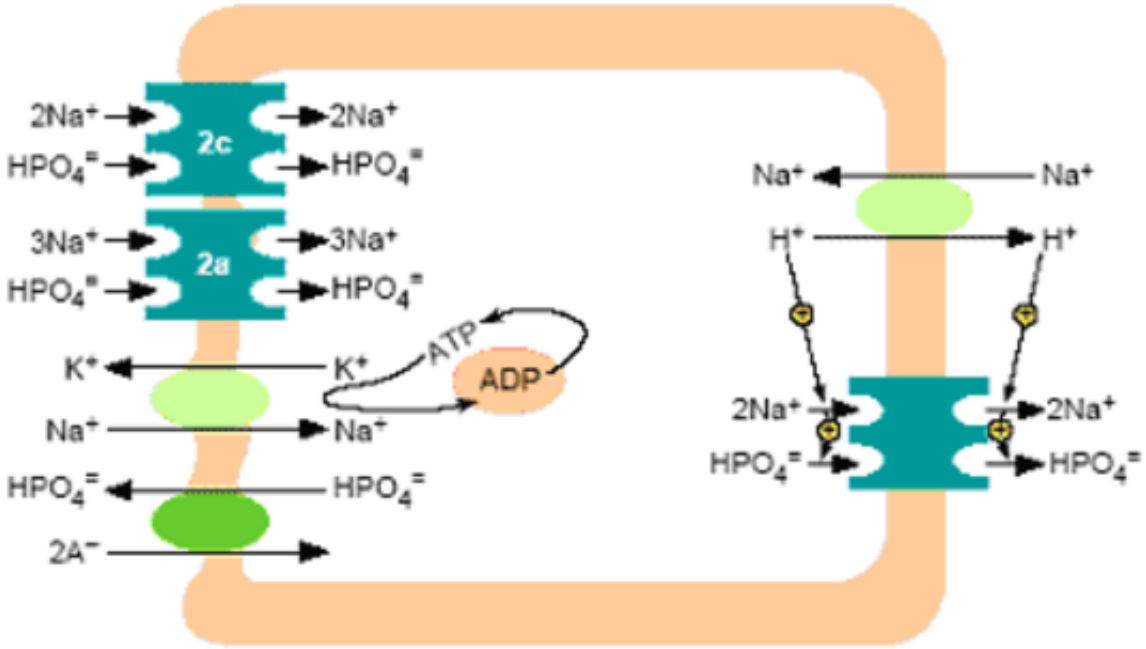


Figure 3. Model of inorganic phosphate transcellular transport in the proximal tubule. At the brush border a Na+/H+ exchanger and NaPi-II co-transporters operate. Nearly all proximal tubular reabsorption can be accounted for by the SLC34 (type II) family of sodium-dependent Pi

transporters. The more abundant NaPi-IIa transporter is electrogenic with a 3:1 (Na: PO4) stoichiometry, preferentially transporting the divalent phosphate anion. The lesser abundant NaPi-IIc transporter is electroneutral with a 2:1 (Na: PO4) stoichiometry, but also prefers the divalent phosphate species. The HPO4- that enters the cell across the luminal surface mixes with the intracellular pool of Pi and is transported across the basolateral membrane. This process is poorly understood, but anion exchange mechanisms have been suggested. A Na+/K+ ATPase located on the basolateral membrane pumps Na+ out of the cell maintaining the inward downhill Na gradient, which serves as the driving force for luminal entry of Na+.

The phosphate that enters the tubule cell plays a major role in governing various aspects of cell metabolism and function and is in rapid exchange with intracellular phosphate. Under these conditions the relatively stable free Pi concentration in the cytosol implies that Pi entry into the cell across the brush border membrane must be tightly coupled with either subcellular compartmentalization, organification, or exit across the basolateral membrane (Figure 3). The transport of phosphate across the basolateral membrane is poorly understood, however, several P transport pathways have been postulated, including Na+-Pi cotransport via type III Na-Pi cotransporters, passive diffusion, and anion exchange. In any case, the basolateral Pi transport serves at least two functions: 1) complete transcellular Pi reabsorption when luminal Pi entry exceeds the cellular Pi requirments; and 2) basolateral Pi influx if apical Pi entry is insufficient to satisfy cellular requirements (15).

Pi entry into renal epithelium is primarily performed by the type II class of Na-Pi cotransporters (SLC34 family members), although recently the finding of type III transporters (SLC20 family members, Pit1 and Pit2) in kidney have raised the possibility of a potential role for this class as well (16).These two families of Na-Pi cotransporters share no significant homology in their primary amino acid sequence and exhibit substantial variability in substrate affinity, pH dependence and tissue expression. The NaPi-II class of transporters account for the bulk of regulated phosphate transport in kidney, and disruption of this regulation may result in significant disease, documenting their physiological importance (17, 18). Interesting physiologic differences exist between these various Pi transporters and their functional diversity speaks to the necessity of the body to be able to transfer Pi between compartments in a variety of situations. Of the class II transporters NaPi-IIa and NaPi-IIc transporters are the predominant actors in the proximal renal tubule. NaPi-IIa, the more abundant species, is electrogenic with a 3:1 (Na: PO4) stoichiometry, preferentially transporting the divalent phosphate anion, and has a high affinity for Pi (all features of the NaPi-IIb member of this family, the predominant intestinal sodium-dependent Pi transporter, see above). NaPi-IIc differs from its type IIa/b family members in that is electroneutral with a 2:1 (Na: PO4) stoichiometry, but also prefers the divalent phosphate species. It has a much lower affinity for Pi, but is an efficient transporter due to its electroneutrality. An aspartic acid residue (Asp 224 in human NaPi-IIa) in a sodium binding site within a conserved amino acid cluster in the electrogenic transporters NaPi-IIa and NaPi-IIb, appears to be critical for electrogenicity. It is replaced with a glycine residue (Gly 196 in human NaPi-IIc) in the electroneutral type IIc transporter (19).

Initial attention focused on NaPi-IIa, as it was determined to be the most abundant Na-Pi cotransporter in kidney. Molecular and/or genetic suppression of NaPi-IIa supports its role in mediating brush-border membrane Na-Pi cotransport. Intravenous injection of specific antisense oligonucleotides reduces brush-border membrane Na-Pi cotransport activity in accord with a decrease in NaPi-IIa protein (20). In addition, disruption of the NaPi-IIa gene in mice leads to a 70% reduction in brush-border Na-Pi cotransport rate and complete loss of the protein (21,22). More recent attention has focused on another type II transporter, NaPi-IIc. This transporter may have a relatively more important role for Pi transport in humans as compared to rodents, and appears to have a more widespread tissue distribution. The identification of a unique form of hypophosphatemia, Hereditary Hypophosphatemic Rickets with Hypercalciuria (HHRH) as a loss-of-function mutation in NaPi-IIc has demonstrated an important physiologic role in humans for this transporter (23).

The roles of type III transporters in this process is not established at this time, and the previously described class of type I sodium-dependent phosphate transporters (of the SLC17 family) are not specific Pi transporters and do not appear to be central to the regulation of phosphate homeostasis.

Regulation of Renal Tubular Phosphate Handling

Several hormones and metabolic pertubations are able to modulate phosphate reabsorption by the kidney. Among these PTH, PTHrP, calcitonin, TGFb, glucocorticoids and phosphate loading inhibit renal phosphate reclamation. In contrast, IGF-1, insulin, thyroid hormone, 1,25(OH)2D, EGF and phosphate deprivation (depletion) stimulate renal phosphate reabsorption. More recently the study of disorders of renal phosphate wasting has revealed important functions of FGF23, a novel member of the fibroblast growth factor family, with respect to renal Pi homeostasis. The bulk of evidence indicates that PTH and FGF23 are the two most important regulators of renal tubular phosphate handling, and are discussed in greater detail below. The common target for regulation by these factors is the renal proximal tubular cell.

PTH

Investigations of classical PTH effects on proximal tubule phosphate transport indicate that both the cAMP-protein kinase A (PKA) and the phospholipase C-protein kinase C (PKC) signal transduction pathways modulate this process. The PTH mediated inhibition of phosphate reabsorption operates through the PKC system at low hormone concentrations (10-8 to 10-10 M) and via PKA at higher concentrations. PTH, after interaction with its receptor, PTHR1, effects a rapid and irreversible endocytosis of NaPi-II transporters to the lysosomal compartment, where subsequent proteolytic degradation of the transporters occur (24). Recovery of Na-Pi cotransport activity following PTH inhibition requires protein synthesis. In addition, the abundance of NaPi-IIa -specific mRNA is not changed by parathyroidectomy but is minimally decreased in response to PTH administration. These data implicate PTH as a regulator of renal Na-Pi cotransport in an acute time frame, and that the regulation is determined by changes in the abundance of NaPi-IIa protein in the renal brush border membrane (25). Certain aspects of Pi homeostasis at the renal level, however, are not explained by actions of PTH. For instance,

even in the setting where parathyroid glands have been removed, regulation of renal P transport by dietary P content still exists, implying that other mediators of this process are at work.

FGF23

In addition to PTH, FGF23 has recently been identified as an important physiologic regulator of renal Pi excretion (26). This novel member of the fibroblast growth factor (FGF) family is produced by osteocytes and osteoblasts, and appears to modulate renal Pi homeostasis on a more long-term basis than PTH. FGF23 serves as a mechanism by which skeletal mineral demands can be communicated to the kidney, thereby influencing phosphate economy of the entire organism, as to meet the needs for skeletal mineralization. In rodents and humans, after days of dietary phosphate loading, circulating FGF23 levels increase, and similarly, with dietary Pi deprivation, FGF23 levels decrease (27). FGF23 activates FGF receptors on the basolateral membrane of renal tubules resulting in decreased expression of type II sodium-dependent Pi transporters on the apical surface of the tubular cell. FGF23 interacts with its receptor via a novel mechanism: in order for the FGF23 signal to be transduced through its cognate receptor, a ternary complex of FGF23, FGFR, and the klotho protein is required (28). This results in downstream ERK phosphorylation, and subsequently reduced expression of NaPi-IIa and NaPi-IIc, and CYP27B1 (1-hydroxylase), with an increase in expression of CYP24A1 (24-hydroxylase).

FGF23 contains a unique C-terminal domain, thought to be the site of the interaction with klotho. The FGF-like domain, N-terminal to a furin protease recognition site, is the basis for the interaction of FGF23 with FGFR. Klotho appears to be able to associate with “c” isoforms of FGFR1 and FGFR3, and also FGFR4 (28). Renal signaling is thought to occur via FGFR1c, thereby rendering the reduced expression of the apical membrane NaPi-II transporters. The physiologic importance of this system has been demonstrated in several ways. First, mice overexpressing FGF23 demonstrate increased renal Pi clearance and concomitant hypophosphatemia (29). Secondly, FGF23 null mice retain P at the kidney and are hyperphosphatemic (30). Thirdly, administration to mice of an FGF23 neutralizing antibody increases serum Pi (31).

Nevertheless, gaps in our understanding of this pathway remain. Klotho appears to be expressed in the distal renal tubules rather than proximal tubular sites. Thus the mechanism by which this pathway effects the transporters in the proximal tubule is unclear. Although membrane-bound klotho is thought to be the species intereacting with FGF23 and FGFRs, a soluble form is secreted, and the membrane bound form can be cleaved to generate a second circulating species, either of which could play a role in this process. Most recently klotho alone has been shown to be able to reduce renal tubular phosphate reabsorption, independent of FGF23 (32). This finding is consistent with a unique case of hypophosphatemia associated with a mutation in the klotho region resulting in overexpression of the protein and an abundance of circulating klotho (33).

The actions of FGF23 and other related proteins as mediators of disease are discussed in detail in the section on Pathophysiology of XLH (see below). Other potential regulators of renal Pi

handling have been suggested. These include fragments of matrix extracellular glycoprotein (MEPE), secreted frizzled related protein-4 (sFRP4), stanniocalcin, and other FGFs, including FGF2, and FGF7 (34-37).

In sum, repeated observations have confirmed that the balance between urinary excretion and dietary input of Pi is maintained in normal humans, in patients with hyper- and hypoparathyroidism, and under man conditions. This is predominantly due to the ability of the renal tubule to adjust Pi reabsorption rate according to the body's Pi supply and demand. Thus Pi reabsorption is increased under conditions of greater need, such as rapid growth, pregnancy, lactation and dietary restriction. Conversely, in times of surfeit, such as slow growth, chronic renal failure or dietary excess, renal Pi reabsorption is curtailed. Such changes in response to chronic changes in Pi availability are characterized by parallel changes in Na-phosphate cotransporter activity, the NaPi-II mRNA level and NaPi-II protein abundance. These changes are likely mediated by FGF23, as well as other possible factors. Removal of NaPi-II cotransporters from the apical membrane of renal tubular cells is an acute process, mediated by PTH.

CLINICAL DISORDERS OF PHOSPHATE METABOLISM

A variety of genetic diseases and disorders due to therapeutic agents affect phosphate homeostasis. Not surprisingly, since the kidney is the primary regulatory site for phosphate homeostasis, aberrant phosphate metabolism results most commonly from altered renal Pi handling. Moreover, the majority of the primary diseases are phosphate losing disorders in which renal Pi wasting and hypophosphatemia predominate and osteomalacia and rickets are characteristic. Osteomalacia and rickets are disorders of calcification characterized by defects of bone mineralization in adults and bone and cartilage mineralization during growth. In osteomalacia, there is a failure to normally mineralize the newly formed organic matrix (osteoid) of bone. In rickets, a disease of children, there is not only abnormal mineralization of bone but defective cartilage growth plate calcification at the epiphyses as well. Apoptosis of chondrocytes in the hypertrophic zone is reduced, typically resulting in an expanded hypertrophic zone, delayed mineralization and vascularization of the calcification front, with an overall appearance of a widened and disorganized growth plate (38).

The remainder of this chapter reviews the pathophysiology of hypophosphatemic rachitic and osteomalacic disorders, and provides a systematic approach to the diagnosis and management of these diseases. The discussion will focus on disorders in which primary disturbances in phosphate homeostasis occur, emphasizing X-linked hypophosphatemic rickets/osteomalacia (XLH). Other disorders including hereditary hypophosphatemic rickets with hypercalciuria (HHRH); autosomal dominant and autosomal recessive hypophosphatemic rickets (ADHR and ARHR); Dent's disease; and tumor induced osteomalacia (TIO) will be discussed.

Mineralization Of Bone And Cartilage

Mineralization of bone is a complex process in which a calcium-phosphate mineral phase is deposited in a highly ordered fashion within the organic matrix (39). Apart from the availability of

calcium and phosphorus, requirements for normal mineralization include: 1) adequate metabolic and transport function of chondrocytes and osteoblasts to regulate the concentration of calcium, phosphorus and other ions at the calcification sites; 2) the presence of collagen with unique type, number and distribution of cross-links, distinct patterns of hydroxylation and glycosylation and abundant phosphate content, which collectively facilitate deposition of mineral at gaps (or "hole zones") between the distal ends of collagen molecules; 3) a low concentration of mineralization inhibitors (such as pyrophosphates and proteoglycans) in bone matrix; and 4) maintenance of an appropriate pH of approximately 7.6 for deposition of calcium-phosphate complexes.

The abnormal mineralization in the hypophosphatemic disorders, is due most likely to phosphopenia at calcification sites and, in some cases, paracrine inhibitory factors, which result in accumulation of unmineralized osteoid, a sine qua non for the diagnosis of osteomalacia. Since the resultant abundant osteoid is not unique to osteomalacia, establishing the diagnosis of osteomalacia requires dynamic histopathologic demonstration that abnormal mineralization, and not increased production, underlies the observed excess accumulation of osteoid (40, 41). Static histomorphometrical parameters seen in osteomalacia include an an increase in osteoid volume and thickness, an increase in bone forming surface covered by incompletely mineralized osteoid, and a decrease in the mineralization front (the percentage of osteoid-covered bone-forming surface undergoing calcification). The critical dynamic parameter used to confirm that osteoid accumulation is due to osteomalacia is the mineral apposition rate.

Inadequate growth plate cartilage mineralization in rickets is primarily observed in the hypertrophic zone of chondrocytes. Irregular alignment and more extensive disorganization of the growth plate may be evident with increasing severity of disease. Calcification in the interstitial regions of this hypertrophic zone is defective. Grossly, these changes result in increased thickness of the epiphyseal plate, and an increase in transverse diameter that often extends beyond the ends of the bone and causes characteristic cupping or flaring.

Clinical Disorders

X-LINKED HYPOPHOSPHATEMIC RICKETS/OSTEOMALACIA

X-linked hypophosphatemic rickets/osteomalacia is the most common "vitamin D resistant" disease in man. The disorder is inherited in X-linked dominant fashion and is manifest by renal phosphate wasting and consequent hypophosphatemia (Table 1). Additional characteristic features of the disease include growth retardation, osteomalacia and rickets in growing children. The clinical expression of the disease is widely variable, ranging from a mild abnormality, the apparent isolated occurrence of hypophosphatemia, to severe bone disease. Evidence of a gene dose effect has been controversial, although most would agree that a wide spectrum of phenotypic severity occurs in both males (with a mutated gene on their only X chromosome) and females (who are heterozygous for the defective X-linked gene). Evidence of disease may be detected at or shortly after birth, however may not become apparent until age 12 months or older (42). The most common clinically evident manifestations of XLH are short stature and limb deformities. Growth abnormalities and limb deformities are both more evident in the lower

extremities, since they represent the fastest growing body segment before puberty.

TABLE 1.

	CALCIUM METABOLISM			PHOSPHATE METABOLISM				VITAMIN D METABOLISM	
	Serum Calcium	Urine Calcium	Serum PTH	GI Calcium Absorption	Serum Pi	TmP/ GFR	GI Pi Absorption	Serum 25(OH) D	Serum 1,25(OH) 2D
XLH	N		N,					N	( )
ADHR	N		N					N	( )
ARHR	N		N	?			?	N	( )
TIO	N		N					N	
XLRH	N		N,					N	
HHRH	N		N,					N	( )

XLH, X-linked hypophosphatemia; ADHR, Autosomal dominant hypophosphatemic rickets; ARHR, Autosomal recessive hypophosphatemic rickets; TIO, Tumor-induced osteomalacia; XLHR, X-linked recessive hypophosphatemia (Dent's Disease); HHRH, Hereditary hypophosphatemic rickets with hypercalciuria. N, normal; , decreased; , increased, ( ), decreased relative to the serum phosphorus concentration; ?, unknown.

The majority of affected children exhibit clinical evidence of rickets (Figure 4), varying from enlargement of the wrists and/or knees to severe malalignment defects such as bowing or knock-knee deformities. (Figure 4). Such defects may result in waddling gait and leg length abnormalities (43). X-ray examination reveals expanded areas of non-mineralized cartilage in epiphyseal regions and lateral curvature of the femora and/or tibia. Severe secondary hyperparathyroidism as occurs in vitamin D deficiency is not present, however less striking in elevations in circulating PTH occur in many patients naive to therapy. Other non-specific but typical findings include elevated serum alkaline phosphatase activity and osteocalcin levels. Serum alkaline phosphatase activity, although usually elevated to 2-3 times the upper limit of normal in childhood, is generally less than the levels observed in overt vitamin D- and calcium-deficiency rickets.

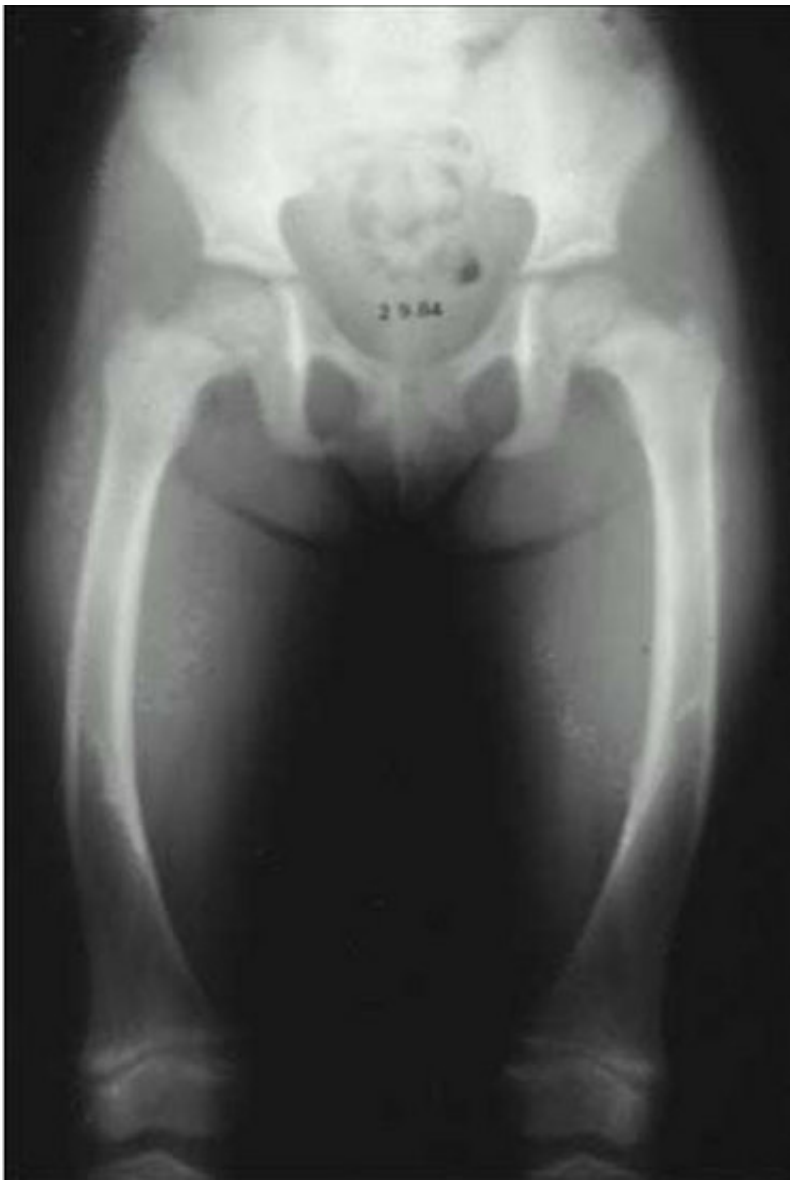


Figure 4.Radiograph of the lower extremeties in a patient with X-linked hypophosphatemia. Bowing of the femurs is evident bilaterally. The distal femoral metaphysis is cupped, frayed and widened, radiographic features of an expanded and disorganized growth plate.

Additional signs of the disease may include delayed dentition and dental abscesses (44, 45), which are thought to arise from the limited mineralization of the dentine compartment of the tooth. An enlarged pulp chamber is evident on dental radiographs. Strikingly absent are common features observed in vitamin D deficiency rickets, such as muscle weakness, tetany and convulsions.

Adults with XLH may be asymptomatic or present with severe bone pain. On clinical examination they often display evidence of post-rachitic deformities, such as bowed legs or

short stature. However, radiographic or biochemical abnormalities typical of active bone disease are usually absent. In contrast, some adult patients present with “active” osteomalacia, characterized radiographically by pseudofractures, coarsened trabeculation, rarified areas and/or non-union fractures, and biochemically by elevated serum alkaline phosphatase activity. Symptoms at presentation may reflect the end-result of chronic changes, and may not correlate with apparent current activity of the disease. In spite of marked variability in the clinical presentation of the disease, bone biopsy in affected children and adults nearly always reveals low turnover osteomalacia without osteopenia (Figure 5). Histomorphometry of biopsy samples invariably demonstrates a reduced rate of formation, diffuse patchy hypomineralization, a decrease in mineralizing surfaces and characteristic areas of hypomineralization of the periosteocytic lacunae (46).

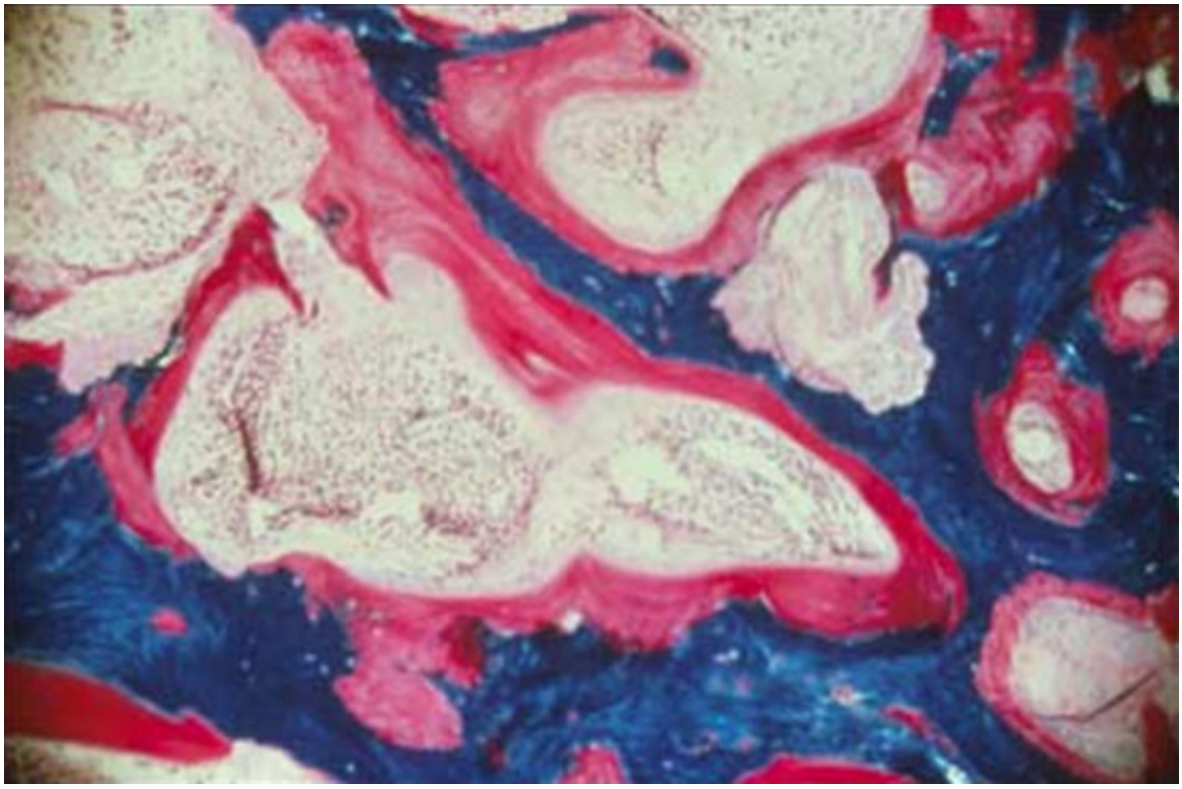


Figure 5.Section from an undecalcified bone biopsy in an untreated patient with X-linked hypophosphatemia. The Goldner stain reveals mineralized bone (blue/green) and an abundance of unmineralized osteoid (red) covering a substantial portion of the surfaces. The width of the osteoid seams is substantially increased.

Osteophytes, enthesopathy (47) and craniosynostosis are not uncommon. A great deal of the morbidity of XLH in adults arises from the high incidence of arthritis, calcified entheses, and osteophytes. Enthesopathy generally is first detectable radiographically by late in the second decade, or early in the third decade. Older subjects have more sites of involvement, and generally increasing involvement with age; the frequency of involvement is greater in males.

With progressive enthesopathy and bony overgrowth, excruciating pain may occur, particularly with fusion of the sacroiliac joint(s) and spinal stenosis (48). It is peculiar that XLH represents a deficiency of mineralization at many skeletal sites, and pathologic ectopic mineralization elsewhere. This paradoxical situation raises the possibility that aberrant humoral factors, in addition to the ambient hypophosphatemia, may play a role in the discordant mineralization abnormalities observed.

Clinical Biochemistry

As previously noted, the primary biochemical abnormality of XLH is hypophosphatemia due to increased urinary phosphate excretion. Moreover, mild gastrointestinal phosphate malabsorption is present in the majority of patients, which may contribute to the evolution of the hypophosphatemia (Table 1) (49, 50).

In contrast, the serum calcium concentration in affected subjects is normal despite gastrointestinal malabsorption of calcium. However, as a consequence of this defect, urinary calcium is often decreased. Circulating PTH levels may be normal to modestly elevated in naive patients, but treatment with phosphate salts may aggravate this tendency such that persistent secondary hyperparathyroidism may occur. As noted above, serum alkaline phosphatase activity is usually elevated in children, although to lesser levels than seen in nutritional forms of rickets. Elevations in the measure are not often evident in adults, and the measure is not a reliable marker of disease involvement in the older age group. Prior to the initiation of therapy, serum 25-OHD levels are normal, and serum 1,25(OH)2D levels are in the low normal range (51, 52). The paradoxical occurrence of hypophosphatemia and normal serum calcitriol levels in affected subjects is consistent with aberrant regulation of both synthesis and clearance of this metabolite (due to increased 25-OHD-24-hydroxylase activity) (53, 54). Circulating levels of FGF23 are generally elevated in individuals with XLH. FGF23 levels do not appear to differ between normal adults and older childen, nor between children and adults affected with XLH. Caution should be used in using this measure as a strict diagnostic criterion for the diagnosis of XLH, as some subjects have been shown to have normal FGF23 levels, and commercially available assays (which recognize “intact” species or both intact and C-terminal species) do not always provide concordant results.

Genetics

With the recognition that hypophosphatemia is the definitive marker for XLH, Winters et al (55) and Burnett et al (56) discovered that this disease is transmitted as an X-linked dominant disorder. Analysis of data from 13 multigenerational pedigrees identified PHEX (for ph osphate regulating gene with homologies to e ndopeptidases located on the X chromosome) as the gene mutated in XLH (57). PHEX is located on chromosome Xp22.1, and encodes a 749-amino acid protein with three putative domains: 1) a small aminoterminal intracellular tail; 2) a single, short transmembrane domain; and 3) a large carboxyterminal extracellular domain, containing ten conserved cysteine residues and a HEXXH pentapeptide motif, which characterizes many zinc metalloproteases. Further studies have revealed that PHEX is homologous to the M13 family of membrane-bound metalloproteases, or neutral endopeptidases. M13 family members, including

neutral endopeptidase 24.11 (NEP), endothelin-converting enzymes 1 and 2 (ECE-1 and ECE-2), the Kell blood group antigen (KELL), neprilysin-like peptide (NL1), and endothelin converting enzyme-like 1 (ECEL1), degrade or activate a variety of peptide hormones. In addition, like other neutral endopeptidases, immunofluorescent studies have revealed a cell-surface location for PHEX in an orientation consistent with a type II integral membrane glycoprotein (58). It has been demonstrated that certain missense mutations in PHEX that substitute a highly conserved cysteine residue will interfere with normal trafficking of the molecule to the plasma membrane (59). Thus it appears that one mechanism associated with the pathophysiology of XLH is to prevent PHEX from locating to the cell membrane.

Phex is predominantly expressed in bones (in osteoblasts/osteocytes) and teeth (in odontoblasts/ameloblasts) (60-63); mRNA, protein or both have also been found in lung, brain, muscle, gonads, skin and parathyroid glands. Subcellular locations appear to be the plasma membrane, endoplasmic reticulum and Golgi organelle. Immunohistochemistry studies suggest that Phex is most abundant on the cell surface of the osteocyte. In sum, the ontogeny of Phex expression suggests a possible role in mineralization in vivo.

The work of several groups has documented PHEX mutations in >160 patients (64-72). Mutations are scattered throughout the 749-amino acid extracellular domain, encoded by exons 2-22, and are diverse, consisting of deletions, insertions and duplications, as well as splice site, nonsense and missense mutations.

The location of Phex expression in bone cells have led to the hypothesis that diminished PHEX/Phex expression in bone initiates the cascade of events responsible for the pathogenesis of XLH. In order to confirm this possibility, several investigators have used targeted over-expression of Phex in attempts to normalize osteoblast mineralization, in vitro, and rescue the Hyp phenotype in vivo (73-75). Results from these studies have not resulted in a complete skeletal rescue, raising questions as to the role of early developmental expression of PHEX, or at least the success of expression when targeted with osteocalcin or type I collagen promoters. Nevertheless, partial rescue of the mineralization defect in Hyp mice occurs, suggesting that local effects of the PHEX mutation may play some role in the mineralization process, but cannot completely restore the skeleton to normality. Of note, this partial rescue occurs in concert with a reduction in FGF23 levels, although not lowered to a truly normal range (76).

In sum, although a physiologic substrate for PHEX has not been identified, the consequence of loss-of-function of PHEX is an elevation in the circulating FGF23 level. Failure of targeted osteoblastic PHEX overexpression to completely rescue Hyp mice may reflect that critical sites (or developmental timing) for PHEX expression are not effectively generated with these models to effectively rescue the skeletal phenotype; this effect may be dependent upon the resultant capacity in these transgenic models of normal PHEX to reduce FGF23 production in mutant cells.

Pathophysiology

The primary inborn error in XLH results in the impaired renal proximal tubule function of Pi

reabsorption. The immediate cause of this abnormality is the decreased abundance of NaPi-II mRNA and immunoreactive protein in the proximal convoluted tubule cells (77-79). A number of recent findings implicate that the reduction in NaPi-II abundance, and the resultant renal phosphate wasting is mediated by increased circulating levels of the recently identified hypophosphatemic factor, FGF23 (see above, **Regulation of Renal Tubular Phosphate Handling**).

Evidence for humoral mediation of phosphate wasting in XLH was provided by both classical parabiosis experiments, suggested that a cross-circulating factor could mediate renal phosphate wasting (80), and in animal studies of renal cross-transplantation between *Hyp* and normal mice. These experiments demonstrated continued normal renal phosphate handling after transplantation of *Hyp* kidney to a normal host, as well as the failure to correct the mutant phenotype upon introduction of a normal kidney to a *Hyp* host (81). These findings are most consistent with humoral mediation of the Pi wasting in the disease. With the recent data conclusively demonstrating that FGF23 is an important regulator of renal phosphate homeostasis, and that mean circulating FGF23 concentrations are greater in XLH patients than in unaffected control subjects, the hypothesis that FGF23 is the humoral mediator of renal phosphate handling abnormalities in XLH has gained wide acceptance.

It remains unclear as to how the loss-of-function of PHEX results in elevated FGF23 levels. The hypothesis that PHEX (a member of the M13 family of zinc-dependent type II cell surface membrane metalloproteinases) could serve as a processor of a phosphaturic hormone such as FGF23 has not been borne out, and the role PHEX plays in this pathway is not clear. In the setting of (presumably) normal PHEX, Pi wasting occurs when FGF23 is elevated for other reasons. This situation is evident in TIO, where overproduction of FGF23 results in a comparable Pi wasting phenotype (82). In Autosomal Dominant Hypophosphatemic Rickets (ADHR) specific mutations in FGF23 result in gain of function of the protein (83). The specific mutations disrupt an RXXR protease recognition site, and thereby protect FGF23 from proteolysis, resulting in reduced clearance and elevating circulating levels of this protein, with coincident renal Pi wasting. In yet another genetic disorder, Autosomal Recessive Hypophosphatemic Rickets (84), due to homozygous loss of function mutations in dentin matrix protein-1 (DMP1), renal tubular Pi wasting occurs in the setting of increased FGF23 levels. DMP1 is a matrix protein of the SIBLING (s mall i ntegrin b inding li gand N-g lycated) family, and, like PHEX and FGF23 has been primarily identified in osteocytes. Furthermore, FGF23 levels are elevated in mice with biallelic disruption of DMP1. Transgenic mice which overexpress FGF23, exhibit retarded growth, hypophosphatemia, decreased (or inappropriately normal) serum 1,25(OH)2D levels and rickets/osteomalacia, all features of XLH. Indeed, murine models of all of these disorders (XLH, ADHR, TIO, and ARHR) similarly demonstrate elevated circulating FGF23 levels with concomitant renal phosphate wasting

In sum, enhanced FGF23 activity is common to several phosphate-wasting disorders. In particular, those disorders that share the combined defects of inappropriately low circulating levels of 1,25(OH)2D and renal tubular Pi wasting are associated with increased FGF23 levels. This coincidence of findings holds for XLH, ADHR, ARHR, and TIO, and are consistent with the notion that FGF23 is a both a direct regulator of Pi homeostasis at the renal level, a down-regulator of 1a-hydroxylase activity, responsible for the catalysis of 25-OH vitamin D to its active

form, and stimulus for its clearance via the 24-hydroxylation pathway. The teleological appeal to this argument stems from the provision of 2 major Pi regulating hormones in the body: firstly, PTH (primarily responsive to serum Ca levels), which also serves to increase Ca levels via an increase in circulating 1,25(OH)2D, and secondly, FGF23 (primarily responsive to Pi), which counters PTH's calcemic effect by reducing 1,25(OH)2D levels (Figure 6).

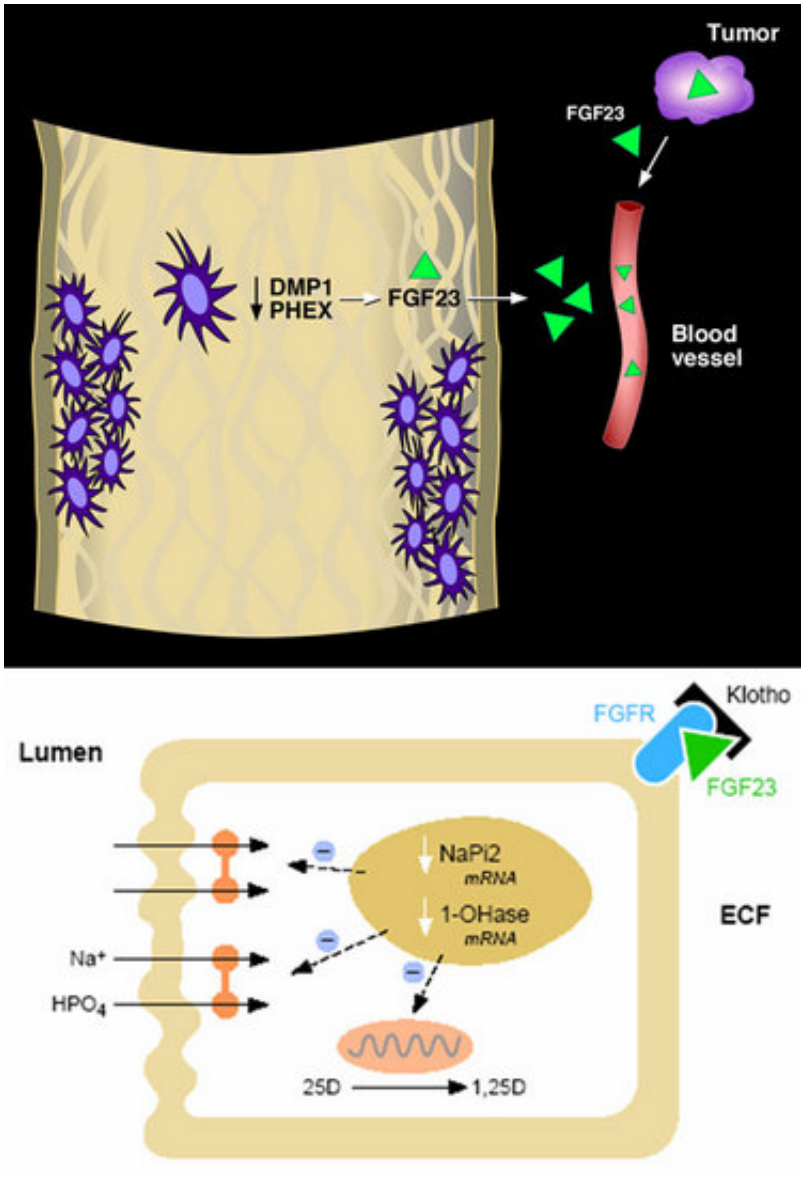


Figure 6.Scheme for the speculated pathophysiology of XLH, ARHR, TIO, and ADHR.Upper panel, osteocytes, comprising a network of connected cells embedded in mineralized bone are the cellular source of PHEX (which is mutated in XLH), DMP1 (which is mutated in ARHR), and FGF23 (which is found in high concentrations in all four of these hypophosphatmic disorders). It follows that loss of PHEX or DMP1 results in increased FGF23 production/secretion by mechanisms that are not currently understood. Circulating FGF23 concentrations may also occur secondary to the increased production associated with various tumors.Lower panel, circulating FGF23 interacts with an FGF receptor (FGFR) on the basolateral surface of the proximal renal tubular cell. Klotho, produced by the distal renal tubule in both membrane bound

and secretory forms, is necessary for the FGF23/FGFR interaction. Signalling through this pathway results in a decrease in NaPi-II mRNAs, thereby reducing the abundance of Pi cotransporters on the apical membrane and the well-described impairment of renal tubular Pi reabsorption. Likewise synthesis of 1,25(OH)2D is impaired, while its clearance is augmented. In XLH and ARHR, increased production of FGF23 occurs in the skeleton; in TIO, increased production of FGF23 occurs in tumors; in ADHR, enhanced activity of FGF23 occurs as a result of the specific mutations that retard its metabolic clearance.

Other recent findings have provided support for the role of klotho in the FGF23-mediated hypophosphatemia pathway. An unusual patient with renal tubular Pi wasting and abnormally increased serum klotho has been described (33). Investigation revealed a translocation breakpoint disrupting the region upstream of that encoding klotho. Indeed, mice with disruption of the klotho gene manifest *hyper* phosphatemia and *elevated* circulating 1,25(OH)2D levels (85). The proof that klotho is distal to PHEX in this regulatory pathway was shown by crossing the klotho disrupted mice with Hyp (PHEX-deletion) mice. The double mutant (Hyp/Kl-/-) mice were hyperphosphatemic, with elevated 1,25(OH)2D levels, despite having extremely elevated circulating FGF23 levels due to PHEX loss-of-function (86).

Indeed further evidence for the central role of FGF23 in the Pi-regulating process comes from the investigation of another group of rare disorders of Pi homeostasis in which renal Pi conservation is excessive in the setting of increased circulating Pi levels. This group of disorders, known as hyperphosphatemic tumoral calcinosis (HTC), is manifest clinically by precipitation of amorphous calcium-phosphate crystals in soft tissues. This phenomenon is thought to result from an increase in the ambient Ca x Pi solubility product, and occurs as a direct result of enhanced renal tubular reabsorption of Pi (87). In addition, circulating 1,25(OH)2D levels are in the high-normal to high range. Thus the precise converse of primary metabolic derangements occurs, as compared to the XLH-related group of diseases. Initially, HTC was been shown to directly result from loss of function mutations in GALNT3, a glycosylating enzyme that appears to be necessary for appropriate O-glycosylation of proteins. Subsequently the disorder was also shown to occur in the setting of loss-of-function mutations of FGF23; patients with HTC have low intact FGF23 levels in both cases (88-90). More recent studies have demonstrated that GALNT3 is necessary for appropriate processing of FGF23, and its loss-of-function essentially disrupts the ability of full-length FGF23 to be secreted (91). Loss of function of klotho has also been described in a case of HTC, despite the finding of elevated FGF23 levels, thus rendering the FGF23 inactive at the renal proximal tubule (92). As with hypophosphatemia syndromes, animal models have confirmed the physiologic implications of these clinical scenarios: FGF23 null mice develop a hyperphosphatemic, calcifying phenotype with elevated 1,25(OH)2D levels (30), similar to mice with disruption of the klotho gene (85, 93). As noted above, the klotho protein is now known to be an essential co-factor in FGFR1c activation when FGF23 serves as the activating ligand (28).

The overall physiologic importance of this regulating system requires further study. It is not clear how PHEX or DMP1 result in elevated FGF23 levels. The intriguing aspect of the osteocyte as a

potential central cell in this pathway also bears further study. One possible interpretation of these findings is that the osteocyte network throughout the skeleton may be a central sensor of skeletal mineral demand. The coordination of certain specific matrix proteins may play a role in the local regulation of phosphate supply and mineralization. It follows that genetic disruption of this pathway may result in the profound systemic disturbances observed in the diseases described above.

Treatment

A generation ago, physicians employed pharmacological doses of vitamin D as the cornerstone for treatment of XLH. However, long-term observations indicate that this therapy fails to cure the disease and poses the serious problem of recurrent vitamin D intoxication and renal damage. Indeed, such treatment results only in incomplete healing of the rachitic abnormality, while hypophosphatemia and impaired growth remain. Similar unresponsiveness prevails upon use of 25(OH)D.

With the recognition that phosphate depletion is an important contributor to impaired skeletal mineralization, physicians began to devise treatment strategies that employed oral phosphate supplementation to compensate for the renal phosphate wasting and thereby increasing the available Pi to the mineralizing skeleton. Pharmacologic amounts of vitamin D were used in combination with phosphate supplements to counter the exacerbation of hyperparathyroidism observed in this setting. Such combination therapy was found to be more effective than either administering vitamin D or phosphate alone. With the recognition that circulating 1,25(OH)2D levels are not appropriately regulated in XLH, the use of this metabolite in combination with phosphate was subsequently used to treat the disease (51, 94-96). The current treatment strategy directly addresses the combined calcitriol and phosphorus deficiency characteristic of the disorder. Although this combination therapy has become the conventional therapy for XLH, complete healing of the skeletal lesions is usually not the case, and late complications of the disease are persistent and often debilitating.

In children the goal of therapy is to improve growth velocity, normalize any lower extremity defects, and heal the attendant bone disease. Generally the treatment regimen includes a period of titration to achieve a maximum dose of 1,25(OH)2D3 (Rocaltrol® or calcitriol), 20-50 ng/kg/day in two divided doses, and phosphorus, (20-75 mg/kg/day, to a maximum of 1-2 gms/day) in 3-5 divided doses. Occasionally patients will prove refractory to this therapy and maximally tolerated amounts of 1,25(OH)2D3 and phosphorus are required with daily dose limits of 3 mcg and 2.5 gms, respectively.

Use of 1,25(OH)2D3/phosphorus combination therapy involves a significant risk of toxicity. Hypercalcemia, hypercalciuria, renal calcinosis, and hyperparathyroidism can be sequelae of unmonitored therapy. Detrimental effects on renal function were particularly common prior to the frequent monitoring now generally employed with this therapy. Indeed, hypercalcemia, severe nephrocalcinosis and/or diminished creatinine clearance necessitates appropriate dose adjustment, and in some cases discontinuation of therapy. Throughout the treatment course careful attention to renal function, as well as serum and urine calcium is extremely important.

Nevertheless, in spite of these varied complications of therapy, treatment of XLH often proceeds with limited interruptions. Moreover, the improved outcome of this therapeutic intervention, compared to that achieved by previous regimens, justifies the aggressive approach that constitutes this current therapy.

While such combined therapy often improves growth velocity, refractoriness to the growth-promoting effects of treatment can be encountered in children who present with markedly short stature prior to 4 years of age. For that reason the use of recombinant growth hormone as additional treatment has been suggested (97), however this approach has not been universally recommended in view of the lack of definitive benefits in controlled studies, and a risk of resultant worsening of the disproportional stature (98).

Indications for combined therapy in adults with XLH are less clear. The occurrence of intractable bone pain and refractory non-union fractures often respond to treatment with calcitriol and phosphorus (99). However, data remain unclear regarding the effects of treatment on fracture incidence (which may not be increased in untreated patients), enthesopathy and dental abscesses. Therefore, the decision to treat affected adults must be individualized. In general it is beneficial to offer adults with significant symptomatology a trial of this therapy, but only if routine biochemical monitoring can be performed.

Given the limitations with even currently advised treatment for XLH, the quest for new and better therapies for XLH continues. The recent description of correction of serum P levels and improved bony growth in *Hyp* mice treated with a neutralizing antibody to FGF23 raise the possibility that measures to inhibit action of this suspected mediator of disease will have a role in the treatment of XLH in the future (31). Currently clinical trials are underway to asses this approach in humans.

AUTOSOMAL DOMINANT HYPOPHOSPHATEMIC RICKETS (ADHR)

Several studies have documented autosomal dominant inheritance of a hypophosphatemic disorder similar to XLH (100, 101). The phenotypic manifestations of this disorder include the expected hypophosphatemia due to renal phosphate wasting, lower extremity deformities, and rickets/osteomalacia. Affected patients also demonstrate normal serum 25(OH)D levels, while maintaining inappropriately normal serum concentrations of 1,25(OH)2D, in the presence of hypophosphatemia, all hallmarks of XLH (Table 1). PTH levels are normal. Long-term studies indicate that a few of the affected female patients demonstrate delayed penetrance of clinically apparent disease and an increased tendency for bone fracture, uncommon occurrences in XLH. In addition, among patients with the expected biochemical features documented in childhood, rare individuals lose the renal phosphate-wasting defect after puberty. As noted above, specific mutations in FGF23 in the 176-179 amino acid residue sequence are present in patients with ADHR (83). These mutations disrupt an RXXR furin protease recognition site, and the resultant mutant molecule is thereby protected from proteolysis, and resultant elevated circulating levels of FGF23 are the likely cause of the renal Pi wasting. Interestingly, circulating FGF23 levels can vary and reflect the activity of disease status (102).

An apparent forme fruste of ADHR (autosomal dominant) hypophosphatemic bone disease has many of the characteristics of XLH and ADHR, but recent reports indicate that affected children display no evidence of rachitic disease. Because this syndrome is described in only a few small kindreds, and radiographically evident rickets is not universal in children with familial hypophosphatemia, these families may have ADHR. Further observations are necessary to discriminate this possibility.

AUTOSOMAL RECESSIVE HYPOPHOSPHATEMIC RICKETS (ARHR)

Recently families with phosphate wasting rickets inherited in an autosomal recessive manner have been described (84, 103). Affected patients have been found to have the same constellation of progressive rachitic deformities seen in both XLH and ADHR. Moreover the biochemical phenotype is manifest by the same measures of hypophosphatemia, excess urinary Pi losses, and aberrant vitamin D metabolism (normal circulating 25-OHD and 1,25(OH)2D levels, despite ambient hypophosphatemia) as observed in both XLH and ADHR. In addition to the expected phenotypic features, and in contrast to XLH, spinal radiographs of patients with ARHR reveal noticeably sclerotic vertebral bodies. In addition to the enlarged pulp chamber characteristic of teeth in individuals with XLH, enamel hypoplasia can be evident in heterozygotes. Of particular interest is the identification of elevated levels of FGF23 in the affected individuals. Experience with long-term follow-up is not widespread in ARHR and therapeutic response or guidelines have not been definitively established.

The identification of a progressive mineralization defect associated with hypophosphatemia in DMP1 knockout mice led to the consideration of homozygous loss of function in this candidate gene as the cause of ARHR. Indeed this has proven to be the case. Thus the role of the osteocyte product, DMP1, appears as either part of the PHEX-FGF23 pathway, or at least can affect circulating FGF23 levels, perhaps independently of PHEX. These observations again suggest that the osteocyte plays a central role in mineral homeostasis.

Hypophosphatemic rickets in association with renal Pi wasting has been recently described in the setting of the extremely rare disorder, generalized arterial calcification of infancy (GACI) (104). This disorder occurs with homozygous loss-of-function mutations of ectonucleotide pyrophosphatase/phosphodiesterase-1 (ENPP1). This protein plays a role in the local generation of extracellular pyrophosphate, which serves as a mineralization inhibitor. Presumably, loss-of-function of ENPP1 results in the inability to generate pyrophosphate, which limits the ability to control mineralization and ectopic (e.g., vascular) calcification. GACI is often fatal, but in a recent report, hypophosphatemia in the setting of elevated FGF23 levels was evident in an adult with a homozygous ENPP1 mutation (105). Moreover his son was affected with both GACI and hypophosphatemia. The mechanism by which this enzyme influences renal tubular phosphate wasting is not evident, and further study is necessary to understand this intriguing problem. One speculated mechanism may reflect a bone cell response to a relatively hypermineralized (or high-phosphate/low pyrophosphate) milieu which results in a compensatory, prolonged secretion of FGF23. Such a mechanism may effectively signal the kidney to reduce the body's mineral load, but apparently cannot be down-regulated to protect

against excessive Pi losses.

TUMOR-INDUCED OSTEOMALACIA

Rickets and/or osteomalacia have been associated with various types of tumors (87). In many cases, the metabolic disturbances improved or completely disappeared upon removal of the tumor, indicating a causal role of the tumor. Affected patients generally present with bone and muscle pain, muscle weakness, rickets/osteomalacia and occasionally recurrent fractures of long bones. Biochemistries include hypophosphatemia secondary to renal phosphate wasting and normal serum levels of calcium and 25(OH)D. Serum 1,25(OH)2D is often overtly low or is otherwise inappropriately normal in the setting of hypophosphatemia (Table 1). Aminoaciduria and/or glucosuria may be present. Radiographic abnormalities include generalized osteopenia, pseudofractures and coarsened trabeculae, as well as widened epiphyseal plates in children. The histologic appearance of trabecular bone in affected subjects most often reflects the presence of a low turnover osteomalacia. In contrast, bone biopsies from the few patients who have tumors that secrete a nonparathyroid hormone factor(s), which activates adenylate cyclase, exhibit features of enhanced bone turnover, including an increase in osteoclast and osteoblast number.

The large majority of patients with this syndrome harbor tumors of mesenchymal origin, including primitive-appearing, mixed connective tissue lesions. These tumors are often classified as osteoblastomas, nonossifying fibromas and ossifying fibromas. In addition tumors of epidermal and endodermal derivation have been implicated as causal of the disease. Indeed, the observation of tumor-induced osteomalacia concurrent with breast carcinoma, prostate carcinoma oat cell carcinoma, small cell carcinoma, multiple myeloma and chronic lymphocytic leukemia have been reported.

Although this syndrome is relatively rare compared to XLH, the importance in its understanding of hypophosphatemia has been very important. The study of these tumors eventually led to the identification and isolation of FGF23 (29, 106), which has become a central factor in the entire class of hypophosphatemic disorders and represents a novel regulatory system affecting Pi homeostasis.

Regardless of the tumor cell type, the lesions at fault for the syndrome are often small, difficult to locate and present in obscure areas which include the nasopharynx, jaw, sinuses, the popliteal region and the suprapatellar area. In any case, a careful and thorough examination is necessary to document/exclude the presence of such a tumor. Indeed, CT and/or MRI scan of a clinically suspicious area should be undertaken. Recently newer imaging techniques such as octreotide scintigraphy or PET scans have been used to successfully identify tumors that remained unidentified by other means of localization. Selective venous sampling has been suggested as a complementary approach to diagnosis. This technique can provide confirmation of local FGF23 secretion in suspicious areas identified by imaging (as to avoid unnecessary operations from false-positive imaging studies). The technique may serve to direct local imaging to anatomic regions defined by step-ups in FGF23 concentrations, although the relatively long half-life of FGF23 may result in limitations to this effort if the sampling is not in very close

proximity to the offending tumor.

In the related clinical settings of widespread fibrous dysplasia of bone, neurofibromatosis and linear nevus sebaceous syndrome, osteomalacia/rickets could be related to a similar mechanism as with the more classic mesenchymal cell tumors. Although proof of a causal relationship in these latter disorders has been precluded in general by an inability to surgically excise the multiplicity of lesions, in one case of fibrous dysplasia, removal of virtually all of the abnormal bone did result in appropriate biochemical and radiographic improvement. Indeed variable degrees of decreased renal tubular phosphate reabsoroption, as assessed by TMP/GFR assessments, occur in series of patients with fibrous dysplasia of bone. Other primary skeletal disorders in which elevated FGF23 levels have been reported include osteoglophonic dysplasia (due to mutations in the FGFR1 receptor) (107) and in Jansen metaphyseal chondrodysplasia, (due to activating mutations of the PTH1 receptor) (108). The mechanism(s) by which elevations in FGF23 occur in these settings is not certain at this time.

Pathophysiology

TIO is a result of Pi wasting secondary to circulating factor(s) secreted by causal tumors. FGF23 has proven to be the primary factor identified in most patients where examination of serum levels or tumor material has occurred. Nevertheless, a variety of other factors have been considered as a potential part of the cascade that can lead to renal Pi wasting including: 1) FRP4 (frizzled related protein 4) (35), a secreted protein with phosphaturic properties, 2) FGF7, which has been identified in TIO tumors and has been shown to inhibit renal Pi transport (37), 3) the SIBLING protein, MEPE (matrix extracellular phosphglycoprotein), which has been reported to generate fragments (ASARM peptide) with potential Pi wasting capacity (34), 4) the SIBLING protein, DMP1, which has now been implicated in ARHR, and has been shown to be in particularly high abundance in TIO tumors (29, 84, 106, 109), and 5) the high molecular weight isoform of FGF2, which when expressed transgenically in mice, results in hypophosphatemic rickets (110). It is also possible that these or other tumor products may have direct effects on the mineralization function of the skeleton.

In contrast to these observations, rare patients with TIO often secondary to hematogenous malignancy manifest abnormalities that would suggest a different pathophysiologic mechanism. In these subjects a nephropathy induced with light chain proteinuria or other immunoglobulin derivatives appears to result in decreased renal tubular reabsorption of phosphate. Thus, light-chain nephropathy must be considered a possible mechanism for the TIO syndrome.

Treatment

The first and foremost treatment of TIO is complete resection of the tumor. However, recurrence of mesenchymal tumors, such as giant cell tumors of bone, or inability to resect completely certain malignancies, such as prostatic carcinoma, has resulted in development of alternative therapeutic intervention for the syndrome. In this regard, administration of 1,25(OH)2D alone or in combination with phosphorus supplementation has served as effective therapy for TIO. Doses of calcitriol required range from 1.5-3.0 µg/d, while those of phosphorus are 2-4 g/d. Although

little information is available regarding the long-term consequences of such treatment, the high doses of medicine required raise the possibility that nephrolithiasis, nephrocalcinosis and hypercalcemia may frequently complicate the therapeutic course. Indeed, hypercalcemia secondary to parathyroid hyperfunction has been documented in several subjects. All of these patients received phosphorus as part of a combination regimen, which may have stimulated parathyroid hormone secretion and exacerbated the path to parathyroid autonomy. Thus, as with treatment of XLH, careful assessment of parathyroid function, serum and urinary calcium and renal function are essential to ensure safe and efficacious therapy. Should effective neutralization of FGF23 be possible with antibody therapy for XLH, extension of this approach to inoperable TIO may be a very useful option.

DENT'S DISEASE (X-LINKED RECESSIVE HYPOPHOSPHATEMIA; XLRH)

The initial description of X-linked recessive hypophosphatemic rickets involved a family in which males presented with rickets or osteomalacia, hypophosphatemia, and a reduced renal threshhold for phosphate reabsorption. In contrast to patients with XLH, affected subjects exhibited hypercalciuria, elevated serum 1,25(OH)2D levels (Table 1), and proteinuria of up to 3 g/day. Patients also developed nephrolithiasis and nephrocalcinosis with progressive renal failure in early adulthood. Female carriers in the family were not hypophosphatemic and lacked any biochemical abnormalities other than hypercalciuria. Three related syndromes have been reported independently: X-linked recessive nephrolithiasis with renal failure, Dent's disease, and low-molecular-weight proteinuria with hypercalciuria and nephrocalcinosis. These syndromes differ in degree from each other, but common themes include proximal tubular reabsorptive failure, nephrolithiasis, nephrocalcinosis, progressive renal insufficiency, and, in some cases, rickets or osteomalacia. Identification of mutations in the voltage-gated chloride-channel gene CLCN5 in all four syndromes has established that they are phenotypic variants of a single disease and are not separate entities (111,112). However, the varied manifestations that may be associated with mutations in this gene, particularly the presence of hypophosphatemia and rickets/osteomalacia, underscore that environmental differences, diet, and/or modifying genetic backgrounds may influence phenotypic expression of the disease.

HEREDITARY HYPOPHOSPHATEMIC RICKETS WITH HYPERCALCIURIA (HHRH)

This rare autosomal recessive disease is marked by hypophosphatemic rickets with hypercalciuria (113). Initial symptoms of the disorder generally manifest between 6 months to 7 years of age and usually consist of bone pain and/or deformities of the lower extremities. Such deformities may include genu varum or genu valgum or anterior bowing of the femur and coxa vara. Additional disease features include short stature, and radiographic signs of rickets or osteopenia. In contrast to XLH, muscle weaknesss may be elicited as a presenting symptom.

Many of the distinguishing characteristics of HHRH, especially when compared to majority of causes of hypophosphatemia discussed in this chapter, stem from the fact that HHRH is not a

disorder of FGF23-mediated hypophosphatemia. In fact levels tend to be somewhat decreased compared to the normal population. And consenquently, in contrast to other diseases in which renal phosphate transport is limited, patients with HHRH exhibit increased 1,25(OH)2D production. The resultant elevated serum calcitriol levels enhance the gastrointestinal calcium absorption, which in turn increases the filtered renal calcium load and inhibits PTH secretion. Collectively these events produce the hypercalciuria observed in affected patients (Table 1). Although initially not thought to be part of the syndrome, the propensity for kidney stones to occur has been reported in several patients.

In general, the severity of the bone mineralization defect correlates inversely with the prevailing serum Pi concentration. Relatives of patients with evident HHRH may exhibit an additional mode of disease expression (114). These subjects manifest hypercalciuria and hypophosphatemia, but the abnormalities are less marked and occur in the absence of discernible bone disease, which would suggest a mild phenotype in the heterozygous state with certain mutations.

After mutations in the candidate NaPi-IIa gene, were excluded as causal to HHRH, the genetic defect was identified in NaPi-IIc (23, 115), previously thought to be of less importance than the type IIa transporter. As would be predicted by the isolated loss of function of a Pi transporter, reduced serum Pi and increased renal Pi losses occur, independent of FGF23 status. However unlike the findings in XLH, Pi wasting does not coexist with limitations in 1,25(OH)2D production, and the system retains its capacity to increase 1,25(OH)2D levels in response to the ambient hypophosphatemia. Recently it has been suggested that specific mutations in NaPi-IIc may be associated with sodium wasting and potentially the tendency to form urinary tract stones (116).

Patients with HHRH have been treated successfully with high-dose phosphorus (1 to 2.5 g/day in five divided doses) alone. In response to therapy, bone pain disappears and muscular strength improves substantially. Moreover, the majority of treated subjects exhibit accelerated linear growth, and radiologic signs of rickets are completely absent within several months. Despite this favorable response, limited studies indicate that such treatment does not completely heal the associated osteomalacia. Indeed there is no collective experience with long-term follow-up of this rare disorder. Curiously an accompanying osteoporosis appears to occur in concert, a finding that is also quite different from the usual picture in XLH.

Clearly further studies are necessary to determine if phosphorus alone is truly sufficient for this disorder.

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