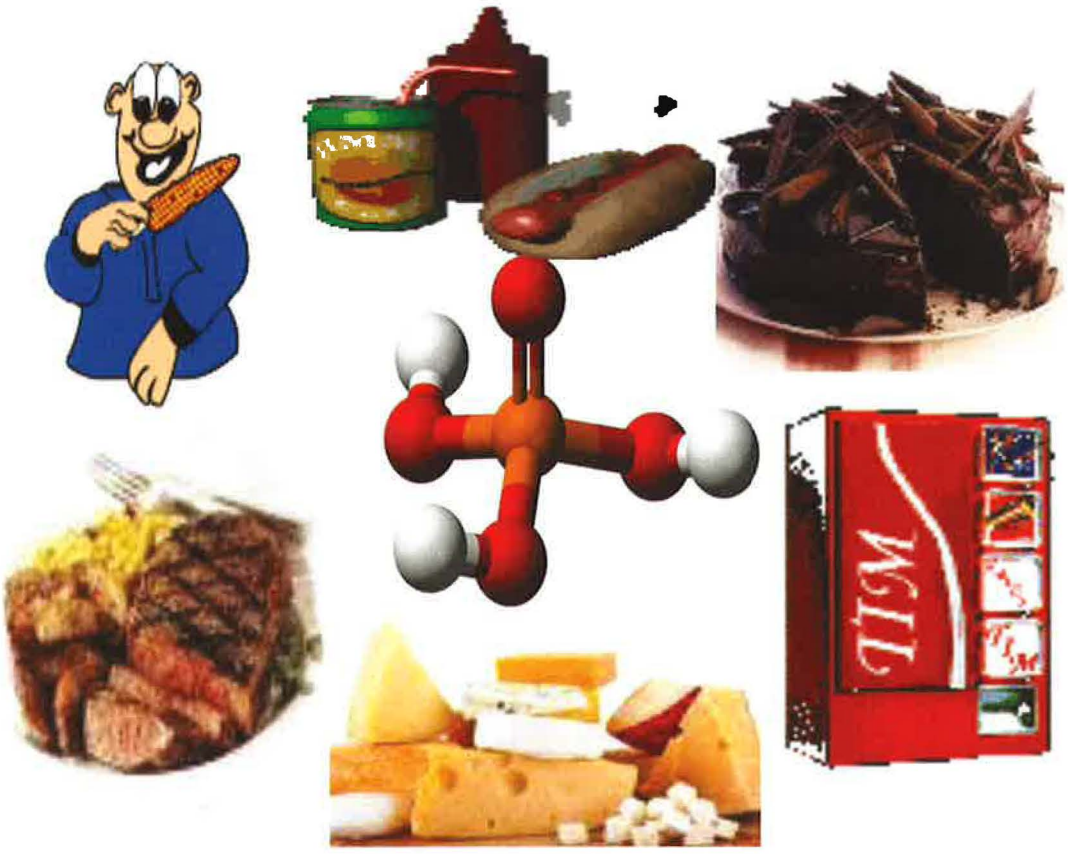
Dietary Phosphate:

Is it toxic?



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This is to acknowledge that Essam Elsayed, MD has disclosed any financial interests or other relationship with commercial concerns related directly or indirectly to this program. Dr Elsayed will not be discussing off-label uses in his presentation.

Research interest:
I am interested in examining effect of obesity, cardiovascular disease and hypertension on the development and progression of chronic kidney disease. Also, in collaboration with mineral metabolism division, we are studying the effect of diet on hypertension and examining the concept of "phosphotoxicity" and the effect of phosphate on oxidative stress measurements in humans.

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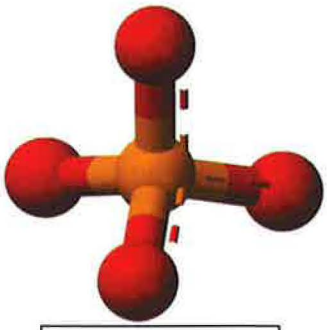
Phosphorus in the human body:

Phosphorus is an essential mineral that is required by every cell in the body for normal function (1). The majority of the phosphorus in the body is found as phosphate (PO_4), which is a salt of phosphoric acid. It is an important and major intracellular anion in mammals with the total body phosphorus in a 70-kg man is about 750 mg. 80-85% of which is in the skeleton as hydroxyapatite and the remaining 15% is in soft tissues (14%) and extracellular fluids (1%). Almost all of the phosphorus found in the extracellular fluid space is in the form of inorganic phosphate. [1] Phosphorus is an element (P), and phosphate is a molecular anion (PO4_3-), part of phosphoric acid (H3PO4). Plasma phosphate mostly is either monovalent or divalent. The latter form is about 80% at a normal plasma ph of 7.4. Normal concentration is 2.5-4.5mg/dL with some diurnal variation in its concentration. Serum levels expressed in milligrams can be converted to millimoles per liter by multiplying by 0.323.



Phosphate Ion

Is phosphate important?

Phosphate is involved in numerous essential biochemical reactions, including cell signaling process and energy metabolism. Phospholipids are essential components of cell membranes. All energy production and storage are dependent on phosphorylated compounds, such as adenosine triphosphate (ATP). of enzymes, hormones, and cell signaling molecules depend on phosphorylation for their activation. Phosphorus also helps to maintain normal acid-base balance in its role as one of the body's most important buffers. Both DNA and RNA, consist of long chains of phosphate-containing molecules. 2,3-diphosphoglycerat hemoglobin and affects oxygen delivery to all the tissues of the body. Abnormal regulation of phosphate homeostasis can cause myopathy, cardiac dysfunctions, hematological abnormalities, and vascular/soft tissue calcifications. [3-5] Theref Understanding the molecular regulation of phosphate homeostasis is very important.

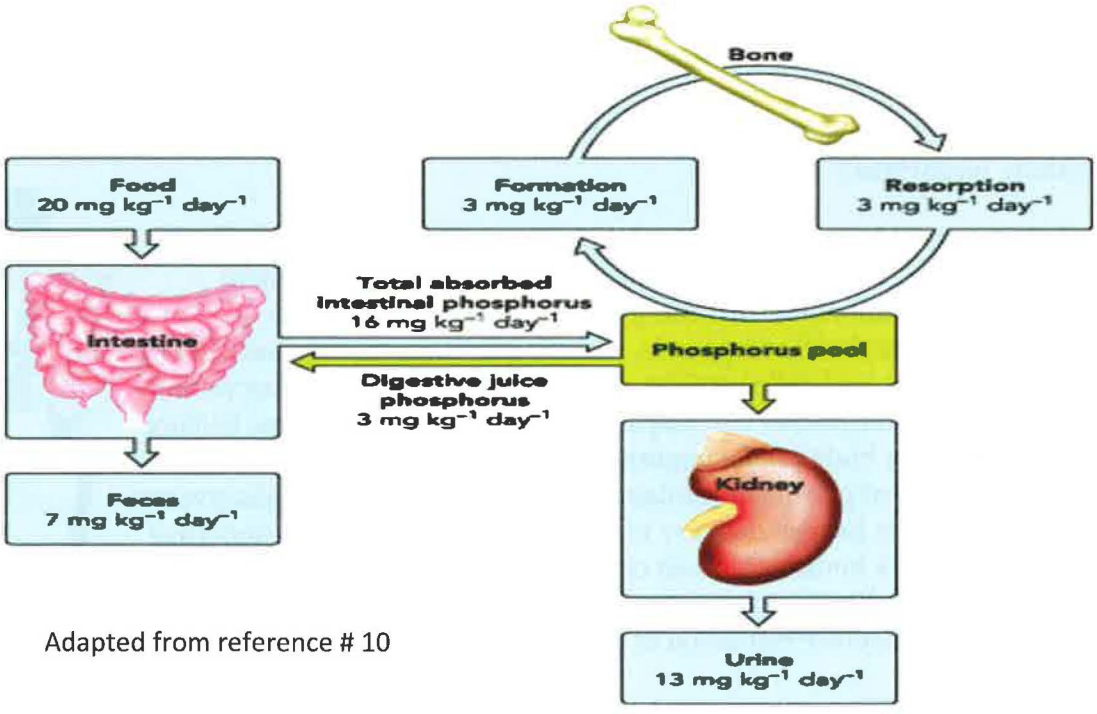
	Phosphorus
Total body content	~700 g
In bone	~80%
In viscera	~10.9%
In skeletal muscle	~9%
In extracellular fluid	~0.1%
Intracellular-to-extracellular ratio	~100:1

Distribution of phosphorus in a healthy 70kg adult

Adapted from reference[5]

Phosphate homeostasis:

Significant amount of intestinal dietary phosphorus absorption partly occurs at the distal portion of the duodenum. [6] Vitamin D enhances intestinal phosphorus absorption by facilitating active uptake. [7] While the intestine absorbs phosphorus from the diet, the kidney plays an important role in the excretion of phosphorus in the urine. [8-10] Changes in serum concentrations of phosphorus are detected by sensors that elicit changes in cellular function. Short-term responses occur independently of hormones previously thought to be important in phosphorus homeostasis, play an important role in the regulation of phosphorus homeostasis. Several hormones and regulatory factors such as the vitamin D, endocrine system, parathyroid hormone, and the phosphatonins (FGF-23, sFRP-4, MEPE), play an important role, mainly, in the long-term regulation of phosphorus homeostasis.[11]

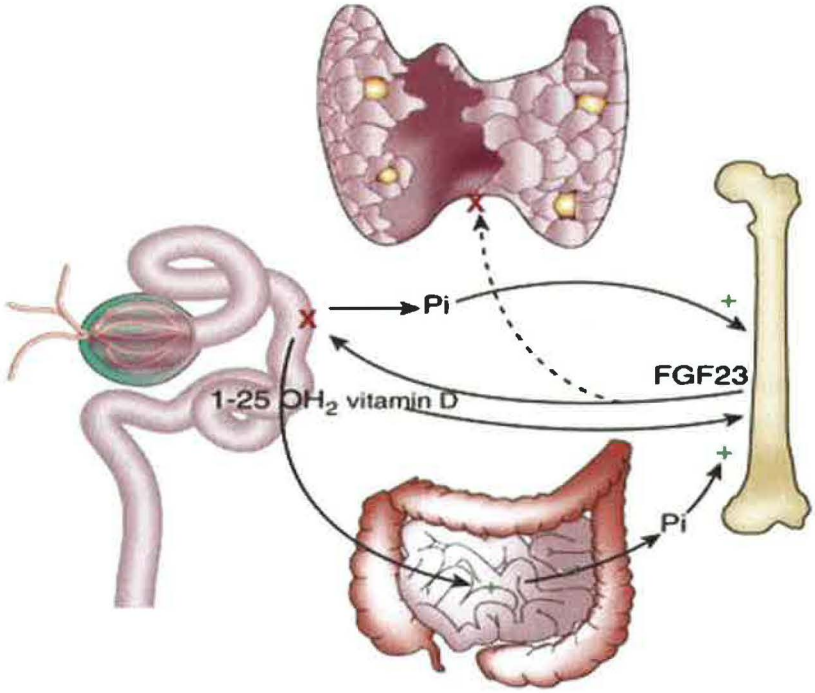


Adapted from reference # 10

Role of intestine, kidney and bone in phosphate homesostasis

Phosphate is absorbed throughout small intestine, mainly in the jejunum, where phosphate absorption is vitamin D dependent. The major route of excretion is by the kidneys, where 80% of the plasma protein is ultrafiltrable. Normally, about 85 to 90% of filtered phosphorus is reabsorbed. The reabsorption of phosphate occurring primarily in the proximal tubules through a transcellular route via a sodium-phosphate cotransporter. The reabsorption of phosphate by proximal tubules is not homogeneous as a large component of filtered phosphate is reabsorbed in the early portions of the tubules. Distal convoluted tubules reabsorb about 10% of the filtered phosphate. Micropuncture studies did not show the loop of Henle as a site of transport. Phosphate reabsorption in the proximal tubule appears to be closely linked to sodium transport, but is independent of sodium in response to glucose load and insulin administration.

phosphate level and amount excreted phosphate are affected by various pathological conditions such as hyperparathyroidism, chronic diarrhea, Impaired kidney function, hypothyroidism, diuretics, malnutrition and alcoholism. Aluminum-containing antacids reduce the absorption of dietary phosphorus by forming aluminum phosphate, which is unabsorbable. High doses of calcitriol, the active form of vitamin D, or its analogs may result in hyperphosphatemia. Also the phosphate level in blood can suddenly fall dangerously low in people recovering from severe metabolic conditions such as: severe undernutrition (refeeding), diabetic ketoacidosis, alcoholism and severe burns.



Bone-kidney-endocrine axis for Pi homeostasis

Phosphate homeostasis in mammals is primarily regulated by the bone-kidney-endocrine axis.[12, 13] High phosphate level increases FGF23 (a phosphaturic hormone) from bone and leads to increase phosphate excretion by the kidney. Moreover, FGF 23 suppresses 1,25-

dihydroxy vitamin D production by the kidney, which results in decrease phosphate absorption from intestine leading to a negative phosphate balance. One critical feature of FGF23 is that it requires Klotho, a single-pass transmembrane protein expressed in renal tubules, as an obligate co-receptor to bind and activate cognate FGF receptors.[14-17]. In animal studies, abnormalities in this bone-kidney endocrine axis result in a syndrome similar to premature aging.[18, 19]

Several factors modify the reabsorption of phosphate include hormonal (insulin, vit D, parathyroid hormone, thyroid hormone, growth hormone and glucocorticoids) and non hormonal (diet, calcium, intravascular volume, kidney function).

Increased reabsorption	Decreased reabsorption
Hormonal	***Hormonal***
Insulin	Parathyroid hormone
Growth hormone	Calcitonin
Vitamin D or its metabolite (acute administration)	Glucocorticoids
	Thyroid
Nonhormonal	***Nonhormonal***
Hypercalcemia	Extracellular volume expansion
Hypermagnesemia	Diuretics
Dietary restriction of phosphate intake	Alcohol ingestion
	High dietary intake
	Urinary alkalinization
	Kidney disease

Factors Affecting the Tubular Reabsorption of Phosphate
Adapted from reference # [20]

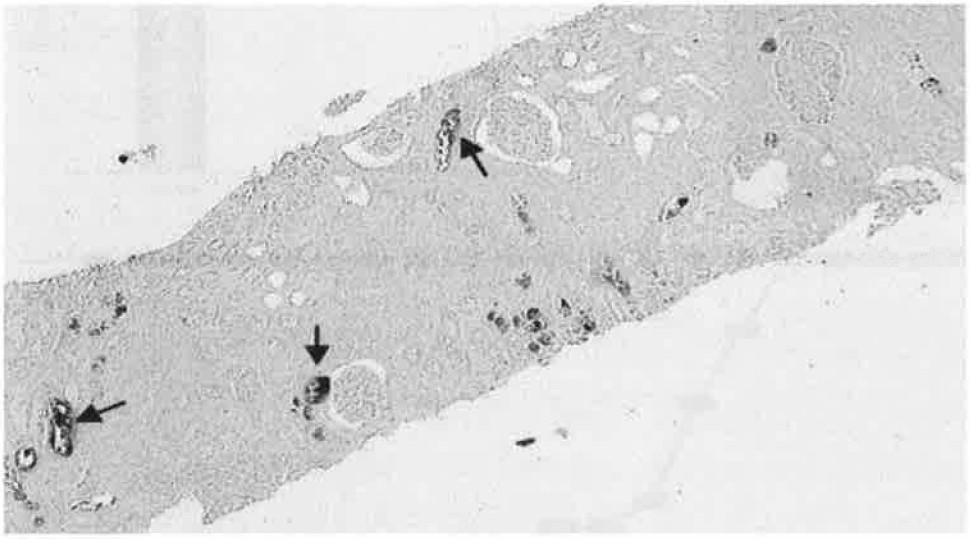
Phosphate toxicity:

In the past few years data that came out suggest that phosphate could be toxic. Many studies in animals, and few in humans, showed an association between phosphate retention and bad outcomes, such as mortality and malignancy but with no clear mechanisms to explain such association.[21, 22] Most of data about phosphate toxicity comes from animal studies or chronic kidney disease (CKD) patients.

Acute phosphate nephropathy:

Acute phosphate nephropathy has been described with the use of bowel prep, prior to colonoscopy, when using sodium phosphate (Fleet Phospho-soda). In late 1990s it became a popular colon prep because of smaller required volume, which results in better patient compliance[23]. United States Food and Drug Administration (FDA) issued a warning regarding the potential for acute kidney injury in patients who received oral sodium phosphate (OSP) prep.

Renal biopsy of these cases, showed diffuse deposition of calcium phosphate in tubular lumina, tubular epithelia, and the interstitium (nephrocalcinosis), which has positive staining with the "von Kossa stain" and lack of birefringence under polarized light.[24]

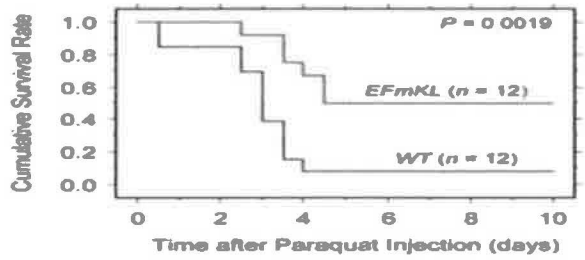


Nephrocalcinosis

The calcium phosphate deposits (stained with von Kossa stain)

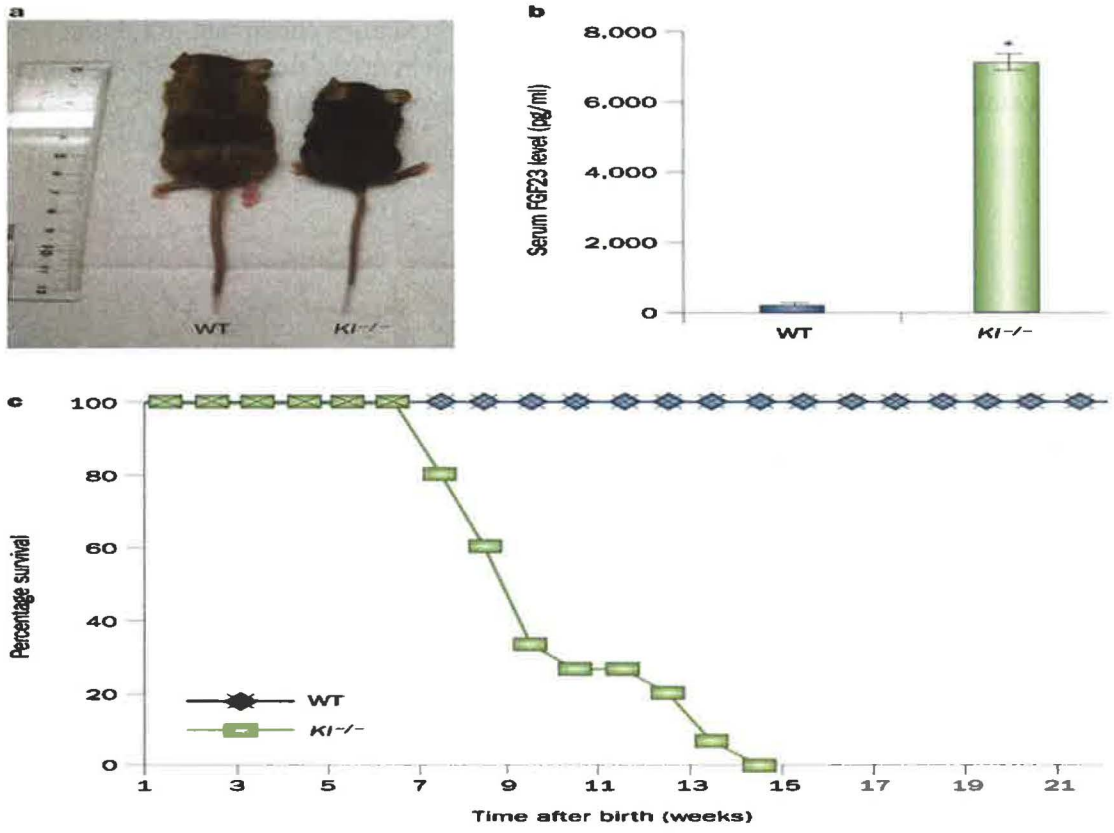
Klotho and phosphate toxicity:

The *klotho* gene product functions as a co-receptor for FGF23. [25] Defects in either FGF23 or Klotho expression lead to phosphate retention in mice and humans. [16, 26] The *klotho*$^{-/-}$mice develop severe hyperphosphatemia by three weeks of age, and remain hyperphosphatemic throughout their life. [27] The hyperphosphatemia in *klotho*$^{-/-}$ mice is associated with increased renal expression of the sodium-phosphate 2a (NaPi2a) co-transporter protein in the proximal tubular epithelial cells.[27, 28] Klotho $^{-/-}$ mice have aging-like phenotypes, including shortened life span, and growth arrest, hypogonadism , premature thymic involution, skin atrophy, muscle atrophy, vascular calcification, osteoporosis, pulmonary emphysema, ectopic calcification, motor neuron degeneration, hearing loss. while klotho overexpressing transgenic mice have significantly longer life span, compared to wild-type mice. [27, 29]



EFmKL: Klotho overexpressing Transgenic mice
WT: wild-type mice

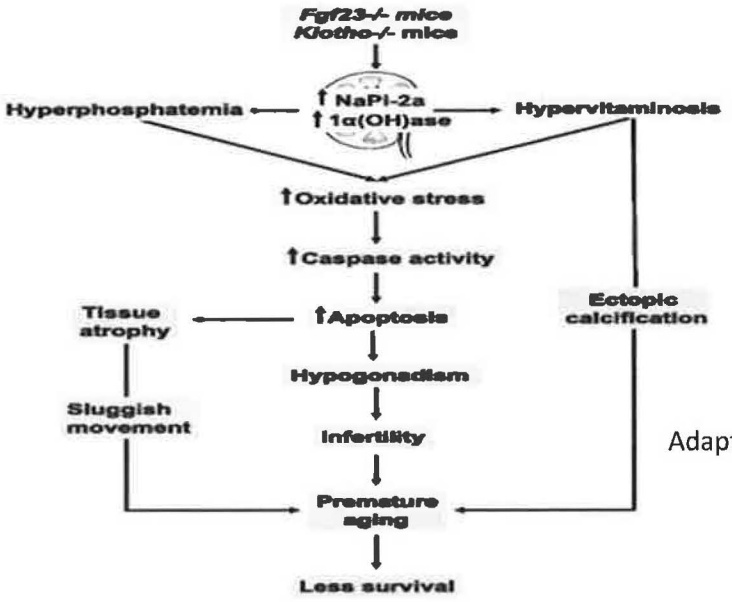
Klotho overexpressing transgenic has longer life span compared to wild type mice



FGF-23 primarily serves as a systemic factor to regulate phosphate homeostasis and vitamin D metabolism by acting on the renal proximal tubules to down-regulate NaPi2a and 1α(OH)ase activity, respectively.[30] FGF23 binds to FGF receptor1 (FGFR1) -Klotho complex in the kidney and thereby induces phosphaturia and suppresses 1,25-dihydroxyvitamin D synthesis.

Fgf23$^{-/-}$ and *klotho*$^{-/-}$ Model:

In *Fgf23*$^{-/-}$ and *klotho*$^{-/-}$ mice, the genetic inactivation of either *Fgf23* or *klotho* results in an increased renal expression of NaPi2a cotransporter and 1-α-hydroxylase [1α(OH)ase], which leads to hyperphosphatemia and hypervitaminosis D in these mutant mice. These changes in serum phosphate and calcitriol in *Fgf23*$^{-/-}$ and *klotho*$^{-/-}$ mice can induce oxidative stress to induce apoptosis and generate hypogonadism and generalized tissue atrophy. Also, hyperphosphatemia and hypervitaminosis D can induce vascular calcification. All of these effects can result in premature aging-like phenotypes and early death in *Fgf23*$^{-/-}$ and *klotho*$^{-/-}$ mice.[31]



Adapted from reference # 31

***Klotho*$^{-/-}$*/NaPi2a*$^{-/-}$ model:** The extensive calcification noted in *klotho*$^{-/-}$ mice by 6 weeks of age was completely eliminated in *klotho*$^{-/-}$*/NaPi2a*$^{-/-}$ mice and was not detected even in 12-week-old double mutant mice. These results indicated that high serum phosphate is an important determinant of calcification, and lowering serum phosphate can reduce/eliminate calcification, even in presence of higher serum $1,25(OH)_2D_3$ levels.

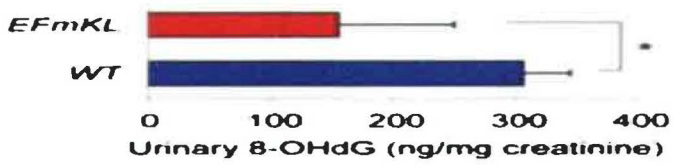
Genetically reducing serum phosphate levels in *klotho*$^{-/-}$ mice by generating a *NaPi2a* and *klotho* double-knockout (*NaPi2a*$^{-/-}$*/klotho*$^{-/-}$) strain resulted in amelioration of premature aging-like features. The NaPi2a(-/-)/klotho(-/-) double-knockout mice regained reproductive ability, recovered their body weight, reduced their organ atrophy, and suppressed ectopic calcifications, with the resulting effect being prolonged survival. More important, when hyperphosphatemia was induced in NaPi2a(-/-)/klotho(-/-) mice by feeding with a high-phosphate diet, premature aging-like features reappeared, clearly suggesting that phosphate toxicity is the main cause of premature aging in klotho(-/-) mice[32]

Klotho and oxidative stress:

Data suggest that phosphate intake increase intracellular uptake of phosphateThis positive correlation between extracellular phosphorus and intracellular phosphate uptake was observed in various types of cells. This increase of extracellular phosphate induces expression of the Pit1 and Pit2 genes, which are members of the type III Na-phosphate cotransporters. These cotransporters are primarily responsible for cellular phosphate uptake from extracellular space in mammalian cells [33, 34]

The increase of intracellular phosphate concentration, even if transient, may increase mitochondrial membrane potential, which is positively correlated with production of reactive oxygen species (ROS) in the electron transport chain.[35] Di Marco *et al.* reported that human

vascular endothelial cells cultured in a high phosphate medium had higher levels of cellular ROS, compared to cells cultured in normal phosphate medium.[36] *klotho*$^{-/-}$mice have higher levels of oxidative stress measurements, while Klotho-overexpressing transgenic mice less levels of oxidative stress as evidenced by lower levels of urinary 8-OHdG, a marker of oxidative damages to DNA[37]



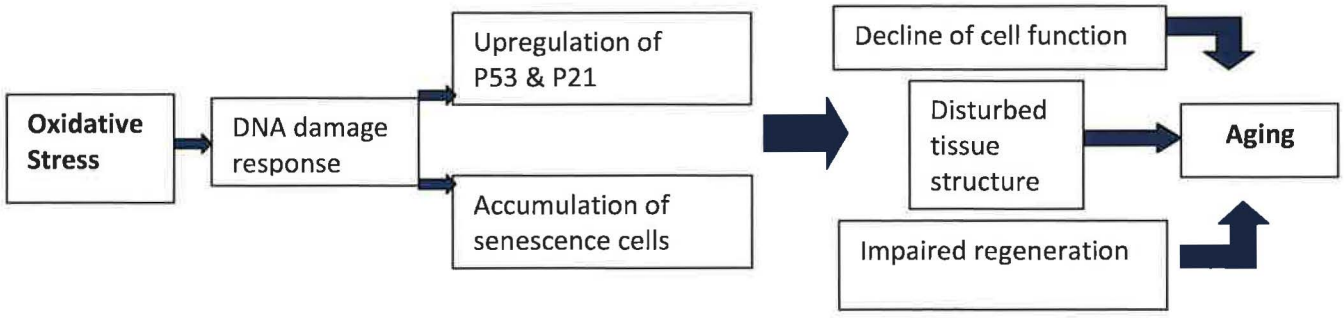
EFmKL: Klotho overexpressing Transgenic mice
WT: wild-type mice

Oxidative stress level in klotho overexpressing and wild-type mice

It is believed that phosphate retention caused by klotho deficiency in mice results in cognition impairment due to increased oxidative damage and apoptosis in the brain. [38] Endothelium-dependent vasodilatation in response to acetylcholine is attenuated in aorta and arterioles from Klotho-deficient mice, indicating that Klotho deficiency causes a reduction of NO synthesis in vascular endothelial cells. Consistent with this finding, Klotho-deficient mice exhibit impaired angiogenesis, which is dependent on endothelium-derived NO. Systemic NO synthesis is also reduced in Klotho-deficient mice as measured by urinary excretion of NO metabolites [39, 40]

The effect of phosphate on oxidative stress markers could be one of the mechanisms by which high phosphate induce cell injury. Oxidative stress causes DNA damage, which is followed by upregulation of P21 and P53 and cell cycle arrest.[41] Persistently high oxidative stress levels interfere with DNA damage repair, which trigger premature senescence. [42] Also, oxidative stress affects the S-phase of cell cycle, leading to more induction of premature senescence. Factors that can increase oxidative stress levels would be expected to induce premature senescence, which is intimately linked with cellular aging, as seen in many studies. Cultured human cells derived from progeroid patients exhibited premature senescence in vitro. Keyes *et al.* established a causative link between premature senescence, induced by oxidative stress, and premature aging in vivo.[43]

Phosphate, oxidative stress and aging

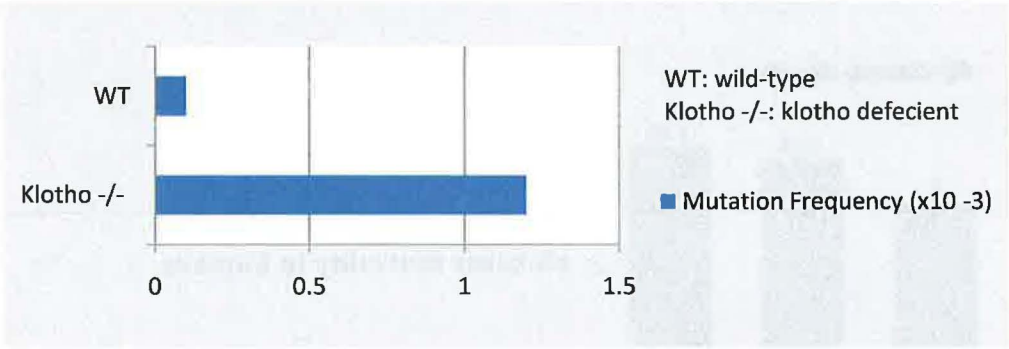


As lowering serum phosphate levels in *klotho*$^{-/-}$ mice significantly rescued soft tissue anomalies, helped restore fertility, and markedly reduced extensive soft tissue and vascular calcifications in *klotho*$^{-/-}$/*NaPi2a*$^{-/-}$ mice. This provides *in vivo* evidence that phosphate toxicity can accelerate the aging process and suggests a novel role for phosphate in mammalian aging. [32]

Moreover, In Saccharomyces cerevisiae, phosphate starvation induces cell cycle arrest and extension of chronological life span. Also, low phosphate diet extends lifespan of fruit flies, while high phosphate diet shortened it.

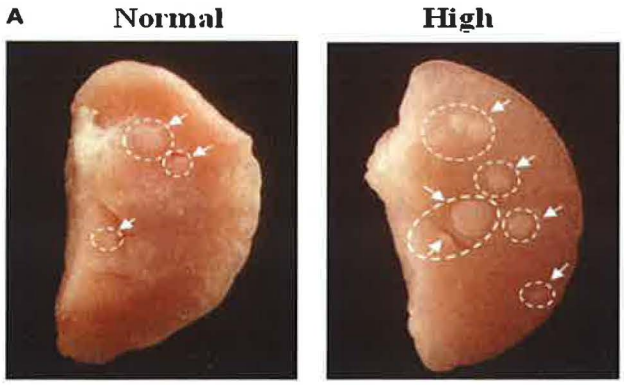
Phosphate and tumorogenisis:

Preliminary data indicates that Klotho $^{-/-}$ mice had more than a 20 fold higher somatic mutation frequency than wild-type mice.



Spontaneous somatic mutation is increased in Klotho $^{-/-}$ mice

Jin et al. showed that high dietary phosphate might cause lung cancer in mice. When Five-week-old K-*ras*^{LA1} male mice were fed a normal (0.5% Pi) and high Pi diet (1.0% Pi) for 4 weeks, the high dietary phosphate increases lung tumorigenesis and Alters Akt Signaling and significantly increased pulmonary NPT-2b protein expression.[21] The effects of high dietary Phosphate on lung tumorigenesis were confirmed in a second model of lung tumorigenesis, the urethane-induced lung cancer mouse. The mean number of tumor and the mean tumor diameter (at least 1.0 mm in diameter) were significantly increased by high dietary phosphate.



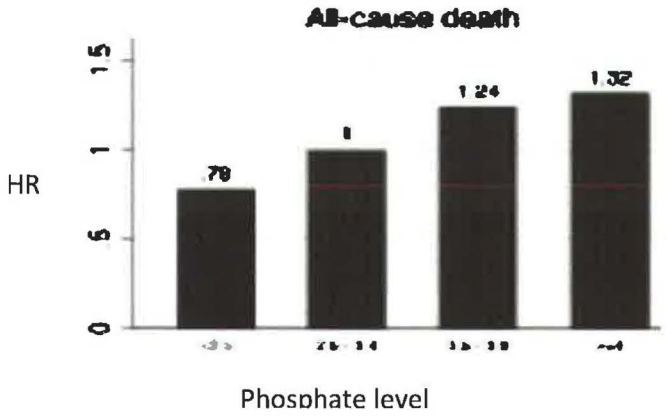
K-*ras*^{LA1} mice lungs showing numerous visible lesions (*arrows* and *dotted circles*) in normal diet (left) and high Pi diet group (*right*)

Adapted from reference # 21

In another mice model, the high-phosphate diet increased skin papilloma number by approximately 50% without changing caloric intake or body weights. The high phosphate diet increases the activation of N-ras and its downstream targets. Also high phosphate intake increased cell proliferation and expression of protumorigenic genes such as Fra-1 and osteopontin in a preosteoblast cell line.[44] In this study, the authors calculated that the human dietary equivalent of a mouse's high phosphate diet is 1,800 milligrams per day, an intake level that many humans match or exceed. The low phosphate diet was equivalent to 5oo mg per day for humans.

Phosphate toxicity in humans:

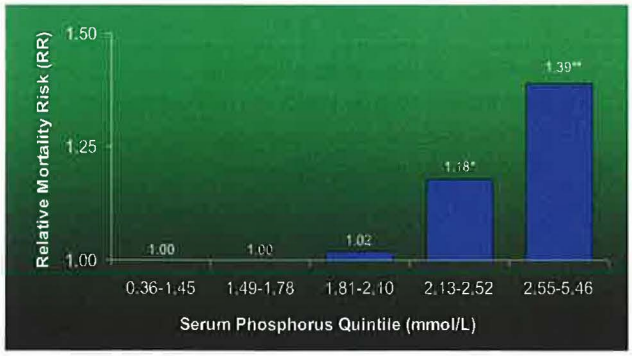
Epidemiological data supports, but does not prove nor explain, the notion of phosphate toxicity in humans. Serum phosphorus levels positively correlate with all-cause mortality even when levels are within the normal range.[45]



Phosphate level and all-cause mortality in humans

Adapted from reference # 45

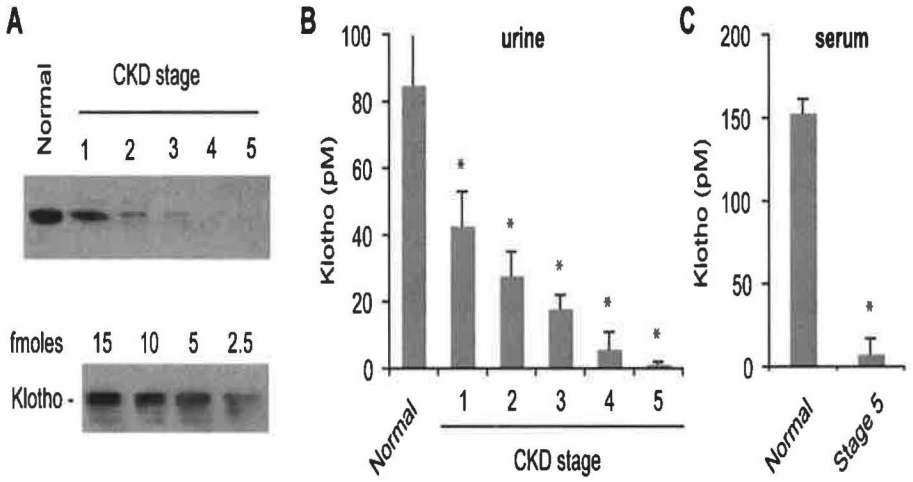
Similar findings were observed in kidney transplant recipients, where high normal levels of serum phosphate were associated with higher mortality, compared to low normal levels.[46] Also, high serum phosphorus and FGF23 levels are independent mediators of mortality in CKD patients.[47] Patients with CKD and hyperphosphatemia have higher risk for death than those with the lower serum phosphate levels.[48] In CKD patients, controlling blood phosphate levels by using phosphate binders has become an important therapeutic goal.[45, 49]



Phosphate level and all-cause mortality in dialysis patients

Adapted from reference # 48

Like Klotho-deficient mice, CKD patients suffer vascular calcification and have elevated serum levels of FGF23 and phosphate. Importantly, Klotho expression is decreased in CKD patients [50]. These observations suggest that Klotho deficiency may contribute to pathophysiology of CKD. Over-expression of Klotho ameliorated progressive renal injury in mouse models of glomerulonephritis and acute kidney injury. [51, 52] Decrease in Klotho expression potentially accelerates renal damage, leading to a deterioration spiral of Klotho expression and renal function.[53]



Klotho expression is decreased in CKD patients

Phosphate retention leading to hyperphosphatemia, together with reduced circulating levels of 1,25-dihydroxyvitamin D, are major biochemical changes detected in patients with CKD. Coronary calcification is a very important pathologic condition that affects the mortality of CKD patients undergoing dialysis. Hyperphosphatemia is thought to be an important risk factor for this vascular calcification. [54, 55]

In CKD patients, circulating FGF23 levels are progressively increased to compensate for persistent phosphate retention and results in reduced renal production of calcitriol. In patients undergoing dialysis, FGF23 levels are markedly elevated in response to hyperphosphatemia and active vitamin D therapy. After kidney transplantation, these patients develop hypophosphatemia and reduced 1,25-dihydroxyvitamin. FGF23 also acts directly on the parathyroid to decrease parathyroid hormone (PTH) synthesis and secretion; however, in end stage kidney disease patients, markedly elevated FGF23 fails to suppress the secretion of PTH. Recent data suggest that this parathyroid resistance to FGF23 may be caused by decreased expression of FGFR1-Klotho complex in the hyperplastic parathyroid glands. [56]

Also many studies suggested that the incidence of renal tumors, including renal cell carcinoma, in CKD patients could be as high as 30 times that of the general population. [57]

Dietary phosphate:

Although the recommended daily allowance of phosphate is 580-700 mg (varies with age); the average American diet has about 1500 mg/day. This is likely due to increased protein consumption and the use of phosphate as a food preservative. One of the challenges when describing a low phosphate diet is that it involves limiting protein intake.

Generally phosphate intake can be calculated using the following regression equation:

Y = 14.134 x + 127.91,

where Y is the phosphate intake and x is the total protein intake. In a 70 kg person consuming 1.2 grams of protein per kg, the phosphate intake will be 1315 mg of phosphorus (just from the protein intake). Food that is high in phosphate includes, but not limited to: Meat, processed meat, dairy products, cornmeal, lentil, oat bran, dried beans, chocolate and dark colored sodas.

USDA National Nutrient Database, Release 17

NDB	Description	Weight (g)	Measure	Content/Measure
20005	Barley, raw	200	1 cup	442
08060	Cereals, KELLOGG'S RAISIN BRAN	61	1 cup	259
01037	Cheese, ricotta,skim milk	246	1 cup	450
20025	Cornmeal, self-rising, enriched, yellow	138	1 cup	860
21092	Cheeseburger, regular, plain	155	1 sandwich	374
	potato, french fries	169	1 large	218
20033	Oat bran, raw	94	1 cup	690
01118	Yogurt, plain, skim milk,	227	8-oz container	356
	Fish, salmon	3 ounces	---	252
	Lentils	---	1/2 cup, cooked	356

Drugs that can help lower phosphate level:

Commonly used phosphate binders:

- Calcium-containing binders: calcium carbonate and acetate

-Sevelamer hydrochloride and carbonate [58]

-Lanthanum carbonate [59]

Phosphate absorption blockade:

-Nicotinamide inhibits intestinal Na-dependent phosphate cotransport [60]

-Phosphatonins such as FGF23can inhibit uptake from food, and by inhibiting sodium-dependant phosphorus re-absorption

-Phosphonoformic acid: Inhibits sodium-dependant phosphate transport [61]

Conclusion:

Phosphorus is an essential mineral that is required by every cell in the body for normal function, but the average American diet contains at least twice the recommended amount of phosphate. Many studies in animals and few in humans have shown an association between high phosphate intake and bad outcomes, such as mortality and malignancy but with no clear mechanisms to explain such association. The concept of phosphotoxicity and shortening of life-span from high Pi intake has been shown mostly in lower model organisms. Phosphotoxicity may be mediated by multiple mechanisms including, but not limited to, interference with DNA repair and induction of high levels of oxidative stress.

Many questions are still need to be answered and different hypotheses will have to be tested. Therefore, prospective randomized trials are needed to further examine if high phosphate intake is toxic to humans and explore all possible mechanisms of such effect. As average American diet is already high in phosphate, limiting phosphate intake might be very challenging by diet alone.

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