Importance of Dietary Phosphorus for Bone Metabolism and Healthy Aging

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Abstract: Inorganic phosphate (P\textsubscript{i}) plays a critical function in many tissues of the body: for example, as part of the hydroxyapatite in the skeleton and as a substrate for ATP synthesis. P\textsubscript{i} is the main source of dietary phosphorus. Reduced bioavailability of P\textsubscript{i} or excessive losses in the urine causes rickets and osteomalacia. While critical for health in normal amounts, dietary phosphorus is plentiful in the Western diet and is often added to foods as a preservative. This abundance of phosphorus may reduce longevity due to metabolic changes and tissue calcifications. In this review, we examine how dietary phosphorus is absorbed in the gut, current knowledge about P\textsubscript{i} sensing, and endocrine regulation of P\textsubscript{i} levels. Moreover, we also examine the roles of P\textsubscript{i} in different tissues, the consequences of low and high dietary phosphorus in these tissues, and the implications for healthy aging.

Keywords: dietary phosphorus; inorganic phosphate (P\textsubscript{i}); hypophosphatemia; hyperphosphatemia; mineralization; absorption; paracellular; transcellular

1. Introduction

Phosphorus is one of the essential elements of the human body and is required for a diverse range of processes, such as ATP synthesis, signal transduction, and bone mineralization. The vast majority (85%) of phosphorus in the body exists as a component of hydroxyapatite [Ca\textsubscript{10}(PO\textsubscript{4})\textsubscript{6}(OH)\textsubscript{2}] in the extracellular matrix of bone and teeth [1]. In contrast, intracellular phosphorus accounts for 14% of total body phosphorus, and only 1% is present, mostly as inorganic phosphate (P\textsubscript{i}), in extracellular fluids [1]. Phosphorus most commonly occurs as a salt of phosphoric acid, which is an essential physiological buffer referred to as P\textsubscript{i}. Although we will focus on this form of phosphorus, it is important to note that phosphorus is also a component of phospholipids, DNA, RNA, ATP, and creatine phosphate (CrP). At physiological pH, P\textsubscript{i} is apportioned 4:1 between its divalent form, HPO\textsubscript{4}\textsuperscript{2−}, and its monovalent form, H\textsubscript{2}PO\textsubscript{4}−, respectively [2]. Moreover, P\textsubscript{i} also forms dimers (such as pyrophosphate) and polymers (such as polyphosphate) [3–5]. Alternatively, P\textsubscript{i} may be covalently bound in organic molecules, including inositol pyrophosphates, membrane phospholipids, phosphoproteins, and ribonucleic acids [3–5].

As a result of its importance in health, the maintenance of extracellular P\textsubscript{i} homeostasis is imperative. Chronic P\textsubscript{i} deficiency can result in both bone loss through resorption [6,7] and contribute to myopathy [8] and frailty. Moreover, severe acute hypophosphatemia may cause cardiac and respiratory failure, leading to death [9]. On the other hand, high extracellular P\textsubscript{i} is similarly associated with adverse health outcomes, including coronary artery calcification, worsening renal function, premature aging, and increased mortality [10–13].
The nutritional environment of Western cultures is, among other features, notable for its very high phosphorus content. This is in no small measure because Pi salts are routinely added to processed foods for a variety of reasons, including taste and food preservation. The dysregulation of extracellular Pi is implicated in skeletal disorders as well as vascular calcification in chronic kidney disease and cardiovascular disease [14,15]. This review will examine how dietary phosphorus is absorbed by the body (with an emphasis on recent insights about endocrine regulation of Pi homeostasis) and the effects of dietary phosphorus as a nutrient in various organ systems. Additionally, we will examine the effects of dietary phosphorus in longevity and how possible adverse effects may indicate a need for closer examination of the use of Pi salts as additives in Western foods. We will highlight areas still poorly understood—for example, the function of Pi transporters in dental health, cardiovascular health, and the nature and molecular basis of paracellular Pi absorption in the gut.

2. Phosphate Absorption from the Diet in the Gut

The concentrations of phosphorus in sea water or soil are in the micromolar range [16]. Accordingly, the normal situation for unicellular organisms, plants, and certain aqueous animals is that of phosphorus deprivation, for which the uptake and sensing of Pi are stimulated by default. It is only when Pi is available in higher quantities that this uptake and sensing system is turned off [17]. In higher species (such as fish and mammals), the reverse is the case, since these species can move to seek food to meet their nutrient needs. Particularly for terrestrial mammals and humans, dietary phosphorus is plentiful, and therefore, homeostatic processes that prevent phosphorus intoxication have evolved.

In Western diets, phosphorus (usually as a Pi salt) is frequently used as an additive in processed foods [18,19]. Recent estimates indicate that phosphorus intakes often exceed the recommended daily allowance (RDA) by one-and-a-half to two-fold [20]; in adults between the ages of 19 and 70, this corresponds to an intake that ranges between 1500 and 1700 mg/day for men and 1000 and 1200 mg/day for women [20]. Intestinal Pi absorption is highest in infancy and childhood and declines with age [21]. However, it remains robust at approximately 50–70% of bioavailable phosphorus [21]. Of this ingested phosphorus, 16 mg/kg/day is unidirectionally absorbed in the proximal intestine, and 3 mg/kg/day is lost through endogenous pancreatic, bile, and gut secretions [22]. The result is a net absorption of 13 mg/kg/day of phosphorus, which can enter the extracellular fluid and be utilized by tissues such as bone (Figure 1) [22]. The majority of dietary phosphorus is absorbed as Pi in the small bowel by two pathways: a passive paracellular pathway and a transcellular absorption pathway. In conditions of abundant dietary Pi intake, 70% of intestinal Pi uptake occurs primarily by passive paracellular diffusion, while only 30% occurs via sodium (Na+-) dependent, carrier-mediated transcellular transport [21,23–26]. However, in experimental animals, it is estimated that the two pathways contribute roughly equally to intestinal Pi absorption [27].
Figure 1. Phosphate homeostasis. (A) The acute response and (B) the chronic response to increases in phosphate intake. (C) The acute response and (D) the chronic response to decreases in phosphate intake. Details are provided in the text. Numbers show hypothetical shifts of phosphorus between body compartments for a 70 kg adult based on [28]. GI, gastrointestinal. ICF, intracellular fluid. PTH, parathyroid hormone. 1,25(OH)$_2$D, 1,25-dihydroxycholecalciferol (calcitriol). FGF23, fibroblast growth factor 23. P, inorganic phosphate. dL, deciliter. mg, milligram. SI conversion: 1 mg phosphorus = 0.32 mmol phosphorus. Adapted from [29].

2.1. Paracellular Phosphate Absorption Pathway

In the paracellular absorption pathway, P$_i$ moves from the diet into the circulation passively through tight junction complexes [30]. However, the specific molecular identities of the components associated with paracellular P$_i$ absorption have not been identified. Dietary P$_i$ absorption through the paracellular pathway occurs primarily in the small intestine, and to a lesser extent in the colon [31,32]. The permeability for P$_i$ in these intestinal segments is similar to Na$^+$ [31,32]. Although both monovalent and divalent P$_i$ exist under physiological conditions, monovalent P$_i$ is slightly preferred for paracellular P$_i$ absorption [31]. Although earlier estimates suggest that the paracellular pathway is the major mechanism for P$_i$ absorption, it is important to note that this pathway is low-affinity. As such, it is sufficient only when phosphorus is present in the diet in high concentrations. The paracellular pathway is also non-saturable, allowing for P$_i$ absorption in potentially toxic quantities.
Paracellular P<sub>i</sub> absorption is susceptible to chemical inhibitors, but it is not known to be regulated by hormonal factors (see Section 3, Endocrine regulation of phosphate homeostasis). It is likewise unknown whether paracellular transport is unidirectional or whether it can result in the ‘leaking’ of P<sub>i</sub> from the extracellular space into the gut under certain conditions.

2.2. Transcellular Absorption Pathway/Transporter-Mediated Phosphate Absorption

The second pathway for intestinal P<sub>i</sub> absorption is mediated primarily by the sodium-dependent phosphate transport protein 2b, NPT2b (encoded by SLC34A2). To a lesser extent, this transcellular transport is also mediated by the type 3 sodium-dependent transporters, PIT1 (encoded by SLC20A1) and PIT2 (encoded by SLC20A2), although the role of the latter is not clear [21,33]. This high affinity and saturable transporter-mediated pathway is present in the duodenum and jejunum [34–37], is stimulated by 1,25-dihydroxyvitamin D [1,25(OH)<sub>2</sub>D<sub>3</sub>, calcitriol], and accounts for 30% of intestinal P<sub>i</sub> absorption when phosphorus is abundant in the diet [21,34,38,39].

NPT2b uses the transmembranous Na<sup>+</sup> gradient to transport P<sub>i</sub> into cells against its own electrochemical gradient at a stoichiometry of 3: 1 Na<sup>+</sup>: P<sub>i</sub> [40]. Both murine [40,41] and swine [42] Npt2b preferentially transport divalent P<sub>i</sub>. The apparent Michaelis–Menten affinity constant (K<sub>m</sub>) for murine Npt2b for P<sub>i</sub> is 10 µM at ~60 mV, as determined via kinetic characterization in Xenopus oocytes [40,41]. This preferential transport of divalent P<sub>i</sub> also explains why P<sub>i</sub> uptake via swine NPT2b expressed in Xenopus oocytes increases with alkaline pH levels and is maximal at pH 8.5 [42].

3. Endocrine Regulation of Phosphate Homeostasis

The normal blood concentration of P<sub>i</sub> in humans is 2.5–4.5 mg/dL and is regulated through the control of intestinal absorption of P<sub>i</sub> from the diet, by the release of P<sub>i</sub> from intracellular stores acutely and from bone remodeling chronically, and renal excretion (reviewed by [14,43–45]). This homeostasis is maintained by parathyroid hormone (PTH), fibroblast growth factor 23 (FGF23), calcitriol, and other factors discussed below. In turn, P<sub>i</sub> feeds back to regulate the secretion of these hormones. This process is often referred to as endocrine P<sub>i</sub> sensing [46] but is still poorly understood (for several excellent reviews, see [46–48]).

3.1. Clinical Chemistry of Phosphate

Clinical laboratories use differing methods to either measure P<sub>i</sub> (colorimetric assays) or phosphorus (flame photometry). However, P<sub>i</sub> measurements are converted to phosphorus (1 mg/dL P<sub>i</sub> contains 0.32 mmol/L phosphorus, which is equal to 0.32 mmol/L phosphorus). Additionally, it is important to collect fasting samples to determine serum P<sub>i</sub> levels, since feeding causes hyperinsulinemia, which reduces serum P<sub>i</sub> levels by inducing intracellular shifts of P<sub>i</sub> [49]. Moreover, it is also important to consider that the serum concentration of phosphorus follows a circadian rhythm in addition to that modulated by dietary phosphorus; serum phosphorus levels vary throughout the day and are lowest in the early morning [50]. Currently, analysis of serum P<sub>i</sub> is not a routine measurement in clinical practice; given the potential for adverse health effects caused by high levels of dietary phosphorus and serum P<sub>i</sub> (discussed later in this review), and the high prevalence of P<sub>i</sub> additives in Western foods, it may be necessary to reevaluate the importance of such testing.

3.2. Regulation of Phosphate Absorption in the Gut

3.2.1. Calcitriol

1,25(OH)<sub>2</sub>D<sub>3</sub>, or calcitriol, is the active form of vitamin D and the key hormone that regulates transcellular P<sub>i</sub> absorption in the intestine by stimulating the expression of NPT2b. A deficiency of P<sub>i</sub> upregulates calcitriol [6], which will be discussed further in Section 4.3 below. Calcitriol binds to its nuclear hormone receptor vitamin D<sub>3</sub> receptor (VDR) [51]. This calcitriol/VDR complex forms nuclear heterodimers with retinoic acid X-receptor (RXR), which then can bind the vitamin D-responsive elements of target genes [51] to regulate gene expression [52–54]. Thereby, calcitriol promotes
intestinal P\textsubscript{i} absorption both directly by inducing NPT2b expression in the gut and indirectly by increasing calcium absorption, thereby improving P\textsubscript{i} absorption by preventing the formation of insoluble CaP\textsubscript{i} in the gut lumen [54,55].

3.2.2. Phosphorus Depletion

Similar to 1,25(OH)\textsubscript{2}D\textsubscript{3}, dietary phosphorus depletion is considered one of the predominant physiological stimuli of intestinal P\textsubscript{i} absorption [25,39,56]. Chronic adaptation to a low-phosphate diet in wild-type (WT) rats appears to go along with the upregulation of Npt2b in the jejunum and upregulation of Npt2a in the proximal tubules, where the bulk of filtered P\textsubscript{i} is reabsorbed [25,37,57–59]. However, it appears that low-P\textsubscript{i}-diet-induced Npt2b upregulation is independent of the 1,25(OH)\textsubscript{2}D\textsubscript{3}–VDR axis [60]. This was shown in mice that had a normal upregulation of Npt2b by a low-P\textsubscript{i} diet despite being VDR- and 1-α hydroxylase-deficient [60]. Additionally, low dietary phosphorus increases the activity of NPT2b by post-transcriptional mechanisms [18,21] and mobilizes P\textsubscript{i} from the bone mineral via increased resorption [7].

3.3.3. Estrogen

Similar to calcitriol, estrogen increases Na\textsuperscript{+}-dependent P\textsubscript{i} absorption in the gut. In response to estrogen treatment, Xu et al. showed increased brush border membrane vesicle (BBMV) P\textsubscript{i} uptake accompanied by an increased abundance of Npt2b protein in rat intestine [61]. Further, estrogen treatment also increases Npt2b mRNA levels in rats, suggesting transcriptional upregulation of the gene encoding Npt2b in response to estrogen [61]. A similar result was found using human intestinal epithelial (Caco-2) cells [61].

3.3.4. Glucocorticoids

Glucocorticoids (GCs) inhibit P\textsubscript{i} uptake in the intestine. Methylprednisolone injection in suckling animals resulted in a 3–4-fold reduction of NPT2b protein and mRNA levels, which reduced Na\textsuperscript{+}–P\textsubscript{i} uptake [62]. This suggests a possible regulatory role for GCs at the transcriptional level. However, other work done demonstrated that GC injection resulted in increased fucosyl transferase activity in suckling rat intestine [62,63]. This finding suggests a role for corticoids in intestinal maturation and the regulation of NPT2b glycosylation in a non-genomic fashion [62,63].

3.3.5. Epidermal Growth Factor

Epidermal growth factor (EGF) similarly inhibits intestinal P\textsubscript{i} absorption. EGF decreased promoter activity in Caco-2 cells transfected with human NPT2b promoter constructs and also decreased NPT2b mRNA abundance in both Caco-2 and rat intestinal cells by 40–50%, indicating that EGF transcriptionally downregulates NPT2b [64]. The molecular basis for this effect is that EGF reduces the binding affinity of the v-myb avian myeloblastosis viral oncogene homolog (c-myb) for the EGF responsive element in the NPT2b gene promoter [65], resulting in decreased promoter activity and therefore reduced NPT2b mRNA abundance [65]. Additionally, Xu et al. found that this regulatory effect involves EGF receptor-mediated activation of the mitogen-activated protein kinase (MAPK), protein kinase C (PKC), and protein kinase A (PKA) pathways [65].

4. Regulation of Systemic Phosphate Homeostasis

4.1. PTH

PTH is a peptide of 84 amino acids that is secreted by the parathyroid glands and signals through the PTH and PTH-related protein receptor [(also known as parathyroid hormone receptor 1 (PTH1R)], which is expressed in osteoblasts, osteocytes, chondrocytes, and proximal tubular cells [46]. P\textsubscript{i} stimulates the secretion of PTH in the parathyroids, which in turn stimulates the synthesis of calcitriol in the proximal tubules and thereby indirectly stimulates intestinal P\textsubscript{i} absorption [66]. Additionally, PTH stimulates bone turnover, resulting in the release of P\textsubscript{i} from the skeleton [67,68].
However, the net effect of PTH is to lower blood levels of P; because PTH also reduces the stability of the type II Na−-Pi co-transporters (NPT2a and NPT2c) at the renal brush border membrane, which reduces the reabsorption of P; from the urine [69,70]. This process is mediated by Na+/H+ exchange regulatory cofactor 1 (NHERF-1), which exists as a complex with NPT2a at the apical membrane of the proximal tubular cells [69,71]. NHERF-1 is phosphorylated via the cyclic adenosine monophosphate-PKA and phospholipase C-PKC signal transduction pathways following the activation of PTHR1 [21,69,71].

4.2. FGF23

FGF23 is a member of the fibroblast growth factor (FGF) family that is produced by osteocytes and osteoblasts in the skeleton [72–74], and it stimulates P; excretion in the kidneys [75]. This most recently identified physiologic regulator of renal P; excretion [76] provides a mechanism by which skeletal mineral demands can be communicated to the kidney. Thus, the skeleton can influence the P; economy of the entire organism through FGF23. Dietary P;, particularly when present in excess amounts, stimulates the synthesis of FGF23 [77]. In turn, FGF23 reduces the expression of NPT2a and NPT2c and thereby reduces P; reabsorption [78]. This process requires binding of the C-terminal tail of FGF23 to the klotho 1 and klotho 2 domains of α-Klotho (KL, which functions as a co-receptor) [79]. Additionally, binding of the FGF-like N-terminal domain to isoform c of FGF receptor 1 (FGFR1c) is also required [79]. Dimerization of this FGF23-KL-FGFR1c heterotrimer by heparan sulfate [79] is required for the activation of the extracellular signal-regulated kinases 1 and 2 (ERK1/2) [80]. Then, activation of ERK1/2 results in NHERF-1 dependent internalization of NPT2a and NPT2c from the apical membrane of proximal tubular cells in the kidneys [81]. Additionally, FGF23 reduces the expression of CYP27B1 (encoding CYP27B1, the 25-hydroxy-vitamin D 1-α hydroxylase that synthesizes calcitriol) and stimulates the expression of CYP24A1 (encoding CYP24A1, the vitamin D 24-hydroxylase that degrades calcitriol) [82,83]. As such, FGF23 inhibits the synthesis of calcitriol [82,83]. The net effect of this NPT2a/c internalization and decreased circulating calcitriol levels is lower blood levels of P;.

4.3. Calcitriol

Calcitriol is the active metabolite of vitamin D as mentioned above. Its synthesis by CYP27B1 and degradation by CYP24A1 are regulated by PTH, FGF23, calcium, and P; [54]. PTH stimulates calcitriol synthesis by inducing the expression of CYP27B1 and by suppressing the expression of CYP24A1 [54]. FGF23 decreases calcitriol levels by suppressing the expression of CYP27B1 and stimulating the expression of CYP24A1 [46,84]. In turn, calcitriol increases FGF23 levels, forming a negative feedback loop. The actions of P; and calcium on calcitriol synthesis are predominantly mediated by FGF23 and PTH, respectively [85–89]. Additionally, calcitriol inhibits PTH both directly by transcriptionally repressing the gene encoding PTH and indirectly by upregulating the calcium-sensing receptor (CASR, a protein that modulates PTH secretion by calcium in the parathyroid cells) [51]. Calcitriol, as mentioned already above, stimulates NPT2b expression in the intestine and thereby increases both the absorption of P; in the gut and consequently circulating blood P; levels [55]. Since it suppresses PTH, calcitriol also indirectly reduces P; excretion in the kidneys. Therefore, the net effect of calcitriol is to increase blood P;.

5. Disorders of Phosphate Homeostasis

In this review, we will focus on disorders of intestinal P; absorption, which can be divided into disorders of intake, bioaccessibility, bioavailability, and regulatory hormones. While generally acquired, some of these disorders have underlying genetic causes (as reviewed below). If left untreated, dietary phosphorus insufficiency can result in rickets or osteomalacia [90,91]. Furthermore, a prolonged reduction of serum P; levels <1.0 mg/dL can result in several more health issues, including rhabdomyolysis, cardiac muscle dysfunction with congestive heart failure, and leukocyte dysfunction, among other abnormalities [92–94]. Excess blood P; can lead to tissue
calcifications and excess syndromes caused by hyperparathyroidism and high circulating FGF23 levels. The effects of dietary phosphorus deficiency or excess will be discussed in detail for each tissue in the next chapters.

5.1. Phosphorus Content in the Western Diet

Phosphorus is abundant in the Western diet. The RDA for phosphorus from the diet is 700 mg/day [20]. However, most Americans far exceed this recommendation [20], since a lack of mandated reporting on nutrition facts labels causes phosphorus content to be commonly unidentified on American food labels [95]. Phosphorus additives tend to be common in prepared frozen foods, dry mixes, packaged meats, bread and baked goods, and some yogurts (where P additives may be added in addition to the phosphorus contributed by milk) [96]. These additives mainly consist of phosphoric acid, phosphates, or polyphosphates [97], and they were found on average to add 736 mg more phosphorus/day compared to additive-free diets [96]. Even in balanced 2200 kcal/day diets, phosphorus consumption as averaged over four days totaled 1677 ± 167 mg/day in additive-enhanced diets [98]. In low-additive diets designed to meet U.S. Department of Agriculture recommended guidelines for fat, protein, carbohydrate, and phosphorus intake, the averaged total was 1070 ± 58 mg phosphorus/day [98]. When separately evaluated by gender, males aged 19–70 have an average daily phosphorus intake that ranges between 1500 and 1700 mg, and women aged 19–70 usually consume between 1000 and 1200 mg phosphorus/day [20].

Similarly, phosphorus intake in Europe averages 1000–1767 mg/day for both sexes [99]. This elevated intake may also be due to unclear phosphorus labeling since, in Europe, information about phosphorus is generally only available in the ingredients list [100]. However, even in this case, phosphorus may be listed as different P additives or identified only by E number (a code assigned to food additives in Europe) [100].

National Health and Nutrition Examination Survey data demonstrated an association between high dietary phosphorus intake (>1400 mg/day) and all-cause mortality in U.S. adults after adjusting for other known contributors [101]. This threshold of 1400 mg/day is routinely exceeded by men aged 14–71 in the U.S., suggesting a negative effect of high dietary phosphorus on human longevity [20].

In addition to attention to labeling and phosphorus content in the diet, physicians need to be aware that therapeutic phosphorus preparations often list the mass of the phosphate salt, which includes oxygen, sodium, and potassium. Therefore, phosphorus content varies for the specific preparation being prescribed, and this should be considered in consultation with the pharmacist and hospital formulary (Table 1) [21].

| Phosphate Preparations          | Phosphorus Content | Potassium (K) Content | Sodium (Na) Content |
|---------------------------------|--------------------|----------------------|---------------------|
| Neutraphos-powder               | 250 mg/packet      | 270 mg/packet        | 164 mg/packet       |
| (for mixing with liquid)        |                    |                      |                     |
| Neutraphos-K-powder             | 250 mg/packet      | 556 mg/packet        | 0 mg/packet         |
| (for mixing with liquid)        |                    |                      |                     |
| K-Phos Original-tablet          | 114 mg/tablet      | 144 mg/tablet        | 0 mg/tablet         |
| (to mix in liquid, acidifying)  |                    |                      |                     |
| K-Phos MF-tablet                | 126 mg/tablet      | 45 mg/tablet         | 67 mg/tablet        |
| (mixing not required, acidifying)|                    |                      |                     |
| K-Phos #2                       | 250 mg/tablet      | 90 mg/tablet         | 133 mg/tablet       |
| (double strength of K-Phos MF)  |                    |                      |                     |
| K-Phos Neutral-tablet           | 250 mg/tablet      | 45 mg/tablet         | 298 mg/tablet       |
| (non-acidifying, mixing not required)|                |                      |                     |
| Phospha-Soda solution           | 127 mg/mL          | 0 mg/mL              | 152 mg/mL           |
| (small doses may be given undiluted)|                  |                      |                     |
| Joulie’s solution               | 30 mg/mL           | 0 mg/mL              | 17.5–20 mg/mL       |
| (prepared by compounding pharmacies)|                  |                      |                     |

Table 1. Clinician’s Guide to P/Vitamin D Supplementation.
Vitamin D | Calciferol (Drisdol) | Solution: 8000 IU/mL  Tablets: 25,000 and 50,000 IU
---|---|---
Dihydrotachysterol | DHT (Hytkerol) | Solution: 0.2 µg/5 mL  Tablets: 0.125, 0.2, and 0.4 mg
1,25 dihydroxyvitamin D | Calcitriol (Rocaltril) | 0.25 and 0.5 µg capsules and 1 µg/mL solution
Calcijex | Ampules for IV use containing 1 or 2 µg of drug per mL
1α-hydroxyvitamin D | Alfacalcidol | 0.25, 0.5, and 1 µg capsules
Oral solution (drops): 2 µg/mL  Solution for IV use: 2 µg/mL
Vitamin D analogs | Paricalcitol (Zemplar) | 1, 2, and 4 µg capsules
Doxercalciferol (Hectoral) | 0.5, 1, and 2.5 µg capsules

1 SI conversion: 1 mg phosphorus = 0.32 mmol phosphorus, 1 µg vitamin D = 40 IU vitamin D. IU, international unit. IV, intravenous. From: [102].

5.2. Influence of Dietary Components and Drugs on the Bioaccessibility of Phosphate

Of course, dietary phosphorus deficiency leads to a total body P deficiency. Furthermore, several dietary components can have a pronounced effect on the bioaccessibility of P. When combined with an insulin-mediated cellular change in P during refeeding, total body P deficiency causes hypophosphatemia (defined as serum P levels below 2.5 mg/dL, and it is considered severe at levels below 1.5 mg/dL) [50,103]. Refeeding syndrome can cause severe hypophosphatemia in malnourished individuals with alcoholism as well as in intensive care or institutionalized individuals [49]. However, hypophosphatemia is rare in the general population, since phosphorus is ubiquitous in the Western diet (as already mentioned above) [98]. In turn, high dietary phosphorus intakes as high as 4000 mg/day result in only minor increases in serum P concentrations due to the high efficiency of renal excretion [104], provided that ingestion is spread throughout the day. However, undiluted cow’s milk can provide sufficiently high P to induce hyperphosphatemia in infants [15]. Moreover, bowel preparations that use P-containing laxatives (such as oral phosphosodas) can result in severe hyperphosphatemia and in some cases renal failure due to nephrocalcinosis [105,106].

pH is also an important factor controlling the bioaccessibility of P from the diet in the gut. At pH values above 6.5, dibasic P predominates but has low solubility (30 mg/dL) [107,108]. At pH values below 6.5, monobasic P predominates with a much higher solubility (1800 mg/dL) [107,108]. In a study of infants who were fed an elemental diet and exhibited unexplained hypophosphatemia, a commonality between many children was treatment with gastric proton pump inhibitors (PPIs) [109]. The effect of this treatment would have likely been abnormally high gastric pH [109]. Given that P solubility decreases with increasing pH (particularly above the acidic range), it is possible that increased gastric pH could have resulted in reduced mineral absorption [108,109]. However, it should be noted that the authors did not identify systemic acid–base abnormalities in these children, and P status was corrected with alternative Pi salts rather than an alteration of acid-modifying medications [109]. Similarly, alkaline pH inhibits P transport in rat intestinal BBMVs [110]. Conversely, acidic pH increases P uptake in rat intestinal BBMVs, and this might be partially mediated by an unidentified, Na+-coupled P transporter that prefers monovalent P [110].

Certain antacids, such as magnesium–aluminum hydroxides, sucralfate, and calcium-containing antacids, can also reduce the bioaccessibility of P [111,112]. These antacids reduce P bioaccessibility by binding to dietary P and by forming insoluble P salts in the small intestine that prevent absorption [111,112]. Thus, prolonged use of P-binding antacids can cause hypophosphatemia [111].

Phytate, which is the major form of phosphorus in the seeds of plants, is a compound that can form indigestible mineral phytate salts in humans (who lack the enzyme phytase that can release P from phytate) [113]. At physiological pH, phytate binds calcium with high affinity [114]. If the calcium concentration is high, phytate forms indigestible, multiple calcium phytate salts [113]. However, phytate becomes digestible at low calcium concentrations due to reduced calcium binding [113]. Kim et al. demonstrated that high phytate/low calcium diets increase intestinal P absorption in rats following intestinal phytate hydrolysis that makes P bioaccessible [113]. This P load results in
secondary hyperparathyroidism and renal P{sub}i wasting, which appears to be independent of FGF23 [113]. Calcium supplementation alleviates this effect [113]. Therefore, attention to dietary calcium may be especially important in vegetarians or other individuals who consume high-phytate diets to avoid hyperphosphatemia and its associated effects.

5.3. Influence of Dietary Components, Drugs, and Disorders on the Bioavailability of Phosphate

There exist a variety of molecules containing a P{sub}i moiety that can act as competitive inhibitors of NPT2b and thereby inhibit intestinal P{sub}i transport. Phosphonoformate (PFA, a phosphonocarboxylate) has a K_{i} value of 0.37 mM in rat small intestine BBMVs, which indicates a low affinity of NPT2b for PFA as compared to P{sub}i [115]. 2'-Phosphophloretin (2'-PP), a derivative of the plant chalcone phloretin, more strongly inhibits Na⁺-dependent P{sub}i uptake, with K_{i} values of 38 ± 7 nM in rabbit intestinal BBMVs and 42 ± 8 nM in rat intestinal BBMVs [116]. Pentavalent arsenate can be transported by NPT2b and also acts as a competitive inhibitor of NPT2b (K_{i} = 51 µM for rat Npt2b in Xenopus oocytes) [115]. Of course, the inhibition of intestinal P{sub}i transport would reduce P{sub}i bioavailability.

On the other hand, nicotinamide adenine dinucleotide (NAD) acts as a non-competitive inhibitor of intestinal P{sub}i transport in vivo [117]. Similarly, triazole derivatives also act in a non-competitive fashion, inhibiting up to 61% of intestinal P{sub}i absorption as measured by in vivo experiments [118,119]. Finally, although tenapanor is not a direct inhibitor of NPT2b, repeat administration of tenapanor reduces transcellular P{sub}i absorption by decreasing the apical membrane expression of NPT2b at the small intestine [120]. This effect occurs at the transcriptional level [120]. These molecules and their effects on intestinal P{sub}i absorption are described in further detail in several excellent reviews [31,33,118].

There are no known competitive inhibitors of paracellular P{sub}i absorption in the intestine, which relies on passive diffusion and, differently from transporter-mediated absorption, is non-saturable. However, a notable non-competitive inhibitor of paracellular P{sub}i transport is Tenapanor. Tenapanor increases transepithelial electrical resistance (TEER) of the sodium/hydrogen exchanger isoform 3 (NHE3, encoded by SLC9A3) [120]. This increase in TEER reduces tight junction permeability to P{sub}i and therefore reduces intestinal P{sub}i absorption [120]. Since paracellular P{sub}i absorption is the major mechanism by which P{sub}i is absorbed when it is abundant in the diet, drugs that target paracellular rather than transcellular P{sub}i absorption could be as effective at preventing hyperphosphatemia in conditions such as chronic kidney disease (CKD) as P{sub}i binders. The development of such drugs could be aided by the identification of P{sub}i-specific occludins and claudins. Additionally, it would be interesting to know if leaky gut, whereby P{sub}i is lost through the paracellular route, can occur in humans (perhaps in situations where drugs or other causes reduce the concentration of P{sub}i in the intestinal lumen such that P{sub}i flows outward into the lumen).

Vitamin D intoxication caused by acute doses of vitamin D >10,000 international units (IU)/day and potentially chronically through extended administration of doses >4000 IU/day [121,122] increases intestinal P{sub}i absorption via the upregulation of NPT2b [54,55] and also increases bone resorption [7,123]. Both factors can contribute to the development of hyperphosphatemia. Additionally, high levels of calcitriol inhibit the production of PTH, thereby blunting PTH-mediated removal of NPT2a/c from the renal proximal tubules [66,124]. This effect contributes to hyperphosphatemia due to reduced renal P{sub}i excretion [66,124]. Conversely, vitamin D deficiency causes decreased intestinal P{sub}i absorption, resulting in rickets or osteomalacia as well as secondary hyperparathyroidism [125,126].

Finally, certain diseases and conditions can also affect P{sub}i bioavailability. Inflammatory bowel diseases are often characterized by chronic diarrhea [127] and result in the malabsorption of P{sub}i [128]. Conversely, metabolic acidosis causes increased NPT2b abundance in mouse small intestinal BBMVs, although this increase is not accompanied by a corresponding increase in NPT2b mRNA [129]. As a result, metabolic acidosis increases P{sub}i uptake, potentially to buffer acid equivalents and contribute to the restoration of acid–base homeostasis [129].

Acquired disorders of P{sub}i homeostasis are summarized below in Table 2.
**Table 2. Acquired Disorders of Phosphate Homeostasis**

| Disorder | Mechanism | Ref. |
|----------|-----------|------|
| **Hypophosphatemic** | Total body deficiency, combined with an insulin-mediated cellular shift in P, during refeeding, causes hypophosphatemia. | [50] |
| Dietary phosphorus deficiency | Reduced intestinal Ca and P absorption causes rickets/osteomalacia and secondary hyperparathyroidism. | [130,131] |
| Vitamin D deficiency | High gastric pH reduces P solubility, which potentially results in reduced mineral absorption and hypophosphatemia. | [109,111,112,132,133] |
| Chronic use of P, antacids/high gastric pH (due to PPIs, autoimmune gastritis/pernicious anemia, etc.) | Ferric carboxymaltose blocks FGF23 cleavage, which induces renal P wasting. | [134] |
| Reduced gastrointestinal absorption (due to Inflammatory Bowel and Celiac diseases, diarrhea, vomiting, short gut, intestinal mucosal hypoplasia, jejunal feeding, prematurity, etc.) | Chronic diarrhea and reduced gastrointestinal absorption of P reduce bioavailable P. | [127,128] |
| Parenteral iron administration | Renal Pi wasting causes rickets/osteomalacia and hypercalciuria. | [135–137] |
| Proximal tubular damage (caused by renal tubular acidosis or drugs such as theophylline, foscarnet) | Bone resorption increases serum P, but the net effect is to lower serum P due to increased renal excretion. | [138,139] |
| Hyperparathyroidism | Competitively inhibit Na-P, co-transport of P. | [115,116,118,140] |
| Phosphonocarboxylates (e.g., PFA), phloretin derivatives (e.g., 2′-PP), arsenate | Downregulates NPT2b, inhibits intestinal P transport. | [117,118,141] |
| Niacin/Nicotinamide, NAD, triazole derivatives | Inhibits paracellular P transport and downregulates NPT2b. | [118,120] |
| Tenapanor | Promotes P uptake into tissues. Can result in hypophosphatemia in the context of refeeding. | [103,142] |
| Insulin | Decreased bone resorption can cause hypophosphatemia along with hypocalcemia. | [143,144] |
| Bisphosphonates and other bone resorption blockers | Inhibits P transport by PIC in reconstituted liposomes. | [145] |
| Adriamycin | Low dietary Ca causes P hyperabsorption. The associated | [113] |
| **Hyperphosphatemic** | **Hyperphosphatemic** | **Hyperphosphatemic** |
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5.4. Genetic Disorders of Intestinal Phosphate Absorption

In addition to acquired disorders, there are several genetic disorders of intestinal \( \text{Pi} \) absorption that concern the active, transcellular transport of \( \text{Pi} \). Thus far, no genetic abnormalities affecting the passive, paracellular transport of \( \text{Pi} \) have been reported.

The key protein involved in active intestinal \( \text{Pi} \) transport is NPT2b [35]. NPT2b deletion is embryonic lethal in mice, and homozygous loss of function (LOF) mutations of NPT2b in humans cause pulmonary alveolar and testicular microlithiasis [151,152]. On the other hand, genetic disorders of vitamin D synthesis or action can impair intestinal \( \text{Pi} \) absorption. Hereditary 1,25(OH)\( _2 \)D-resistant rickets (HVDDR) is characterized by mutation in the \( \text{VDR} \) gene, which results in vitamin D resistance [153]. As a result of this vitamin D resistance, HVDDR symptoms include hypophosphatemia, hypocalcemia, secondary hyperparathyroidism, and severe rickets with osteomalacia [153]. Similarly, vitamin D-resistant rickets type 1A is characterized by mutation in \( \text{CYP27B1} \) [154]. Thus, this disorder causes calcitriol deficiency, which results in hypophosphatemia, hypocalcemia, and rickets [15,154].
Although LOF mutations in claudins and occludins that affect the paracellular transport of other minerals (such as magnesium) have been described, no disorders caused by LOF mutations in Pi-specific tight junction proteins have been reported thus far.

5.5. Other Disorders of Phosphate Homeostasis

For the large number of disorders of Pi homeostasis that do not concern Pi as a nutrient (listed in Table 3 and visually in Figure 2), the reader is referred to several excellent recent reviews [15,21,155]. These disorders can be broadly divided into disorders of extracellular Pi homeostasis or disorders of intracellular Pi homeostasis. Disorders of extracellular Pi homeostasis can be further categorized as being FGF23-dependent, PTH-dependent, and FGF23 or PTH-independent. This distinction is mainly helpful diagnostically, since Pi absorption and calcium absorption are differentially affected (Table 3).

Table 3. Human Genetic Disorders of Pi Homeostasis 1.

| Disorder | Abbreviation | Inheritance | Gene | Mechanism | Ref. |
|----------|--------------|-------------|------|-----------|-----|
| Hyperphosphatemic Disorders | HFTC1 | AR | GALNT3 | FGF23 deficiency | [156,157] |
| Hyperphosphatemic Familial Tumoral Calcino... | HSS | AR | | | |
| Hyperphosphatemic Familial Tumoral Calcino... | HFTC2 | AR | FGF23 | FGF23 deficiency | [158,159] |
| Hyperphosphatemic Familial Tumoral Calcino... | HFTC3 | AR | KL | FGF23 resistance | [160] |
| Idiopathic Hyperphosphatasia (Juvenile Paget's Disease) | N/A | AR | TNFRSF11B | OPG deficiency | [161] |
| Pseudohypoparathyroidism | PHP1A | AD (impr.) | GNAS or up-stream regulatory region | PTH resistance, FGF23-independent | [162,163] |
| Familial Isolated Hypoparathyroidism | FIH | AD or AR | CASR | PTH deficiency, FGF23-independent | [164–166] |
| Blomstrand disease | BOCD | AR | PTHR1 | PTH resistance, FGF23-independent | [167,168] |
| Hypophosphatemic Disorders | XLH | X-linked | PHEX | FGF23-dependent | [169] |
| Autosomal Dominant Hypophosphatemic Rickets | ADHR | AD | FGF23 | FGF23-dependent | [170] |
| Autosomal Dominant Hypophosphatemic Rickets | ADHR | AD | KL | FGF23-dependent | [171] |
| Autosomal Recessive Hypophosphatemic Rickets types 1, 2, and 3 | ARHR1, ARHR2, ARHR3 | AR | DMP1, ENPP1, FAM20C | FGF23-dependent | [172–174] |
| Hereditary Hypophosphatemic Rickets with Hypercalciuria | HHRR | AR | SLC34A3 | Proximal tubular Pi wasting, FGF23-independent | [175,176] |
| Vitamin D-resistant rickets type 1A | VDDR1A | AR | CYP27B1 | 1,25(OH)2D deficiency, FGF23-independent | [154,177] |
| Hereditary 1,25(OH)2D-resistant rickets | HVDDR | AR | VDR | 1,25(OH)2D resistance, FGF23-independent | [153,178] |
| Familial hypocalciuric hypercalcaemia/neonatal severe hyperparathyroidism | FHH | AD/AR | CASR | PTH excess, FGF23-independent | [179] |
| Disorder                                              | Mode | Gene   | Description                                      | Reference |
|-------------------------------------------------------|------|--------|--------------------------------------------------|-----------|
| Jansen disease                                        | AD   | PTHR1  | Const. active PTHR1, FGF23-dependent             | [180,181] |
| Normophosphatemic disorders                           |      |        |                                                  |           |
| Pulmonary alveolar microlithias                       | AR   | SLC3A2 | Reduced alveolar epithelial P\textsubscript{i} uptake | [35]      |
| Normophosphatemic familial tumoral calcinosis         | AR   | SAMD9  | Unknown                                          | [182]     |
| Muscle dystrophy and cardiomyopathy                   | AR   | SLC25A3| Reduced mitochondrial P\textsubscript{i} uptake   | [183,184] |
| Primary familial basal ganglial calcification type 1   | AD   | PIT2   | Reduced microglial P\textsubscript{i} uptake     | [185]     |
| Primary familial basal ganglial calcification type 4   | AD   | PDGFRB | Reduced PIT2 expression                          | [186]     |
| Primary familial basal ganglial calcification type 5   | AD   | PDGFB  | Reduced PIT2 expression                          | [186]     |
| Primary familial basal ganglial calcification type 6   | AD   | XPR1   | Reduced vascular P\textsubscript{i} export       | [187]     |
| Primary familial basal ganglial calcification type 7   | AR   | MYORG  | Unclear, astrocyte dysfunction and possible NVU disruption may be causative factors. | [188,189] |
| Primary familial basal ganglial calcification type 8   | AR   | JAM2   | Reduced JAM2 expression                          | [190,191] |

1 Adapted from [21]. Bold font was used to visually denote the boundaries between hyperphosphatemic, hypophosphatemic and normophosphatemic disorder categories. AD, autosomal dominant. AR, autosomal recessive. GALNT3, polypeptide N-acetylgalactosaminyltransferase 3. FGF23/FGF23, fibroblast growth factor 23. KL, klotho. TNFRSF11B, TNF receptor superfamily member 11B. GNAS, guanine nucleotide- binding protein, alpha stimulating. CASR, calcium-sensing receptor. GCMB, glial cell missing gene. PTH/PTH, parathyroid hormone. PTHR1/PTH, parathyroid hormone 1 receptor. PHEX, phosphate-regulating endopeptidase homolog, X-linked. DMP1, dentin matrix acidic phosphoprotein 1. ENPP1, ectonucleotide pyrophosphatase-phosphodiesterase family member 1. FAM20C, golgi-associated secretory pathway kinase. SLC34A3, solute carrier family 34 member 3. CYP27B1, vitamin D 1-alpha hydroxylase. VDR, vitamin D receptor. SLC34A2, solute carrier family 34 member 2. SAMD9, sterile alpha motif domain containing 9. SLC25A3, solute carrier family 25 member 3. PIT2/PIT2, type III sodium-dependent phosphate transporter 2. PDGFRB, platelet derived growth factor receptor beta. PDGFB, platelet derived growth factor subunit B. XPR1, xenotropic and polytropic retrovirus receptor 1. MYORG, myogenesis regulating glycosidase. JAM2/JAM2, junctional adhesion molecule 2. OPG, osteoprotegerin. P, inorganic phosphate. 1,25(OH)\textsubscript{2}D, 1,25-dihydroxyvitamin D. NVU, neurovascular unit.
Figure 2. Human disorders of phosphate homeostasis caused by transporters of inorganic phosphate (Pᵢ). Compounds that inhibit Pᵢ transport are denoted in red, while compounds that stimulate Pᵢ transport are denoted in green. ¹ These compounds may affect their respective Pᵢ transporters in tissues other than the ones that the compounds are listed under. For example, NPT2A inhibitors may also affect Pᵢ transport in the bone. Additionally, tenapanor is not a direct inhibitor of Pᵢ transport through NPT2B. XPR1 small peptide inhibitors were only reported in in vitro studies. Question mark indicates unknown. FGF23, fibroblast growth factor 23. BMD, bone mineral density. NAD, nicotinamide adenine dinucleotide. NPT1, sodium-dependent phosphate transport protein 1. NPT2A, sodium-dependent phosphate transport protein 2A. NPT2B, sodium-dependent phosphate transport protein 2B. NPT2C, sodium-dependent phosphate transport protein 2C. PFA, phosphonoformic acid. PIT1, type III sodium-dependent Pᵢ transporter 1. PIT2, type III sodium-dependent Pᵢ transporter 2. PIC, (SLC25A3, solute carrier family 25 member 3). XPR1, xenotropic and polytropic retrovirus receptor 1. XRBD, soluble ligand that can bind XPR1. Adapted from [46].
6. Metabolic Phosphate Sensing

Before discussing the tissue-specific roles of Pi, it may be helpful to summarize what is known about metabolic Pi sensing. For several excellent reviews as well, see [48,192,193]. Recent evidence suggests that the type 3 Na+-dependent Pi transporters PIT1 and PIT2 have an important role in metabolic Pi sensing. Interestingly, PIT1 and PIT2 might sense extracellular Pi without requiring its translocation, which is a process that is also referred to as transport-independent Pi sensing [194,195]. Therefore, these transporters may serve as sensors for extracellular Pi in addition to regulating intracellular Pi levels. The transport-independent Pi-sensing process might involve co-receptors: for example, FGFRI [48] and the CASR [196]. It is also possible that Pi directly binds and inhibits the CASR [196,197]. Finally, there is recent evidence that intracellular Pi stimulates the synthesis of 5-diphosphoinositol 1,2,3,4,6-pentakisphosphate (IP7) or 1,5-bisdiphosphoinositol 1,2,3,4-tetrakisphosphate (IP8), which are molecules that signal cellular Pi sufficiency and stimulate Pi efflux via Xenotropic and polytropic retrovirus receptor 1 (XPR1) [198–201]. Metabolic Pi sensing has a role separate from the endocrine Pi sensing discussed above, and it is important for maintaining intracellular Pi concentrations and producing intracellular effects such as gene activation, as is discussed in more detail below.

6.1. Extracellular Phosphate Sensing

Extracellular Pi activates the mitogen-activated protein kinases ERK1 and ERK2 (encoded by MAPK1 and MAPK3) in most cell types [202]. This process is evolutionarily conserved between Drosophila melanogaster and humans [202]. ERK1/2 activation is blocked by pharmacological or genetic inhibitors of the type 3 Na+-dependent Pi transporters [203]. Since multiple type 3 transporters can fulfill this role, it might be intracellular Pi that is sensed to activate ERK1 and ERK2.

However, several observations suggest that extracellular Pi is sensed in the cell membrane. Using green fluorescent protein-tagged versions of PIT1 and PIT2, these transporters were shown to dimerize in response to Pi, which in turn activates ERK1/2 (Figure 3) [204]. This activation appears to occur even if amino acid mutations that block Pi transport are introduced into PIT1 and PIT2 [194]. However, it is currently unclear which downstream molecules mediate the activation of ERK1/2 when these transporters function as ‘transceptors’.

The exposure of murine bone marrow stromal cells (BMSCs), which are cells with osteoblastic potential, to nanohydroxyapatite (nHAp) crystals increases the expression of osteopontin (Opn) and reduces the expression of alkaline phosphatase (Alp) in a dose-dependent manner [205]. These authors further showed that nHAp activates FGFR substrate 2 (FRS2) and ERK1/2 signaling downstream of Pit and Fgfr, and the inhibition of ERK1/2 blocks the regulation of Opn gene expression [206]. Likewise, pharmacologic inhibition of either Pit or Fgfr in BMSCs decreased the expression of Opn and derepressed Alp [206]. Electron microscopic evaluation showed nHAp at the cell surface of BMSCs, suggesting that nHAp signaling occurs without the internalization of nHAp [206]. Co-localization of immunostaining for Pit, Fgfr, and nHAp further confirmed that nHAp may bridge Pit and Fgfrs in the membrane of BMSCs, and this may be important for mediating the biological effect of nHAp (Figure 3) [206].

Additional evidence that FGFRI functions as a Pi sensor was provided in UMR106 rat osteosarcoma cells. Exposure of these cells to high extracellular Pi causes the autophosphorylation of FGFRI at multiple tyrosine residues [207], although it is currently unclear whether Pi binds directly to FGFRI. The sequential phosphorylation of six FGFRI tyrosine residues (653, 583, 463, 766, 585, and 654) leads to the activation of FGFRI signaling [208,209], which involves the phosphorylation of FRS2 and ERK1/2, and the gene expression of Early growth response 1 (Egr1), ETS variant 4, and ETS variant 5 (Etv5) [48,207]. The expression of EGR1 and ETV5 upregulates the polypeptide N-acetylgalactosaminyltransferase 3 (GALNT3) [48,207], although this upregulation may require other transcription factors [48]. GALNT3 is the enzyme required for O-glycosylation of FGF23 at threonine 178, whereby it stabilizes bioactive iFGF23 [48].

Lastly, crystallographic studies showed the binding of Pi to the CASR [196,197]. The CASR is highly expressed in the parathyroid glands and distal convoluted renal tubules, and it inhibits PTH
secretion by the parathyroids and the reabsorption of calcium from the urine upon the binding of calcium [210]. Conversely, Pi inhibits CASR in a non-competitive fashion, resulting in the stimulation of PTH secretion by parathyroid cells [197]. It is unclear at the moment whether the CASR also regulates FGF23 secretion and bioactivity.

6.2. Intracellular Phosphate Sensing

Upon uptake via NPT2b and PIT1/2, intracellular (IC) Pi stimulates the inositol hexakisphosphate kinases 1 and 2 (IP6K1 and -2) and synthesis of the second messenger IP7 from inositol hexakisphosphate (IP6) [199,200]. IP7 is further converted into IP8 by diphosphoinositol pentakisphosphate kinases (PPIP5Ks) [201]. The binding of IP7 or IP8 to the SPX domain of XPR1 [198,199] triggers Pi efflux from the cell, regulating the IC Pi concentration [200,201].

Cells overexpressing PIT2 showed a concomitant efflux in response to the resulting increase of Pi uptake, possibly to maintain IC ATP and Pi levels [211]. Pi efflux depends on IP7/IP8 signaling, which promotes efflux through XPR1 [211] and is absent in XPR1 KO cells or when IP6Ks are blocked pharmacologically [211]. Thus, IP7/IP8 may be important for intracellular Pi homeostasis controlled by PIT2 and XPR1 [211].

Subcellular compartments might further sequester intracellular Pi. The mitochondrial Pi carrier protein (PIC, encoded by SLC25A3) is part of the multiprotein complex that makes up the mitochondrial permeability transition pore (mPTP) [46]. The mPTP regulates mitochondrial membrane potential and mitochondrial apoptosis [212] and is important for skeletal and cardiac muscle function [213]. PIT1 localizes to the endoplasmic reticulum (ER), where it seems to be involved in regulating the ER stress of growth plate chondrocytes [214]. Finally, large and small conductance chloride channels transport Pi into the sarcoplasmic reticulum of rabbit skeletal muscle [215]. However, whether these compartments participate as intracellular sensors for Pi is currently unknown.

**Figure 3.** Schematic representation of metabolic Pi sensing in mammals (modified from [193]). Pi sensing in mammals can be divided into the distinct processes of extracellular Pi (Pi_e) sensing and intracellular Pi (Pi_i) sensing. (a) Extracellular Pi can be imported into the cell by the sodium-dependent phosphate transport protein 2b (Npt2b), which changes the intracellular Pi concentration. (b) An increase in intracellular Pi stimulates synthesis of 5-diphosphoinositol 1,2,3,4,6-pentakisphosphate (IP7) from inositol hexakisphosphate (IP6) by the inositol hexakisphosphate kinases 1 and -2 [199,200]. IP7 can be further converted into 1,5-bisdiphosphoinositol 1,2,3,4-tetrakisphosphate (IP8) by...
diphosphoinositol pentakisphosphate kinases [201]. P\textsubscript{i} efflux through xenotropic and polytropic retrovirus receptor 1 (XPR1) maintains the intracellular P\textsubscript{i} concentration, and this process is stimulated by the binding of IP\textsubscript{7} and IP\textsubscript{8} to the SPX domain of this P\textsubscript{i} exporter [200,201]. (c) In addition to stimulating IP\textsubscript{7} and IP\textsubscript{8} synthesis, P\textsubscript{i} can also stimulate ATP flux by serving as a substrate for ATP synthesis at complex V of the respiratory chain in the mitochondria and by stimulating the respiratory chain directly [216,217]. ATP inhibits the AMP-activated protein kinase (AMPK) pathway, while AMP and ADP activate it [218,219]. (d) PIT1 and PIT2, similar to Npt2b, function as P\textsubscript{i} transporters, which raise intracellular P\textsubscript{i}. PIT1 and PIT2 also heterodimerize in response to P\textsubscript{i} and activate the extracellular signal-regulated kinases 1 and 2 (ERK1/2) pathway transport-independently [204,220]. (e) The binding of nanohydroxyapatite (nHAp) crystals to the cell surface may bridge PIT1 and FGFR1 [206]. FGFR1 activates the AKT, protein kinase C (PKC), and ERK1/2 pathways. (f) In addition to PIT1, PIT2 and FGFR1, the calcium-sensing receptor (CASR) may also function as an extracellular P\textsubscript{i} sensor, at least in parathyroid cells [196,197]. P\textsubscript{i} acts at arginine residue 62 of the CASR as a non-competitive antagonist [196,197], thereby inhibiting the inhibitory G protein G\textsubscript{i} [221]. Through the actions of AMPK, AKT, PKC, ERK1/2, and G protein, extracellular P\textsubscript{i} can regulate gene transcription, such as the expression of osteopontin in bone cells [18,206,222,223] and vascular smooth muscle cells [224,225]. P\textsubscript{i}, inorganic phosphate. P\textsubscript{e}, extracellular P\textsubscript{i}. P\textsubscript{i}, intracellular P\textsubscript{i}. Na, sodium. NPT2b, sodium-dependent phosphate transport protein 2b. IP\textsubscript{6}K1/2, inositol hexakisphosphate kinases 1 and -2. IP\textsubscript{6}, inositol hexakisphosphate. IP\textsubscript{7}, 5-diphosphoinositol 1,2,3,4,6-pentakisphosphate. IP\textsubscript{8}, 1,5-bis(diphosphoinositol 1,2,3,4-tetrasphosphate. XPR1, xenotropic and polytropic retrovirus receptor 1. SPX, a domain of XPR1. PIC, mitochondrial phosphate carrier. ADP, adenosine diphosphate. ATP, adenosine triphosphate. AMPK, adenosine monophosphate-activated protein kinase. PIT1, type III sodium-dependent P\textsubscript{i} transporter 1. PIT2, type III sodium-dependent P\textsubscript{i} transporter 2. nHAp, nanohydroxyapatite. FGFR1c, fibroblast growth factor receptor 1 isoform c. FRS2, FGFR substrate 2. PLC\textsubscript{γ1}, phospholipase C gamma isoform. SOS, son of sevenless. GRB2, growth factor receptor bound protein 2. ERK1/2, extracellular signal-regulated kinases 1 and 2. AKT, protein kinase B. PKC, protein kinase C. CASR, calcium-sensing receptor. G\textsubscript{i}, inhibitory G protein. (I, II, III, IV, V), complexes I-V of the mitochondrial respiratory chain.

Since the cellular uptake of P\textsubscript{i} lowers extracellular P\textsubscript{i}, the sensing of intracellular P\textsubscript{i} (metabolic P\textsubscript{i} sensing) is likely independent of extracellular P\textsubscript{i} sensing (endocrine P\textsubscript{i} sensing). However, it is possible that endocrine sensing activates intracellular P\textsubscript{i} sensing pathways in endocrine cells.

7. Importance of Dietary Phosphorus for Bone Health

7.1. General Importance of Phosphate for Bone Health

P\textsubscript{i} is required for proper plate growth and bone development, and along with calcium, it comprises the hydroxyapatite that is deposited during mineralization of the vertebrate skeleton. As a result, P\textsubscript{i} is critical for the mineralization process (particularly during the growth spurt at puberty [226]), to maintain bone strength after the closure of the epiphyses [227], and during fracture repair and remodeling [228]. The process of matrix mineralization requires the secretion of matrix vesicles (MVs) by osteoblasts and hypertrophic chondrocytes [229,230]. The phosphatase PHOSPHO1 can liberate P\textsubscript{i} from phosphocholine and other lipids in the MV membrane [21]. P\textsubscript{i} is also thought to be imported into the MVs via PIT1 [21]. MVs induce hydroxyapatite crystal formation [231]. In the presence of sufficient concentrations of extracellular calcium and P\textsubscript{i}, these crystals continue to grow after the dissolution of the MV membrane [231]. The ambient extracellular P\textsubscript{i} concentration in bone is maintained by tissue non-specific alkaline phosphatase (TNAP), which is abundant in MVs [21]. TNAP cleaves pyrophosphate (PP\textsubscript{i}) and other organic bisphosphonates, which generates two P\textsubscript{i} molecules [21]. A high P\textsubscript{i}/PP\textsubscript{i} ratio is generally thought to favor mineralization [232–234]. Clinically relevant hypophosphatemic individuals exhibit an increased activity of alkaline phosphatase [22,235]. This allows bone-specific alkaline phosphatase activity to serve as a marker of P\textsubscript{i} homeostasis in the bone [22,235].
Dietary phosphorus deprivation impairs cell metabolism and causes skeletal demineralization to occur. Moreover, secondary changes due to the adaptive hormonal response (i.e., upregulation of calcitriol, suppression of PTH and FGF23) can be observed. The main process that stimulates bone resorption is calcitriol-mediated activation of osteoclasts through the receptor activator of NF-kB (RANK)–RANK Ligand (RANKL) signaling, as described in more detail below [236,237]. This process is more important with prolonged dietary phosphorus deficiency and can cause rickets and stunted growth in children and osteomalacia in adults [90,91]. In addition, there are several acute effects of phosphorus deprivation, which will be described separately for each tissue below.

Similarly, high dietary phosphorus intake adversely affects bone health. Firstly, high dietary phosphorus can reduce calcium absorption and serum calcium concentrations through the formation of insoluble calcium–P₃ complexes [238]. This reduction in serum calcium causes reduced calcium binding to CASR and thereby reduces the inhibition of CASR [239]. Recently, it was shown that P₃ can bind and directly inhibit CASR [196,197]. As a result, secondary hyperparathyroidism develops, which in turn stimulates bone resorption [197,239,240]. Additionally, P₃ has direct effects on bone cells: for example, P₃ stimulates the expression of bone matrix protein osteopontin, which is a mineralization inhibitor [18,222]. Thereby, high dietary phosphorus is associated with increased risk for bone fractures, as shown in a study of 2420 Brazilian individuals in whom every 100 mg of dietary phosphorus intake increased the risk of fracture by 9% [241]. Recent insights suggest that FGF23 (whose levels are determined by P₃ concentrations) may impair bone matrix mineralization independently of calcium by transcriptionally suppressing TNAP (although this effect also suppresses OPN) [242,243]. High phosphate containing soft drinks may finally affect dental mineralization, as discussed later in this review.

7.2. Role of Phosphate in Chondrocytes

Chondrocytes produce and maintain the extracellular matrix of joint cartilage and permit the longitudinal growth of long bones through endochondral ossification. P₃ is essential for normal hypertrophic differentiation and apoptosis, which was shown in several primary [244–246] and stable chondrocytic cell lines [247,248]. Hypertrophic differentiation and apoptosis require the activation of ERK1/2 and the mitochondrial–caspase-9 pathway [244]. These processes are blocked by ablation or pharmacological inhibition of the PIT1 transporter or of the mitogen-activated protein kinase kinase 1 [246,248]. In addition to the ERK pathway, P₃ induces nitrate or nitrite, which stimulates nitric oxide synthase (NOS) production and, in turn, stimulates chondrocyte apoptosis [248]. Furthermore, the acute chondrocyte-specific deletion of Pit1 in mice results in pronounced cell death in the first two postnatal days, possibly owing to P₃ transport-independent ER stress [214]. Chondrocytes might also regulate systemic P₃ homeostasis by secreting FGF23 [244], but it is unknown whether this is under the feedback control of P₃.

In summary, P₃ stimulates hypertrophic differentiation and apoptosis in chondrocytes via PIT1, ERK1 and ERK2, and possibly via NOS, which is necessary for normal bone growth and possibly articular cartilage function.

7.3. Role of Phosphate in Osteoblasts and Osteocytes

In the vertebrate skeleton, osteoblasts and osteocytes are responsible for the synthesis of the bone matrix [249]. The bone matrix is composed of type 1 collagen, non-collagenous proteins (such as osteocalcin) and small integrin-binding ligand, N-linked glycoprotein (SIBLING) proteins [including dentin matrix acidic phosphoprotein 1 (DMP1), matrix extracellular phosphoglycoprotein (MEPE), and OPN] [250]. When osteoblasts become buried in the bone matrix, they undergo terminal differentiation into osteocytes, which serve as mechanosensors and secrete endocrine and paracrine factors to maintain skeletal homeostasis [251]. P₃ may stimulate osteoblast proliferation and differentiation, as it induces the expression of genes important for cell proliferation, energy metabolism, and mineralization in osteoblast-like cells [47,252,253]. Similarly, P₃ might also stimulate insulin-like growth factor 1 expression in the mouse-derived osteoblast cell line MC3T3-E1, which enhances osteoblast proliferation in an autocrine fashion [223,254]. In MC3T3 cells and primary
murine calvaria-derived osteoblasts, P; induces the expression of Fos-related antigen 1, Opn, and matrix Gla protein (which are genes required for mineralization) [223,255]. This process is dependent on ERK1 and ERK2 [223,255], further supporting the role of P; in mineralization.

P; stimulates osteocyte maturation and matrix formation in the osteocyte lacuna. This process can be modeled in IDG-SW3 osteocyte-like murine cells in vitro, in which 10 mM P; and 10 mM calcitriol induces the gene expression of Galnt3, Dmp1, phosphate-regulating endopeptidase homolog, X-linked, ectonucleotide pyrophosphatase-phosphodiesterase family member 1, and Mepe [256]. Additionally, P; (and calcitriol and PTH) cause osteocytes to secrete FGF23 to regulate systemic P; homeostasis [257–259].

In summary, P; stimulates the differentiation of osteoblasts and osteocytes, matrix maturation, and bone formation. These processes involve the function of P; transporters and ERK1/2 signaling in vitro. The mild bone and mineral metabolism phenotypes of the global Pit1 and Pit2 null mice suggest a high degree of redundancy of these generally co-expressed transporters [46,260]. Bone-specific ablation of Pit1 and Pit2 (individually and in combination) in mice might be required to shed light on their metabolic and endocrine functions.

7.4. Role of Phosphate in Osteoclasts and Bone Resorption

Osteoclasts are large, multinucleated cells derived from the monocyte lineage that are responsible for bone resorption [261], which is necessary for the remodeling and repair of the skeleton. Osteoclasts express NPT2A, PIT1, and PIT2 [262]. A concentration of 4 mM extracellular P; inhibits osteoclast-like cell formation in mouse bone marrow cells [237]. This extracellular P; concentration similarly decreases the number and area of resorption pits formed by mature rat osteoclasts on sperm whale dentine slices, which is a common assay for osteoclast function [263]. This observation presumably reflects a feedback mechanism to limit the degradation of hydroxyapatite. This feedback mechanism might involve the NPT2A-dependent inhibition of RANK–RANKL signaling, the inhibition of osteoclast growth by P; [236], and the suppression of microRNA 223 expression (which was reported in the pre-osteoclast RAW264.7 cell line [264] and in Npt2a-null mice [265]). P; reduces the gene expression of RANKL in osteoclast lineage cells, which results in the suppression of RANK in osteoclasts and the inhibition of osteoclastogenesis and bone resorption [237]. However, some P; is required for normal osteoclast function. Both WT mice fed a low P; diet and Hyp mice (a murine model of X-linked hypophosphatemic rickets, XLH) exhibited decreased osteoclast numbers in osteoclast-like cells derived from bone marrow cells compared with WT mice fed normal P; diets [266]. This defect was reversed by a high P; diet [266]. PFA, an inhibitor of Na+-P; cotransporters, reduces bone resorption in cultured osteoclasts, possibly by inhibiting ATP production (for which uptake of extracellular P; is required) [267]. Additionally, as a result of increased mitochondrial respiration, extracellular P; stimulates the production of reactive oxygen species (ROS), which are signaling factors necessary for osteoclastogenesis and which stimulate bone resorption in RAW264.7 osteoclasts [268]. Furthermore, the generation of ROS increases osteoclast function and survival, which indicates that P; is required for the normal function of osteoclast cells [268].

In summary, osteoclasts express NPT2A, PIT1, and PIT2 transporters. High P; levels limit the survival and differentiation of osteoclasts, which might provide a mechanism of feedback inhibition during bone resorption, which is a process that releases large quantities of P; However, some P; seems to be required for normal osteoclast function.

8. Importance of Dietary Phosphorus for Teeth (or Dental Health)

The teeth are comprised of an enamel that is formed by epithelial cells (called amelobasts) and dentin [269] that is formed by mesenchymal cells (called odontoblasts); both tissues surround the dental pulp, which is a soft connective tissue that contains blood vessels and nerve fibers [269]. Cementum, periodontal ligament, and alveolar bone connect the teeth to the jaw [269]. Similar to the bone, hydroxyapatite is a major component of enamel and dentin. Different from the hydroxyapatite in the skeleton, in teeth, it is fluorinated, contains metal cation substitutions for calcium, and
carbonate substitutions for P [270]. Fluorapatite (FAP, \(\text{Ca}_10(\text{PO}_4)_6\text{F}_2\)) is less soluble than hydroxyapatite [270], which explains why fluoride is an effective agent to improve dental health (though only within a narrow window of 3–4 mg for adults, whereas excessive levels lead to dental fluorosis) [270,271].

Adequate nutritional quantities of calcium and phosphorus are important for dental mineralization. A dietary ratio of 4–5 was necessary for normal bone and dentin calcification in rats that were observed from 23 to 70 days of age [272,273]. Calcium and phosphorus deficiency disturb calcification of the growing dentin and alveolar bone in rats [272,274], although enamel formation and calcification are not impaired [272]. In young rats, dietary phosphorus deficiency in the absence of vitamin D reduces overall incisor tooth mass [273,275], which is not observed in adult rats [273]. Moreover, histomorphometric evaluation shows increased levels of alveolar bone resorption in response to calcium and phosphorus deficiency [274,276].

Excessive phosphorus intake, on the other hand, also produces an inappropriate Ca/P ratio, and it was shown in degus (Octodon degu) to increase amelogenesis and dentinogenesis [277]. The results were a thicker enamel layer, the formation of enamel pearls, and altered dentin structure [277]. The authors also observed enamel depigmentation and hypoplasia, as well as a loss of the superficial enamel layer and a pitted appearance of the enamel layer [277]. This effect on enamel formation might occur during the secretory stage [277]. Moreover, a 1.3-fold increase in dental decay was observed in children consuming diet sodas, which can contain high concentrations of P [278]. Additionally, high phosphorus exposure infamously led to osteonecrosis of the jaw (a condition commonly known as “phossy jaw”) in 19th-century matchmakers [279]. Specifically, these workers developed gingivitis and sequestration of the alveolar crest bone, and they experienced osteonecrosis of the mandibular and maxillary bones [279]. Though “phossy jaw” is not a clinical issue in the present, there is concern that “bisphossy jaw” may be its modern-day equivalent. Oral bisphosphonates often treat disorders such as osteoporosis, but these medications carry a concerning side effect of maxillary and mandibular bone necrosis and sequestration [280]. However, the mechanism through which oral bisphosphonates cause osteonecrosis is unknown [279].

Role of Phosphate in the Tooth

Among the currently known P transporters (Slc34a1, Slc34a2, Slc34a3, Slc20a1, Slc20a2, and Xpr1), SLC20A2/PIT2 is the most highly expressed in teeth [281]. However, knockout mouse models showed that no single transporter is essential for initiation of the mineralization process [281]. PIT1 is expressed in ameloblasts and odontoblasts, while PIT2 is expressed in the subodontoblastic cell layer and the stratum intermedium of ameloblasts [281,282]. PIT2 appears to be involved during the mineralization of dentin, as suggested by the dentin dysplasia described in the global Pit2 knockout [281]. Slc34a1/Npt2a and Slc34a2/Npt2b are expressed in the MRPC-1 rat odontoblast-like mineralizing pulpal cell line [283,284]. Slc34a2/Npt2b is negligibly expressed in ameloblasts during the secretory stage, but it is significantly upregulated in the maturation stage [281,284,285]. However, the role of Npt2b in tooth development and mineralization is unknown [284].

Individuals with XLH (a hypophosphatemic condition characterized by FGF23 overproduction [72]) exhibit various dental abnormalities. These abnormalities include the abnormal mineralization of dentin and increased pulp chambers (resulting in dental fractures), less abundant cementum (resulting in impaired tooth attachment), and an increased risk of periodontal disease and the development of dental abscesses [21,286,287]. Enamel is largely unaffected in XLH [288]. Whether these changes are caused by hypophosphatemia alone or in conjunction with FGF23 excess remains to be shown.
9. Importance of Dietary Phosphorus for Cardiovascular Health

9.1. Role of Phosphate in Cardiac Muscle Function

Hypophosphatemia causes skeletal and cardiac myopathy by reducing intramuscular ATP synthesis and decreasing 2,3-bisphosphoglycerate (2,3-BPG) in erythrocytes (which reduces skeletal muscle oxygenation) [216,217,289,290]. Additionally, ventricular arrhythmia can occur in the context of acute myocardial infarction [291]. These hypophosphatemic effects are largely reversible but can lead to rhabdomyolysis, heart failure, and death in some cases [92,93,289,292–295].

On the other hand, hyperphosphatemia is associated with myocardial hypertrophy in rats [296] and humans with CKD [297–299]. High serum Pi is also associated with increased cardiovascular morbidity and mortality in these patients [299,300]. Finally, hyperphosphatemia can often cause vascular calcification in CKD, and the extent and histoanatomic type of calcification predict subsequent mortality [301,302].

These effects are thought to be mediated by FGF23, which causes endothelial dysfunction and increases arterial stiffness [303], activates the renin-angiotensin system [304], and causes inflammation [305], vascular calcification [306], and left ventricular hypertrophy (LVH) [307,308]. For an in-depth examination of the link between FGF23 and cardiovascular disease, see the excellent review by Stohr et al. [308]. Additionally, secondary hyperparathyroidism is associated with heart failure [309], hypertension [310], LVH [311,312], arrhythmias [312], and calcific valvular disease [311,312].

9.2. Role of Phosphate in Vascular Health

Hyperphosphatemia causes vascular smooth muscle cell (VSMC) apoptosis, osteogenic transdifferentiation, and vascular calcification [313–315]. VSMC apoptosis requires the downregulation of growth-arrest specific gene 6 and its receptor, Axl [316]. This downregulation reduces phosphatidylinositol 3-kinase-mediated phosphorylation of protein kinase B, which is also known as AKT [316]. As a result, Bcl2 (an anti-apoptotic protein) is inactivated, and Bad (a pro-apoptotic mediator) and caspase 3 are activated [316]. The osteogenic transdifferentiation of VSMCs and vascular calcification require PIT1 and PIT2 and the activation of ERK1/2 in a transport-independent manner [224,225,317]. Additionally, the activation of WNT–β-catenin–runt-related transcription factor 2 signaling (an anabolic signaling pathway important for the function of osteoblasts and osteocytes) by Pi can be observed [46,318–321]. Conversely, transdifferentiation is inhibited by secreted frizzled-related protein 5 [46,322]. PIT2 also mediates Pi uptake into the microglia, which inhibits vascular calcification in the basal ganglia in an interplay with the Pi exporter XPR1 [187,323]. Additional sodium-independent transport systems for the intake and efflux of Pi may exist in VSMCs, which have not been well-characterized [198,324,325].

High dietary phosphorus finally reduces endothelium-dependent vasodilation in vitro and was shown to reduce flow-mediated vasodilation in healthy men [326]. In a study of normal U.S. adults, Kendrick et al. showed that high-normal levels of serum Pi are associated with a high ankle-brachial pressure index, which is a marker for arterial stiffness [327]. Thereby, high dietary phosphorus may acutely increase the risk of cardiovascular mortality [326].

9.3. Role of Phosphate in Erythrocyte Function

Pi affects erythrocyte function directly [289,290] and indirectly via FGF23 [328]. Hypophosphatemia reduces the concentration of 2,3-BPG in erythrocytes, since Pi is required for the synthesis of ATP and thus for the glycolytic synthesis of the 2,3-BPG precursor, 1,3-bisphosphoglycerate [290]. For example, Lichtman et al. found a 45% reduction of 2,3-BPG in the erythrocytes of patients with parenteral nutrition-induced hypophosphatemia [290]. Reduced concentrations of 2,3-BPG shift the oxyhemoglobin dissociation curve to the left (increasing hemoglobin affinity for O2), and thereby hypophosphatemia can cause tissue hypoxia [289].
Blood Pi may indirectly affect hematopoiesis by regulating FGF23. FGF23 may stimulate hematopoiesis, as suggested by low erythrocyte counts found in FGF23 null mice [329]. In turn, erythropoietin may stimulate the synthesis and secretion of FGF23 by myeloid lineage LSK cells in the hematopoietic bone marrow [330].

10. Importance of Dietary Phosphorus for Skeletal Muscle Health

Similar to cardiac muscle, Pi is essential in skeletal muscle as a substrate for ATP and CrP synthesis [331,332]. Hypophosphatemia causes a reduction in ATP flux (VATP) in mouse models [217]. Similarly, the ablation of Pit1 and Pit2 in mice is post-natally lethal due to a generalized skeletal muscle myopathy [333]. Likewise, patients with hypophosphatemia develop myopathy in addition to rickets and osteomalacia [332]. Moreover, iatrogenic Pi depletion in patients with chronic renal failure results in proximal myopathy [8], and rhabdomyolysis can occur with severe hypophosphatemia superimposed on simple phosphorus deficiency [92,292,334].

On the other hand, hyperphosphatemia may contribute to the development of muscle weakness and frailty, at least in patients with CKD [335,336]. High-medium Pi concentrations cause protein loss in myotubes from rat L6 cells and stimulate autophagy, resulting in myotube atrophy [337].

Role of Phosphate in Skeletal Muscle

We recently showed that hypophosphatemia causes a reduction in VATP, as shown by a 50% reduction in WT mice fed a low Pi diet [217]. VATP in these mice normalized after intravenous Pi supplementation [217]. Likewise, the simultaneous conditional deletion of all four Pit1/2 alleles in mouse skeletal muscle causes muscular atrophy and myofiber degeneration by post-natal day 10 (P10) [333]. These mice die by P13 [333]. Similarly, the ablation of three of four Pit1/2 alleles in skeletal muscle reduced the running ability of these mice as measured by wheel turns/day [333]. This result suggested that the loss of Pit1/2 causes impaired muscle function [333]. Studies in C2C12 myocyte cultures suggest that Pi acts at complex V of the respiratory chain as a substrate for ATP synthesis during oxidative phosphorylation [216,217].

Little is known so far about the molecular mechanism, but Pi appears to maintain cytochrome b oxidation and cytochrome c reduction [338] and to stimulate the activity of several Krebs cycle dehydrogenases: 2-oxoglutarate dehydrogenase, isocitrate dehydrogenase, and malate dehydrogenase [339–342]. This action of Pi increases the concentrations of mitochondrial electron donors (FADH, NADH, and NADPH) to fuel the electron transport chain. Additionally, Pi is an important cofactor for glyceraldehyde 3-phosphate dehydrogenase, an important rate-limiting glycolytic enzyme that generates NADH [343,344].

The type III Na+/Pi cotransporters Pit1 and Pit2 mediate Pi uptake into the sarcoplasma [345–348], and it is then transported into the mitochondria by PIC [21] and the mitochondrial dicarboxylate carrier [349,350]. PIC is also a component of the mPTP, which regulates calcium transport into the mitochondrial matrix [213]. Human LOF mutations of SLC25A3 [183,184,213,351] or cardiac-specific deletion in mice [352] cause cardiomyopathy.

However, the mechanism whereby blood Pi, PIT1, and PIT2 modify muscle ATP synthesis is not clear, since the hydrolysis of ATP and CrP during exercise may generate sufficient Pi inside myofibers for ATP synthesis. In fact, the sarcoplasmic buildup of Pi may be an important cause of muscle fatigue [353]. Sarcoplasmic Pi can increase from 5–30 mM following intense exercise, which has been shown to reduce peak force by decreasing force per actin-myosin bridge, by increasing the number of low-force bridges in skeletal muscle, by decreasing cytosolic ionized calcium, and by causing calcium–Pi precipitations in the sarcoplasmic reticulum [46,353–357].

Since the activation of the ERK1/2 pathway [194,204] is necessary for normal neuromuscular junctions [358,359], therefore, an intriguing possibility is that the transport-independent signaling functions of PIT1/2 are important for skeletal muscle function [204,333].

In summary, Pi is required to maintain muscle function, but excess Pi leads to calcification (which is best documented in vascular smooth musculature) and skeletal muscle fatigue. This process may involve the functions of the PIT1/2 transporters and of ERK1/2 signaling.
11. Importance of Dietary Phosphorus for Healthy Aging

As examined in the previous chapters of this review, concentrations of P that are either too high or too low both have detrimental health effects. Since P depletion in terrestrial animals and humans is rare due to the ubiquitous nature of phosphorus in the diet (particularly in processed foods [96] and when used in high doses to treat hypophosphatemic disorders [360]), we will focus our discussion of longevity on the effects of high dietary phosphorus.

11.1. High Dietary Phosphate Reduces Longevity in Lower Species

We previously used Drosophila melanogaster to study the effects of high dietary P on longevity. Supplementation of standard medium (SM) with 30 mM P reduced fly lifespan to 38 ± 2.4 days [361]. In contrast, control yae flies had a median lifespan of 42 ± 0.8 days, and flies cultured in SM + 30 mM sodium sulfate had a median lifespan of 44 ± 0.8 days [361]. Median lifespan increased to 49 ± 1.9 days and 47 ± 1.8 days, respectively, by inhibiting P intake through the addition of either sevelamer (which blocks P absorption) or PFA (which inhibits the cellular uptake of P) to the SM [361]. Finally, more recent findings show that PFA and the ablation of MFS2 (a P transporter in fly Malphigian tubules that functions in P excretion) increase blood P, decrease the formation of Malphigian tubule calcium-P; stones, and increase lifespan in Drosophila [362]. These results suggest that the excretion of P and the formation of Malpighian tubule stones reduce the longevity of flies cultured on a high P medium [362].

11.2. High Dietary Phosphorus Reduces Longevity in Higher Species and Humans

Similarly, in higher species such as mice, high dietary phosphorus negatively affects longevity. P; loading in uremic rats dose-dependently induces inflammation in the aorta, heart, and kidneys [363]. Furthermore, Klotho null mice have severe hyperphosphatemia [364,365]. These mice die prematurely due to vascular and renal calcification, as well as atrophy of the skin, muscle, intestinal, and gonadal tissues [364,365].

The aging-like syndrome of Klotho−/− mice is ameliorated when serum P; levels are normalized in Npt2a+/Klotho−/− double-knockout mice, and it is induced again by placing these double-knockout mice on a high P; diet [364,365]. This dietary P; toxicity in Npt2a+ and Klotho−/− double-knockout mice very much resembles the potential of dietary P; to modify mortality in CKD patients, as discussed above [363,366–368].

In addition, P; has recently been implicated in cancer aggressiveness [369]. SLC20A1 may be overexpressed in tongue tumors [370,371], and Npt2b expression is increased in lung cancers [369,371]. Furthermore, high dietary P; stimulates the AKT-mammalian target of rapamycin regulatory pathway, leading to higher lung cancer aggressiveness in K-rasA1 mice [369,371]. The knockdown of Npt2b with siRNA was shown to decrease the number and size of lung tumors in this mouse model, suggesting that the regulation of P; consumption through NPT2b knockdown may be a possible treatment for lung cancer [371,372]. Similarly, a high P; concentration in the tumor microenvironment has been identified as a marker for tumor progression in mouse mammary gland tumors [371,373]. For a more detailed overview of the role of P; transporters in cancer and tumor biology, refer to the excellent review by Lacerda-Abreu et al. [371].

Finally, high dietary phosphorus increases fracture risk [241], which has implications for lifespan, since excess mortality for five years following a proximal non-hip or lower leg fragility fracture, and ≥10 years following a hip fracture, have been reported [374]. These results together further support a role for excess phosphorus in reducing longevity, even in humans who have normal kidney function.
12. Conclusions

Phosphorus is an important nutrient in our diet. \( P \) serves crucial functions as an important physiologic buffer, as a substrate for critical cellular functions, and along with calcium as a component of the bone mineral in the skeleton. \( P \) is absorbed in the small intestine via a paracellular route of passive diffusion or a transcellular route via \( P \) transporters such as NPT2b. Interruption of these absorption processes can result in hypophosphatemia. Since phosphorus has become abundant in Western diets—where it often supplements prepared foods—it is also important to consider that excess levels of dietary phosphorus have adverse health effects. High dietary phosphorus has been implicated in several processes related to accelerated aging; for instance, increased fracture risks, cancer proliferation, cardiac and skeletal muscle dysfunction, and vascular calcification. Currently, it is estimated that dietary phosphorus exceeds the RDA by 1.5–2X, which is of particular concern for individuals with cardiovascular diseases or CKD. A better declaration and regulation of \( P \) additives in the processed foods industries, and more clinical research, is clearly needed to understand how to optimize nutritional phosphorus best while avoiding toxic side effects. Of particular interest for the latter are agents that target NPT2b to block intestinal \( P \) absorption, agents that regulate PIT1/2 (and possibly IP7/IP8/XPR1) to optimize cellular \( P \) homeostasis, and agents that improve renal \( P \) excretion via NPT2a and NPT2c. Furthermore, the contributions of type I (SLC17) and type III (SLC20) \( P \) transporters, and the molecular basis for paracellular diffusion in the intestine and proximal renal tubules, are still largely unknown. Thus, research into these pathways could provide novel targets for the development of therapeutics that improve the outcomes of individuals with cardiovascular diseases or CKD.

Key Points

Phosphorus is abundant in Western diets, and understanding endocrine and metabolic \( P \) sensing is essential to understand human disorders. Both high and low dietary phosphorus can cause adverse health effects and impair longevity, and it may be important to consider implementing phosphorus analysis as a routine measurement in clinical practice. Declaration of \( P \) additive use by the food industry may be helpful, since currently dietary phosphorus intake routinely exceeds the RDA.

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Glossary

| Key Term/Abbreviation | Definition |
|-----------------------|------------|
| I, II, III, IV, V     | Complexes I-V of the mitochondrial respiratory chain |
| 1,25-dihydroxyvitamin D | 1,25(OH)\(_2\)D |
| 2′-PP                 | 2′-Phosphophloretin |
| 2,3-BPG               | 2,3-Bisphosphoglycerate |
| AD                    | Autosomal dominant |
| AKT                   | Protein kinase B |
| Alp                   | Alkaline phosphatase |
| AMP                   | Adenosine monophosphate |
| Term            | Definition                                                   |
|-----------------|--------------------------------------------------------------|
| AMPK            | AMP-activated protein kinase                                  |
| AR              | Autosomal recessive                                          |
| ATP             | Adenosine triphosphate                                       |
| BBMV            | Brush border membrane vesicle                                |
| BPG             | Bisphosphoglycerate                                          |
| CASR            | Calcium-sensing receptor                                     |
| CKD             | Chronic kidney disease                                      |
| c-myb           | V-myb avian myeloblastosis viral oncogene homolog             |
| CrP             | Creatine phosphate                                           |
| CYP24A1         | The vitamin D 24-hydroxylase                                 |
| CYP27B1         | The vitamin D 1-α hydroxylase                                |
| DMP1            | Dentin matrix acidic phosphoprotein 1                        |
| DNA             | Deoxyribonucleic acid                                        |
| EGF             | Epidermal growth factor                                      |
| EGR1            | Early growth response 1                                       |
| Enpp1           | Ectonucleotide pyrophosphatase-phosphodiesterase family member 1 |
| ER              | Endoplasmic reticulum                                        |
| ERK1            | Extracellular signal-regulated kinase 1                      |
| ERK2            | Extracellular signal-regulated kinase 2                      |
| Etv5            | ETS variant 5                                                |
| FADH            | Flavin adenine dinucleotide                                  |
| FAM20c          | Golgi-associated secretory pathway kinase                    |
| FAP             | Fluorapatite mineral                                         |
| FGF             | Fibroblast growth factor                                     |
| FGF23           | Fibroblast growth factor 23                                  |
| FGFR1           | Fibroblast growth factor receptor 1                          |
| FGFR1c          | FGFR1 isoform c                                              |
| FRS2            | FGFR substrate 2                                             |
| GALNT3          | Polypeptide N-Acetylgalactosaminyltransferase 3               |
| GC              | Glucocorticoid                                               |
| GCMB            | Glial cell missing gene                                       |
| GI              | Gastrointestinal                                             |
| GNAS            | Guanine nucleotide-binding protein, alpha stimulating         |
| GRB2            | Growth factor receptor bound protein 2                       |
| HVDDR           | Hereditary 1,25(OH)_2D-resistant rickets                     |
| IBGC            | Idiopathic basal ganglia calcification                       |
| IC              | Intracellular                                                |
| ICF             | Intracellular fluid                                          |
| IU              | International units                                          |
| IP6K1           | Inositol hexakisphosphate kinase 1                           |
| IP6K2           | Inositol hexakisphosphate kinase 2                           |
| IP6             | Inositol hexakisphosphate                                    |
| IP7             | 5-diphosphoinositol 1,2,3,4,6-pentakisphosphate               |
| IP8             | 1,5-bisdiphosphoinositol 1,2,3,4-tetrakisphosphate           |
| IV              | Intravenous                                                  |
| JAM2            | Junctional adhesion molecule 2                               |
| KL              | α-Klotho                                                     |
| Km              | Michaelis-Menten affinity constant                            |
| LOF             | Loss of function                                             |
| LVH             | Left ventricular hypertrophy                                 |
| MAPK            | Mitogen-activated protein kinase                              |
| MAPK1           | Mitogen-activated protein kinase 1                           |
| MAPK3           | Mitogen-activated protein kinase 3                           |
| MEPE            | Matrix extracellular phosphoglycoprotein                     |
| mPTP            | Mitochondrial permeability transition pore                   |
| MV              | Matrix vesicle                                               |
| MYORG           | Myogenesis regulating glycosidase                            |
| Abbreviation | Description |
|--------------|-------------|
| Na⁺          | Sodium ion  |
| NAD          | Nicotinamide adenine dinucleotide |
| NADH         | Reduced nicotinamide adenine dinucleotide |
| NADPH        | Reduced nicotinamide adenine dinucleotide phosphate |
| NF-κB        | Nuclear factor kappa-light-chain-enhancer of activated B cells |
| NHE3         | Sodium/hydrogen exchanger isoform 3 |
| NHERF-1      | Na⁺/H⁺ exchanger regulatory factor |
| NOS          | Nitric oxide synthase |
| NPT1         | Sodium-dependent phosphate transport protein 1 |
| NPT2a        | Sodium-dependent phosphate transport protein 2a |
| NPT2b        | Sodium-dependent phosphate transport protein 2b |
| NPT2c        | Sodium-dependent phosphate transport protein 2c |
| NVU          | Neurovascular unit |
| OPG          | Osteoprotegerin |
| OPN          | Osteopontin |
| P10          | Postnatal day 10 |
| PDGFB        | Platelet-derived growth factor subunit B |
| PDGFRB       | Platelet-derived growth factor receptor beta |
| PFA          | Phosphonoformate/phosphonoformic acid |
| PFBC         | Primary familial brain calcification |
| Phex         | Phosphate-regulating endopeptidase homolog, X-linked |
| PHOSPHO1     | Phosphoethanolamine/phosphocholine phosphatase 1 |
| Pᵢ           | Inorganic phosphate |
| PIC          | Mitochondrial phosphate carrier |
| P1T1         | Type III sodium-dependent phosphate transporter 1 |
| P1T2         | Type III sodium-dependent phosphate transporter 2 |
| PKA          | Protein kinase A |
| PKC          | Protein kinase C |
| PLCγ         | Phospholipase C gamma isoform |
| PPᵢ          | Pyrophosphate |
| PPIs         | Proton pump inhibitors |
| PTH          | Parathyroid hormone |
| PTHR1        | Parathyroid hormone 1 receptor |
| RANK         | Receptor activator of NF-κB |
| RANKL        | Receptor activator of NF-κB ligand |
| RDA          | Recommended daily allowance |
| RNA          | Ribonucleic acid |
| ROS          | Reactive oxygen species |
| RXR          | Retinoic acid X-receptor |
| SAMD9        | Sterile alpha motif domain containing 9 |
| SIBLING      | Small integrin-binding ligand, N-linked glycoprotein |
| SLC17        | Solute carrier family 17 |
| SLC20A1      | Solute carrier family 20 member 1; gene encoding P1T1 |
| SLC20A2      | Solute carrier family 20 member 2; gene encoding P1T2 |
| SLC25A3      | Solute carrier family 25 member 3; gene encoding P1C |
| SLC34A1      | Solute carrier family 34 member 3; gene encoding NPT2a |
| SLC34A2      | Solute carrier family 34 member 2; gene encoding NPT2b |
| SLC34A3      | Solute carrier family 34 member 3; gene encoding NPT2c |
| SM           | Standard medium |
| SOS          | Son of sevenless |
| SPX          | A protein domain named after SYG1/Pho81/XPR1 proteins |
| TEER         | Transepithelial electrical resistance |
| TNAP         | Tissue non-specific alkaline phosphatase |
| TNFRSF11B    | TNF receptor superfamily member 11B |
| VᵢATP        | ATP flux |
| VDR          | Vitamin D receptor |
| VRE          | Vitamin D-responsive elements |
| VSMC         | Vascular smooth muscle cell |
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