FGF-23/ΚΛΟΘΩ, βιταμίνη D, παραθορμόνη στον μεταβολισμό ασβεστίου και φωσφόρου

Αλκυονίδες ημέρες Νεφρολογίας,
Καλαμπάκα 12-15 Φεβρουαρίου 2015

Ιωάννης Γ. Γριβέας MD., PhD.

Νεφρολογικό Τμήμα 417 ΝΙΜΤΣ

Μ.Χ.Α. 'Νεφροιατρική'

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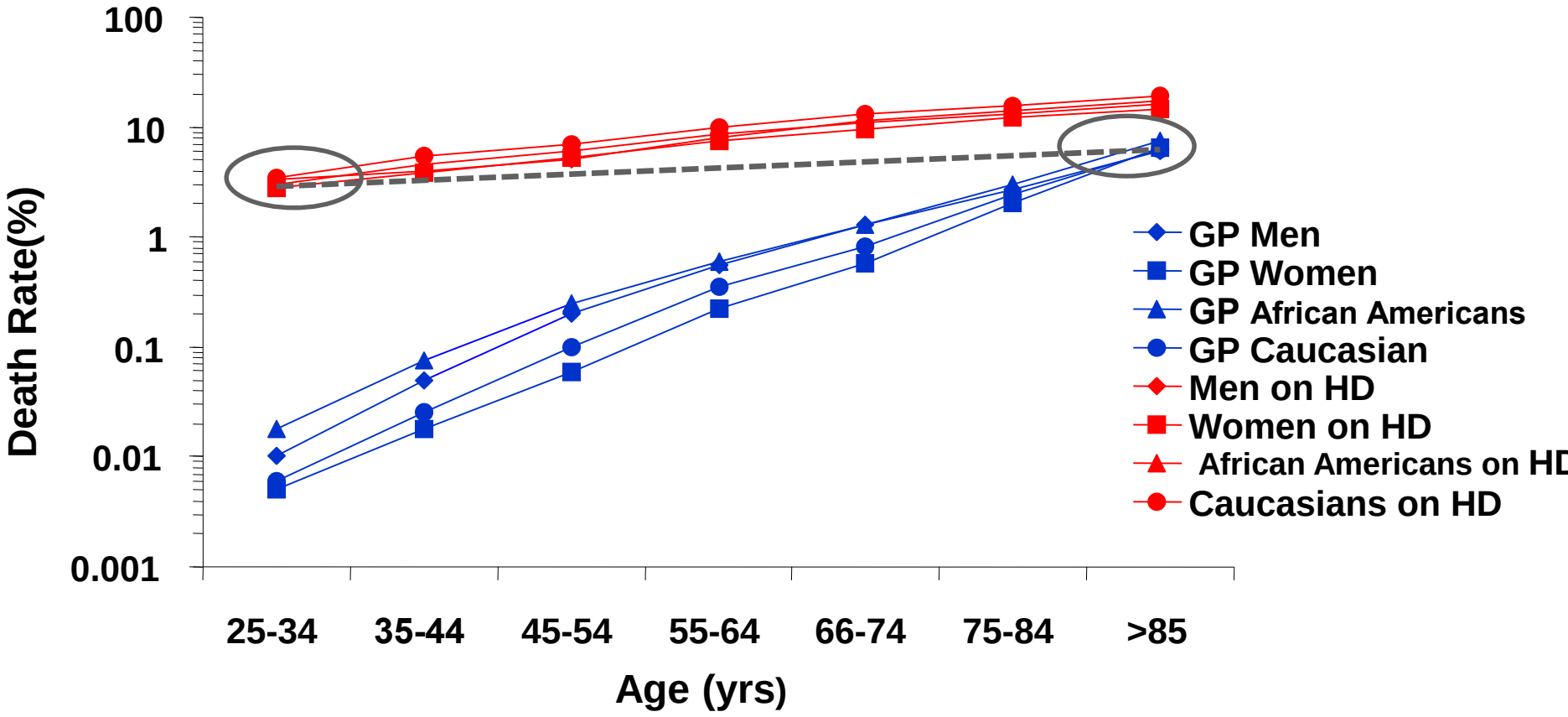
Survival in CKD



Foley RN et al. Am J Kidney Dis. 1998; 32(5 suppl 3): S112-9.

Life-expectancy in USA-population (2002) compared with dialysis patients (2003) by age, race, sex

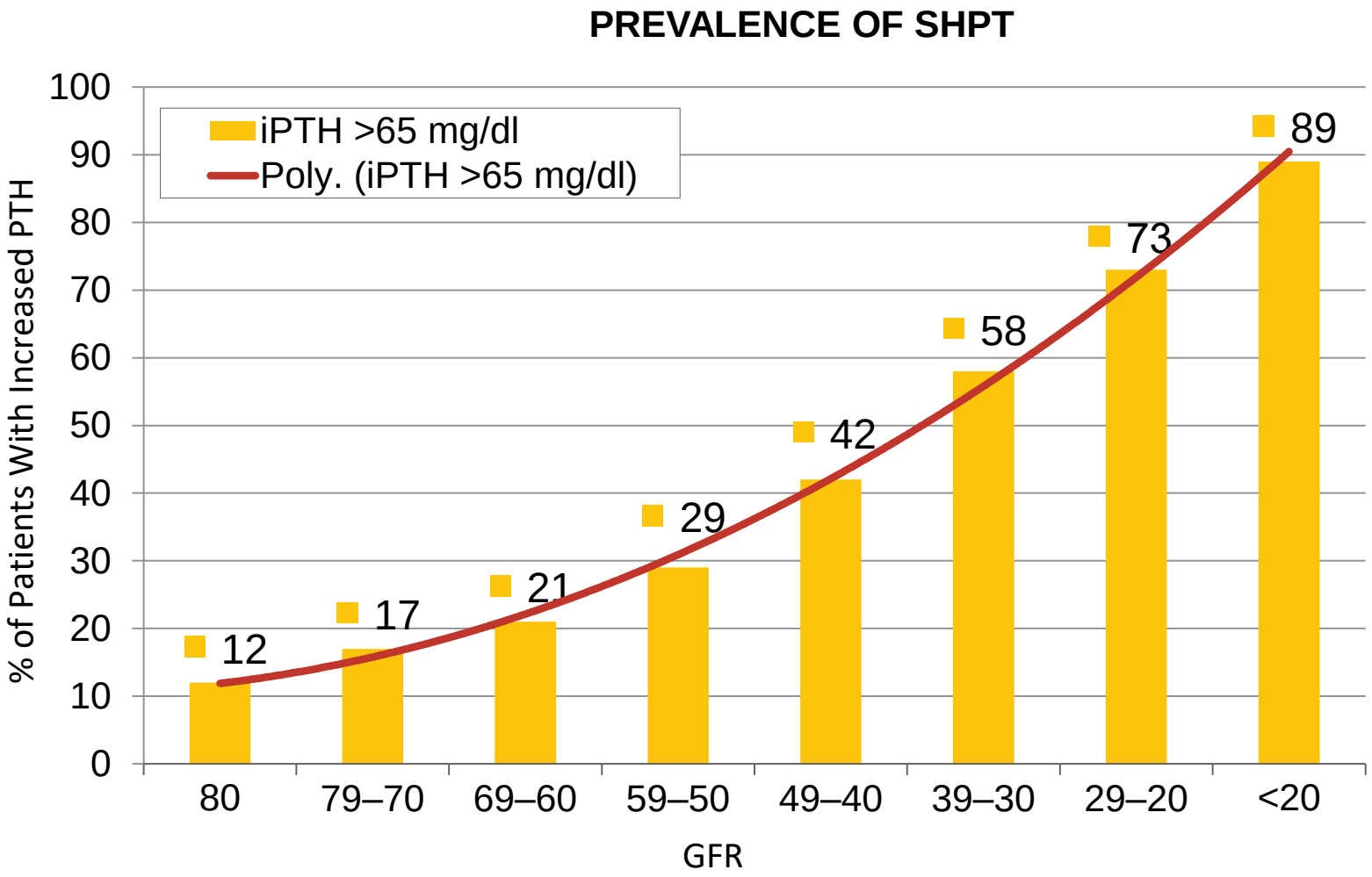
6.b Expected remaining lifetimes (years) of the general U.S. population & dialysis & transplant patients, by age, gender, & race | general U.S. population, 2002, & prevalent dialysis & transplant patients, 2003

General U.S. population, 2002										ESRD patients, 2003						
	All races			White			Black				Dialysis			Transplant		
Age	All	M	F	All	M	F	All	M	F	Age	All	M	F	All	M	F
0-14	70.9	68.2	73.4	71.3	68.7	73.7	66.4	62.9	69.7	0-14	18.3	19.2	17.3	50.0	50.2	49.9
15-19	61.1	58.4	63.6	61.4	58.9	63.9	56.7	53.3	59.9	15-19	16.0	16.9	15.1	39.7	39.6	39.8
20-24	56.3	53.8	58.7	56.7	54.2	59.0	52.0	48.7	55.0	20-24	13.9	14.8	12.9	36.2	36.1	36.4
25-29	51.6	49.1	53.9	51.9	49.5	54.2	47.4	44.3	50.2	25-29	12.0	12.7	11.3	32.3	32.1	32.6
30-34	46.8	44.4	49.0	47.1	44.8	49.3	42.8	39.8	45.5	30-34	10.5	10.8	10.0	28.5	28.3	28.9
35-39	42.1	39.8	44.2	42.4	40.1	44.5	38.3	35.4	40.8	35-39	9.0	9.2	8.7	25.2	24.8	25.8
		35.2	39.5	37.7				31.1	36.3	40-44	7.8				21.5	22.7
45-49		30.8	34.9	33.2	35.1			26.9	32.0	45-49	6.8		6.7		18.5	19.8
		26.6	30.4					23.1	27.8	50-54	5.9				15.7	17.2
55-59		22.5	26.0		22.7			19.6	23.9	55-59	5.0		5.0		13.2	14.7
		18.7	21.9					16.4	20.1	60-64	4.3				11.0	12.5
65-69	16.8	15.2	18.0	16.8	15.2	18.0	15.3	13.5	16.7	65-69	3.7	3.6	3.7	9.6	9.1	10.6
70-74	13.4	12.0	14.4	13.4	12.0	14.4	12.4	10.8	13.5	70-74	3.1	3.1	3.1	7.9	7.4	8.9
75-79	10.4	9.3	11.1	10.3	9.2	11.1	9.9	8.6	10.7	75-79	2.6	2.6	2.7	6.7	6.2	7.7
80-84	7.8	6.9	8.3	7.7	6.9	8.2	7.8	6.8	8.3	80-84	2.2	2.2	2.2			
85+	4.3	3.8	4.5	4.2	3.7	4.3	4.6	4.2	4.8	85+	1.8	1.7	1.8			
overall*	25.2	23.4	26.6	25.3	23.5	26.7	23.0	20.8	24.7	overall*	5.5	5.6	5.4	15.1	14.6	15.9

Most common cancers: colon, breast, prostate

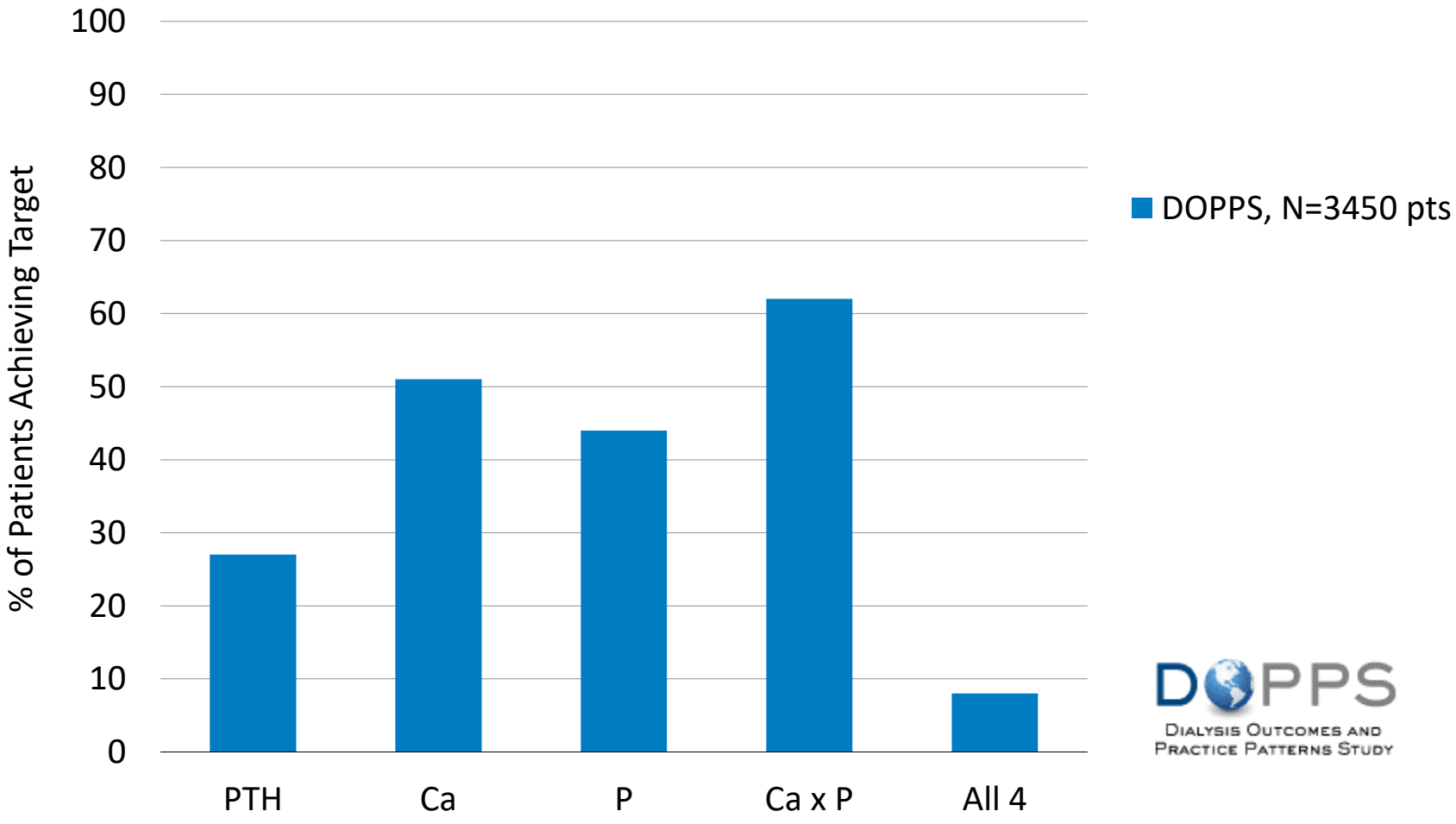
United States Renal Data System (USRDS) 2005 Annual Data Report: Atlas of End-Stage Renal Disease in the United States, NIH, NIDDKD, Bethesda, MD, 2005

Prevalence of SHPT as GFR Decreases



Levin A, et al. *CJAN.* 2009;4:1508-1514.

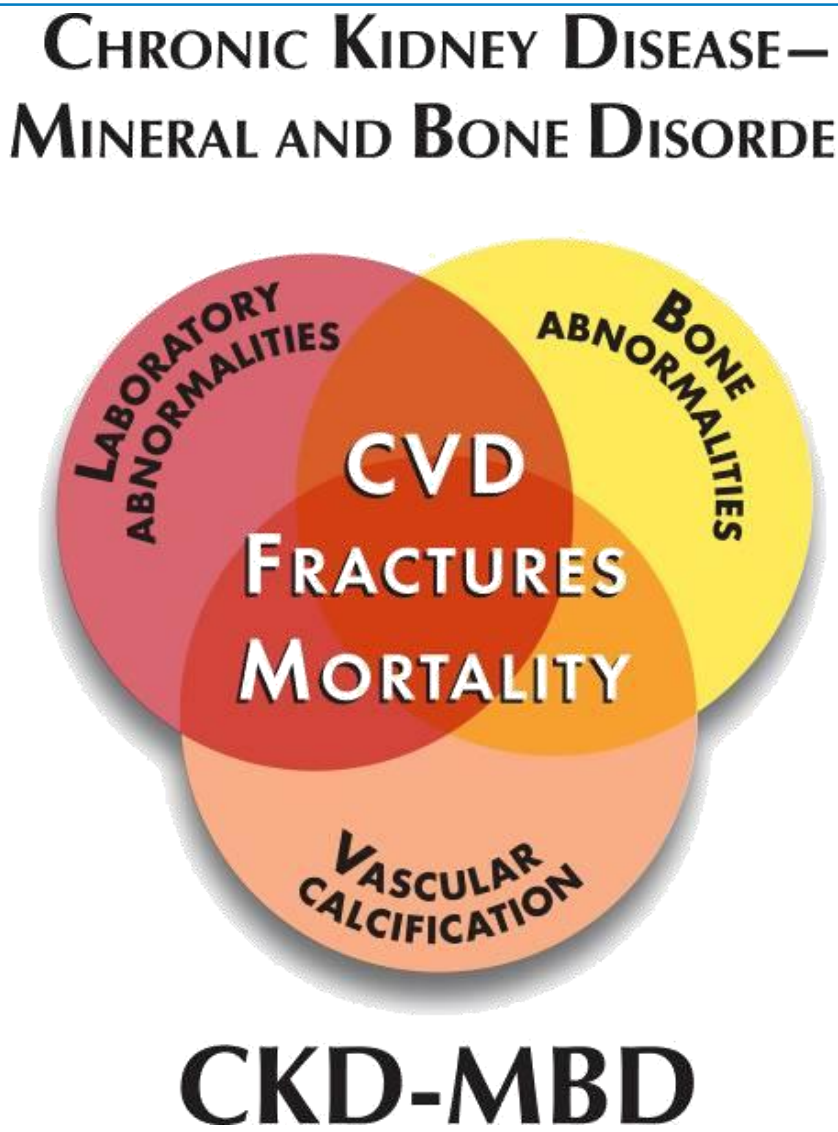
SHPT is Undertreated



DOPPS
Dialysis Outcomes and Practice Patterns Study

1. Kim J, et al. *JASN.* 2003;14:269A.
2. Kong X, et al. *BMC Nephrology.* 2012;13:116.

CKD–MBD: Systemic “syndrome”



Beyond laboratory abnormalities

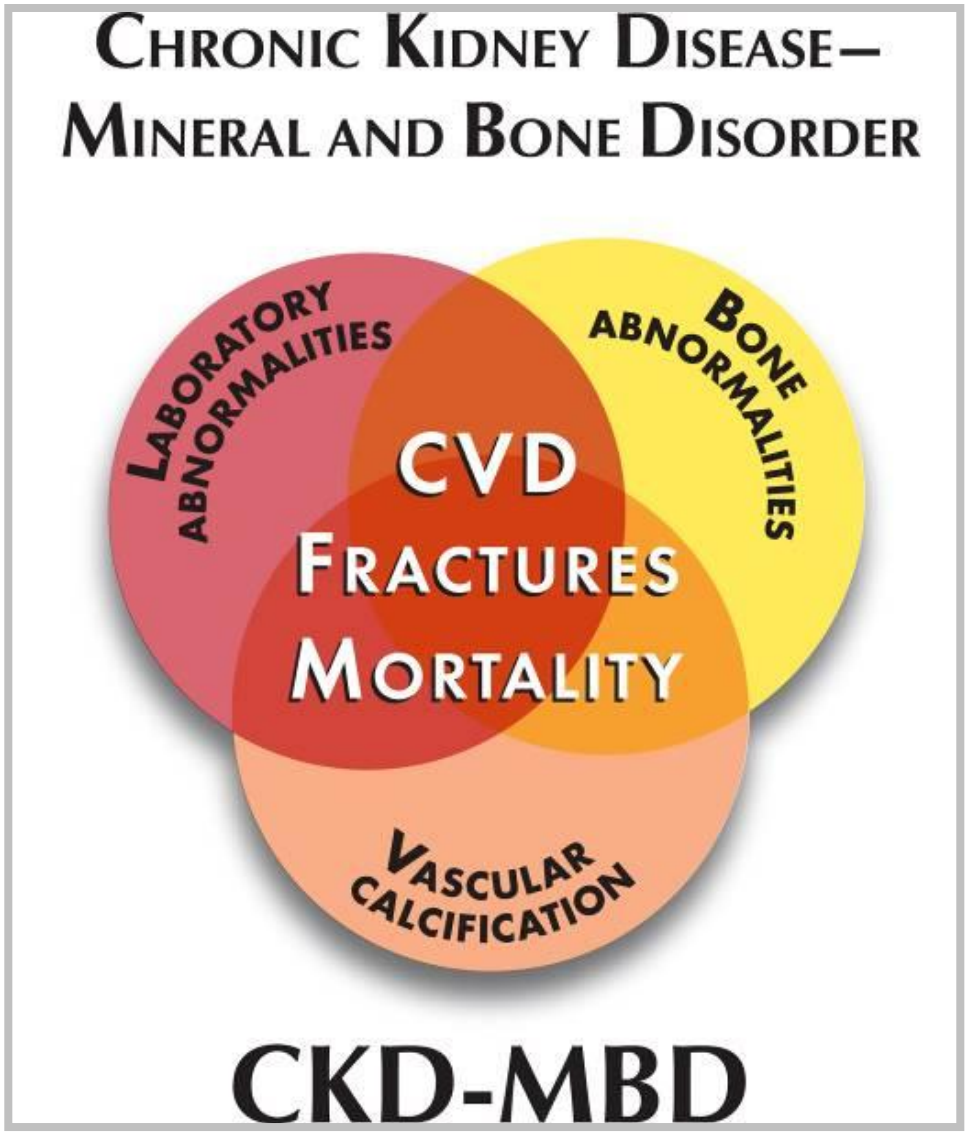
Bone–kidney axis

Bone–vascular axis

18%

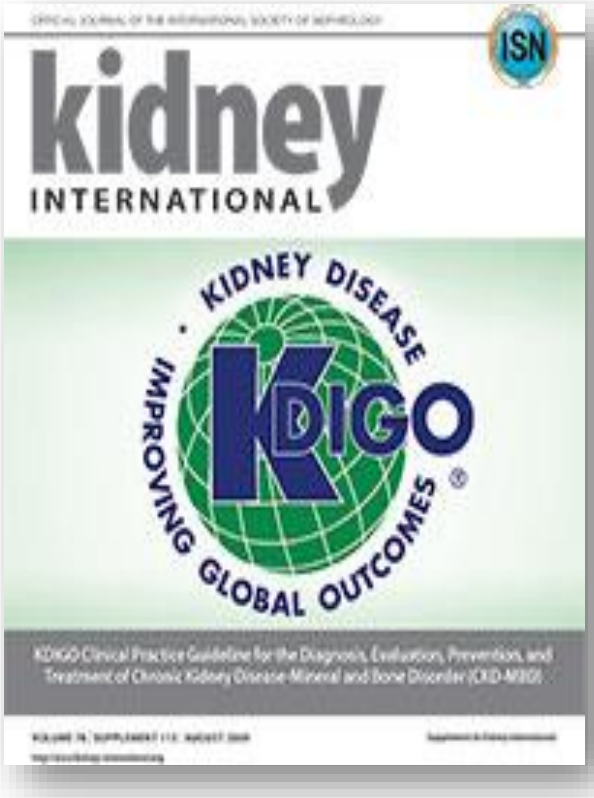
Attributable risk of mortality in dialysis

CKD–MBD: Systemic “syndrome”



Bone–vascular axis

THERE IS NOT AN UNDISPUTABLE “1A” EVIDENCE IN GUIDELINES



“ We suggest…

We might…”

Related Commentary, page 2354

Research article

FGF23 neutralization improves chronic kidney disease–associated hyperparathyroidism yet increases mortality

Victoria Shalhoub,[1] Edward M. Shatzen,[1] Sabrina C. Ward,[1] James Davis,[1] Jennitte Stevens,[2] Vivian Bi,[2] Lisa Renshaw,[2] Nessa Hawkins,[2] Wei Wang,[2] Ching Chen,[2] Mei-Mei Tsai,[2] Russell C. Cattley,[3] Thomas J. Wronski,[4] Xuechen Xia,[4] Xiaodong Li,[1] Charles Henley,[1] Michael Eschenberg,[5] and William G. Richards[1]

[1]Department of Metabolic Disorders, [2]Department of Protein Sciences, [3]Department of Pathology, Amgen Inc., Thousand Oaks, California, USA. [4]Department of Physiological Sciences, University of Florida, Gainesville, Florida, USA. [5]Medical Sciences Biostatistics, Amgen Inc., Thousand Oaks, California, USA.

- FGF-23 is a phosphatonin that increases urinary phosphorus excretion and also suppresses synthesis of 1,25-vitamin D, the active form of vitamin D.

- It has been associated with all kinds of evil consequences associated with:

  chronic kidney disease (CKD),

  including more rapid progression of CKD,

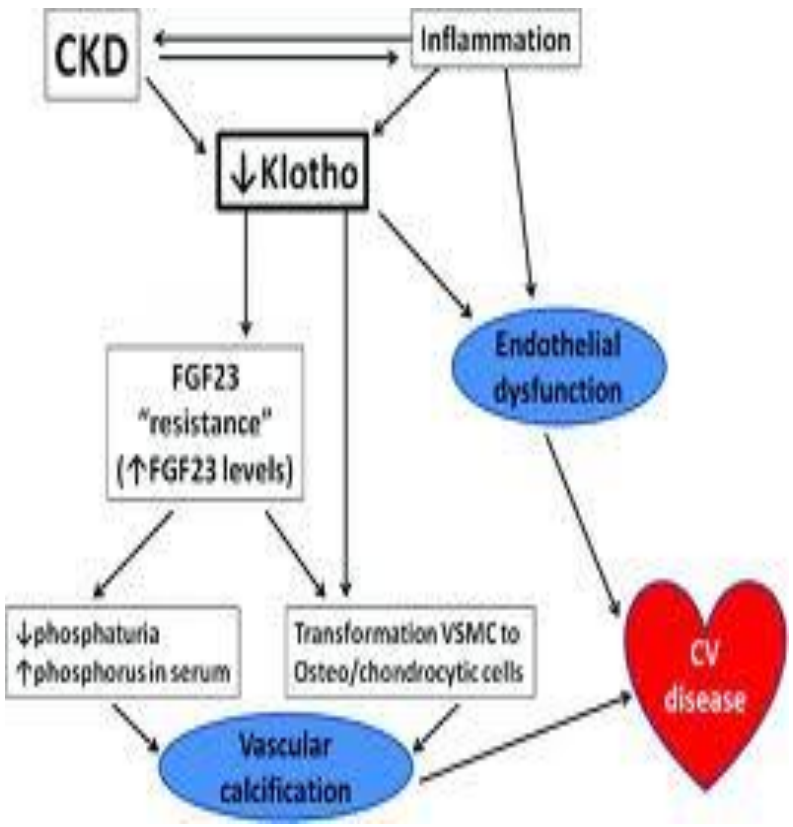
  cardiovascular events,

  mortality,

  development of end-stage renal disease (ESRD), secondary hyperparathyroidism, and

  left ventricular hypertrophy.

- Quite a lot of attention has been paid to FGF-23 recently, including interest in looking at ways to potentially suppress levels of FGF-23 -- with the hope of reversing some of the adverse consequences that have been associated with elevated FGF-23 levels, reported in various clinical epidemiologic studies.

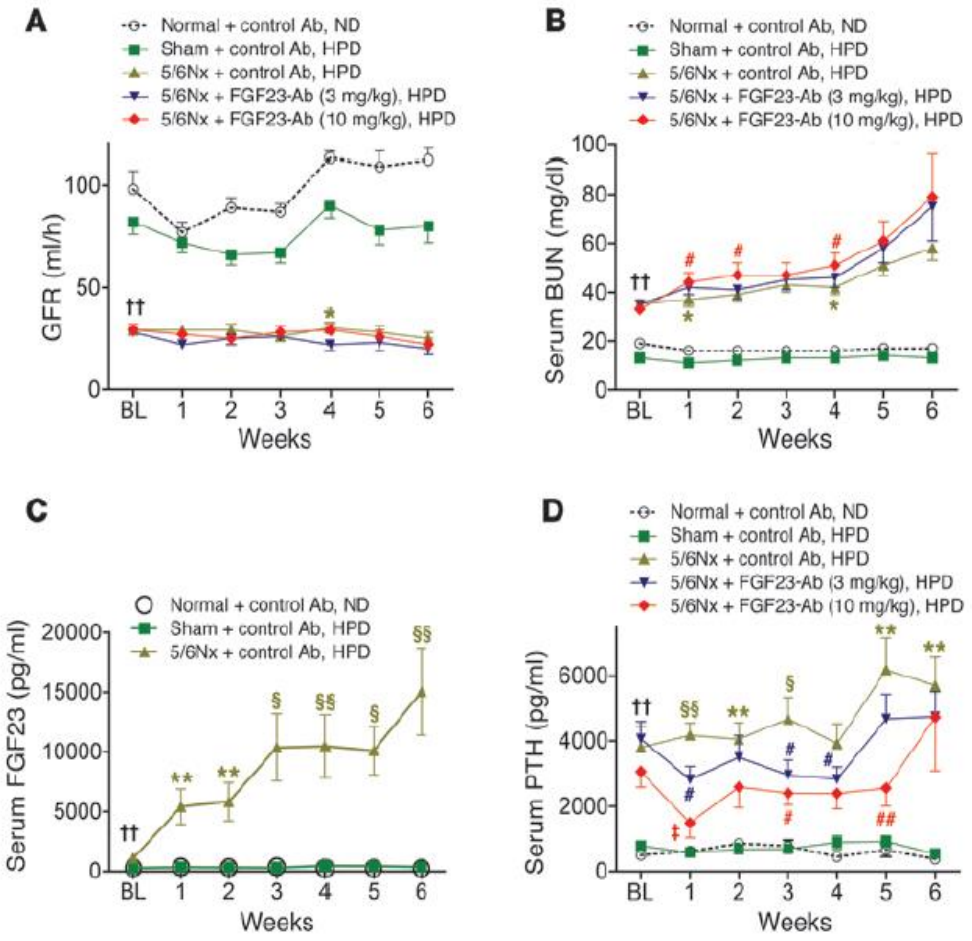


- In this study, investigators used a rat model of CKD and administered monoclonal antibodies at 2 different dose levels to rats for 6 weeks. (A control group of rats received an isotype control antibody.)

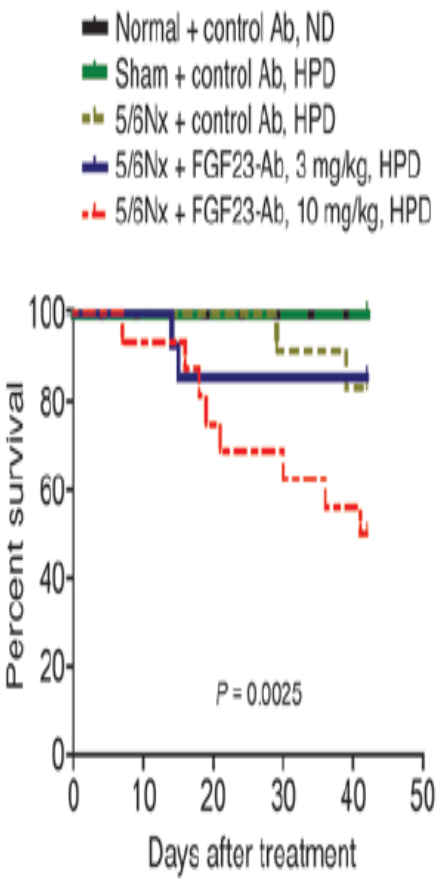
- The rats had what looked like CKD with metabolic bone disease: low vitamin D, high phosphorus, and high parathyroid hormone (PTH) levels, and so forth.

- They administered these antibodies to the rats and found some good effects resulting from this. Vitamin D levels came up and PTH levels went down, and they saw histologic improvement in metabolic bone disease that was associated with this CKD model.

- They found no benefit, however, on loss of glomerular filtration rate (GFR) in these animals, and no change in gene expression for various genes associated with left ventricular hypertrophy



- **But here's the big problem: The mortality rate in the animals that received the antibodies to FGF-23 was also substantially higher compared with the control group**.
- Levels of serum phosphorus were elevated, as you might imagine would happen when you neutralize the effect of a phosphatonin.
- In addition, the animals that received the FGF-23 had much more extensive calcification of the aorta that was dose-dependent, related to how much antibody they got.



- This was quite a surprise to the investigators noting that there is a danger in leaping from epidemiologic studies that associate elevated or abnormal hormone levels, such as FGF-23, with adverse consequences to thinking that if we reduce these levels, we will improve all the consequences.

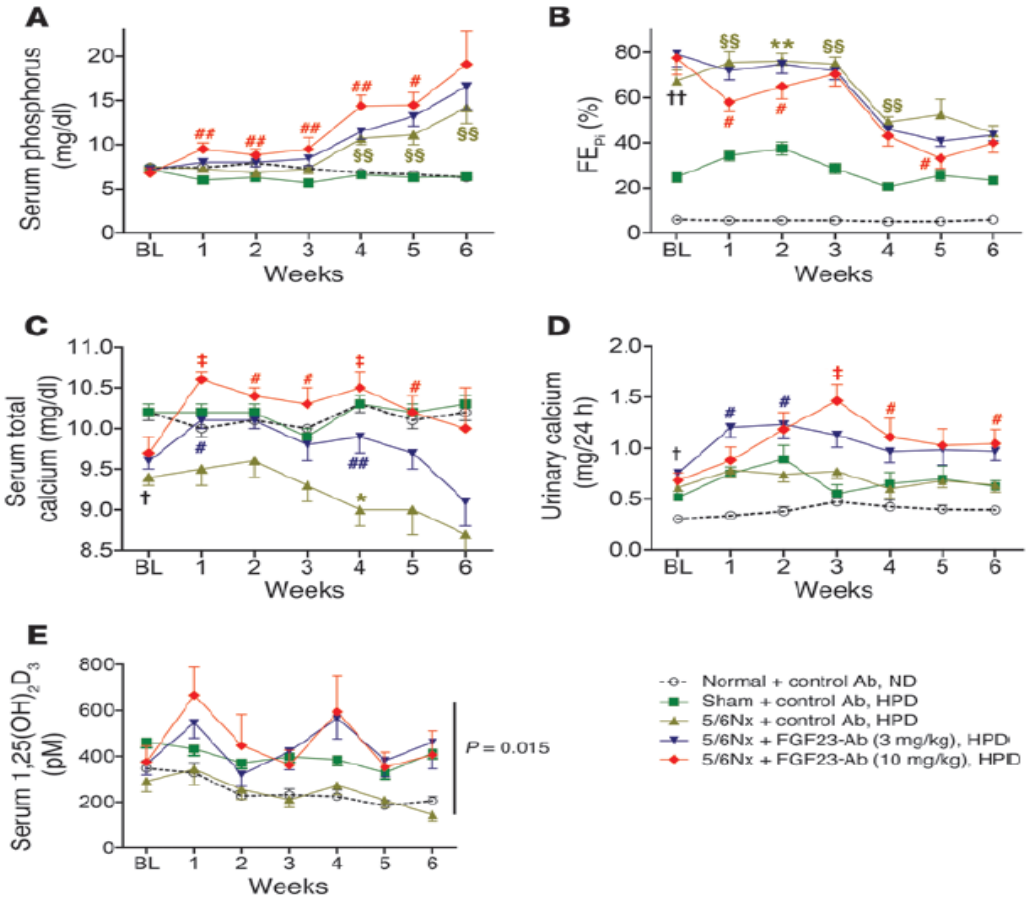
- This study shows that it does not necessarily happen that way.

There is much more to this story.

It may be that the elevated serum phosphorus level, which occurred in response to the antibodies to FGF-23, was "the evil humor," if you will, rather than the FGF-23.

There may be dose effects.

You may need some FGF-23, and oversuppression may be bad.



- Need to take another look at this whole axis and to try to better understand what is causing the ill consequences of low vitamin D, high phosphate, high PTH, and high FGF-23 levels.

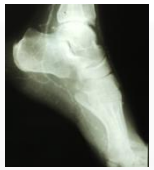
- High phosphorus in the setting of some PTH suppression compared with CKD and higher vitamin D levels may be bad.

- Suppression of FGF-23 increases 1,25-vitamin D levels, which increases gastrointestinal tract absorption of phosphorus.

PTH Action

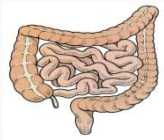
(↑Ca, ↓P)

The overall action of PTH is to **increase plasma Ca^{++} and decrease plasma P**

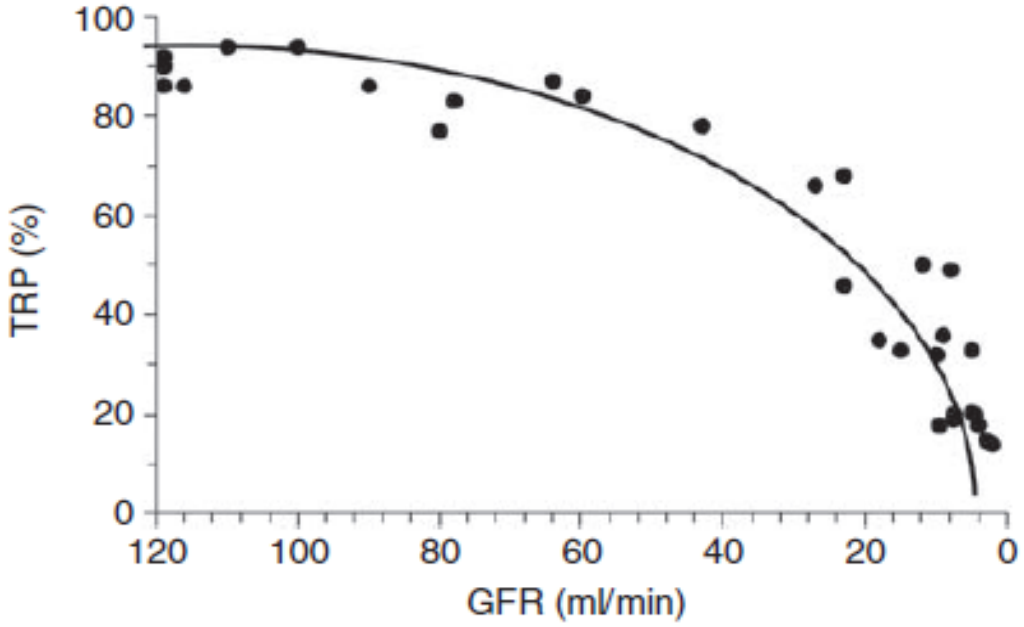


PTH acts on **bone** (directly) to ***stimulate Ca^{++} resorption***

kidney to ***stimulate Ca^{++} reabsorption*** (distal tubule) and ***inhibit reabsorption of P*** (thereby stimulating its excretion)



intestine (indirectly) by stimulating VitD synthesis, leading to ↑Ca uptake



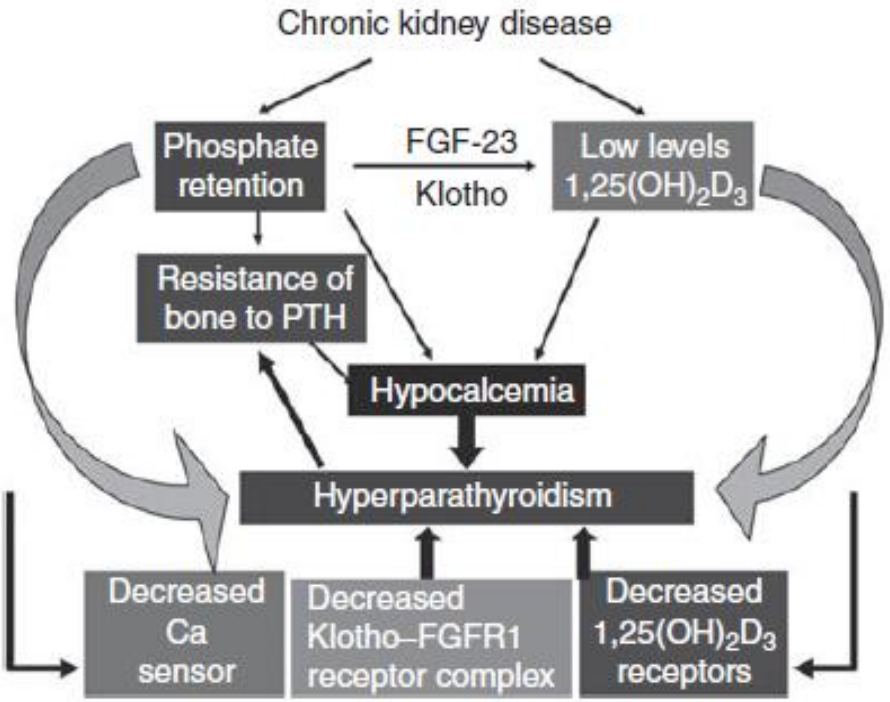
Slatopolsky E, Caglar S, Gradowska L *et al.* On the prevention of secondary hyperparathyroidism in experimental chronic renal disease using 'proportional reduction' of dietary phosphorus intake. *Kidney Int* 1972; **2**: 147–151.

Open

The intact nephron hypothesis: the concept and its implications for phosphate management in CKD-related mineral and bone disorder

Eduardo Slatopolsky[1]

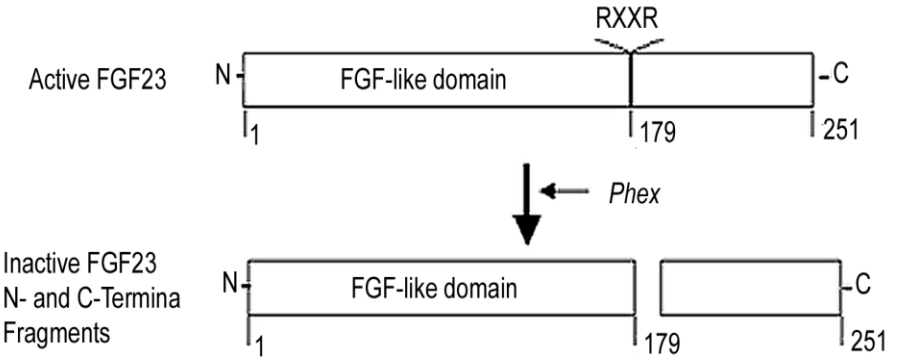
[1]*Renal Division, Department of Internal Medicine, Washington University School of Medicine, St Louis, Missouri, USA*



FGF23: A Candidate for Phosphatonin?

- **FGF23 is a ~32 kDa (251 amino acids) protein with an N-terminal region containing the FGF homology domain and a novel 71 aa C-terminus that has phosphaturic activity *in vivo*.**
- **FGF23 is overproduced by tumors causing tumor-induced osteomalacia (TIO).**
- **Autosomal dominant hypophosphatemic rickets (ADHR) is caused by missense mutations of the 176-RXXR-179 motif in FGF-23 preventing its processing into inactive N- and C-terminal fragments.**
- **FGF23 is proposed to be a substrate for PHEX**

Phex-Dependent Cleavage And Inactivation Of The Phosphaturic Hormone FGF23 Hypothesis



Physiol Rev 92: 131–155, 2012
doi:10.1152/physrev.00002.2011

REGULATION AND FUNCTION OF THE FGF23/KLOTHO ENDOCRINE PATHWAYS

Aline Martin, Valentin David, and L. Darryl Quarles

University of Tennessee Health Science Center, Memphis, Tennessee

Table 1. *Comparative analysis of gene mutations leading to increased FGF23 expression in bone*

Factor for Comparison							
Hereditary hypophosphatemic disorders	ARHR2	ARHR1	XLH	OGD	None	MAS	ADHR
OMIM no.	613312	241520	307800	166250	None	174800	193100
Mutated gene	Enpp1	Dmp1	Phex	Fgfr1	Fgf2-HMW	Gnas2	Fgf23
Type of mutation	Loss of function	Loss of function	Loss of function	Gain of function	Gain of function	Gain of function	Gain of function
Mouse homolog	Enpp1−/−	Dmp1−/−	Hyp	None	Tg-Fgf2-HMW	None	Tg-Fgf23

ARHR, autosomal recessive hypophosphatemic rickets; XLH, *X*-linked hypophosphatemic rickets; OGD, osteoglophonic dysplasia; MAS, McCune-Albright syndrome; ADHR, autosomal dominant hypophosphatemic rickets.

Physiol Rev 92: 131–155, 2012
doi:10.1152/physrev.00002.2011

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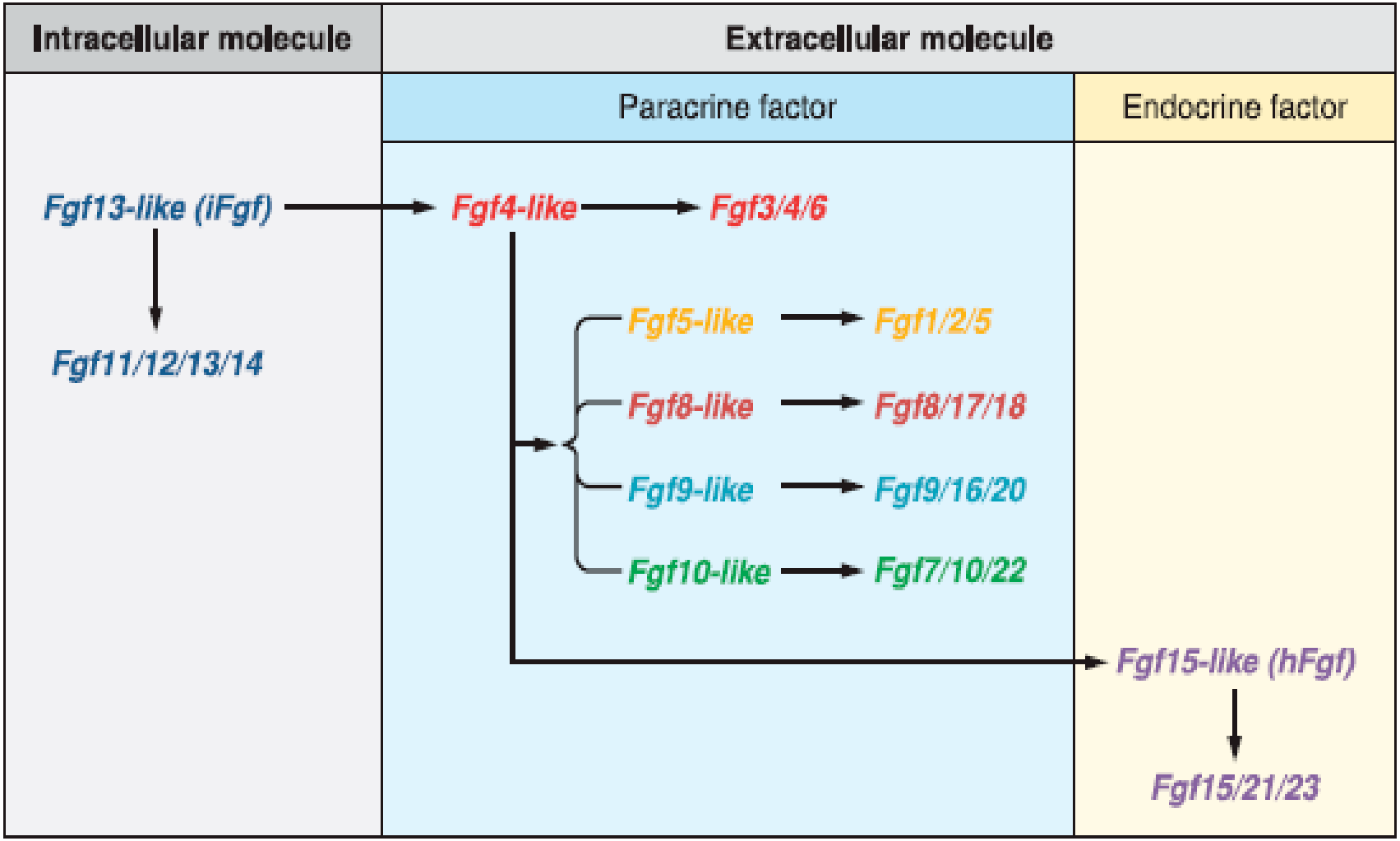
In contrast, the FGF23-bone-kidney axis is part of newly discovered biological systems linking bone to other organ functions through a complex endocrine network that is integrated with the PTH/vitamin D axis and which plays an equally important role in health and disease. The discovery that osteoblasts and osteocytes are the principal site for FGF23 production and secretion identified bone, not only as the major reservoir for calcium and phosphate, but as an endocrine organ that communicates with other organs involved in mineral homeostasis. FGF23 secreted by bone targets the kidney to regulate renal phosphate handing and vitamin D metabolism (225). The FGF23 bone/kidney axis has at least two physiological functions: *1*) to provide a phosphaturic signal emanating from bone to coordinate bone phosphate flux due to alterations in bone turnover and mineralization with renal conservation of phosphate and 2) to provide a counterregulatory hormone to protect the organism from adverse effects of excessive vitamin D exposure by FGF23-mediated suppression of $1,25(OH)_2D$ production and increased catabolism by the kidney. FGF23


Intracellular molecule
Extracellular molecule
Paracrine factor
Endocrine factor
Fgf13-like (iFgf)
Fgf11/12/13/14
Fgf4-like
Fgf3/4/6
Fgf5-like
Fgf1/2/5
Fgf8-like
Fgf8/17/18
Fgf9-like
Fgf9/16/20
Fgf10-like
Fgf7/10/22
Fgf15-like (hFgf)
Fgf15/21/23

Nephrol Dial Transplant (2012) 27: 2650–2657
doi: 10.1093/ndt/gfs160

NDT
Nephrology Dialysis Transplantation

Editorial Review

The emerging role of Klotho in clinical nephrology

Ming Chang Hu[1,2,3], Makoto Kuro-o[1,4] and Orson W. Moe[1,2,5]

[1]Charles and Jane Pak Center for Mineral Metabolism and Clinical Research, University of Texas Southwestern Medical Center, Dallas, TX, USA, [2]Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX, USA, [3]Department of Pediatrics, University of Texas Southwestern Medical Center, Dallas, TX, USA, [4]Department of Pathology, University of Texas Southwestern Medical Center, Dallas, TX, USA and [5]Department of Physiology, University of Texas Southwestern Medical Center, Dallas, TX, USA

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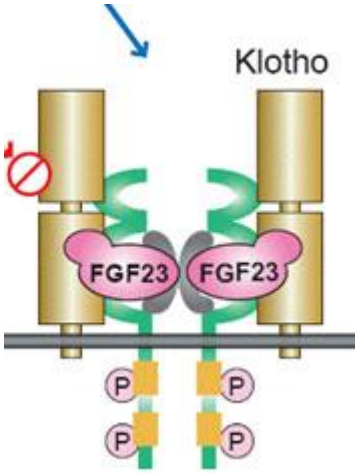
- The Klotho gene encodes
  - Single-pass transmembrane protein
    - Belongs to a family 1 glycosidase
    - Expressed primarily in renal tubules (distal tubules)
  - Present in the circulation and urine



Blood Cells Mol Dis 1998; 24: 83–100

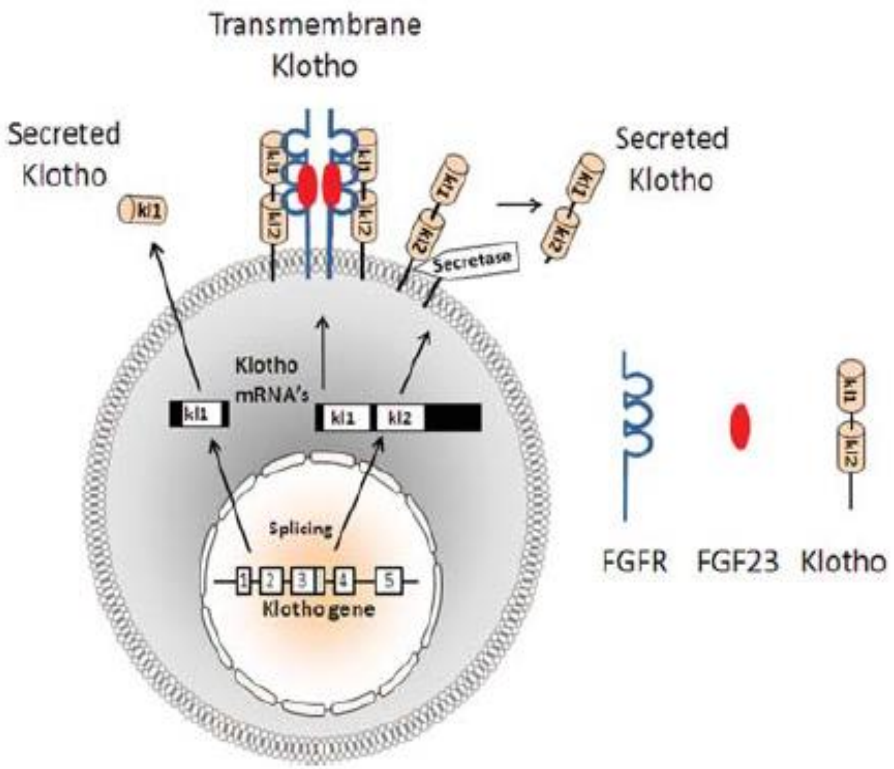


Fig. 1. Schema for Klotho gene, transcripts and proteins. Rodent and human *Klotho* spans 50 kb and consists of five exons. Two transcripts, secreted and membrane forms of Klotho, are generated through alternative RNA splicing. The internal splice donor site is in exon 3. The resultant alternatively spliced transcript after exon 3 (grey) with an in-frame translation stop codon is introduced. The short protein product, secreted Klotho, contains only Kl1 and is released from the cell. The longer Klotho encoded by the membrane form of the Klotho transcript is a single-pass transmembrane protein anchored in the cell surface. The extracellular domain of membrane Klotho containing Kl1 and Kl2 repeats is shed and cleaved by ADAM10/17, and released into bloodstream. Thus, the circulation harbors two forms of Klotho—one is the ectodomain derived from cleavage of the extracellular domain of membrane Klotho and another is the secreted protein derived from an alternatively spliced Klotho transcript. Transmembrane Klotho works with FGFRs as co-receptor for FGF23 signal transduction.

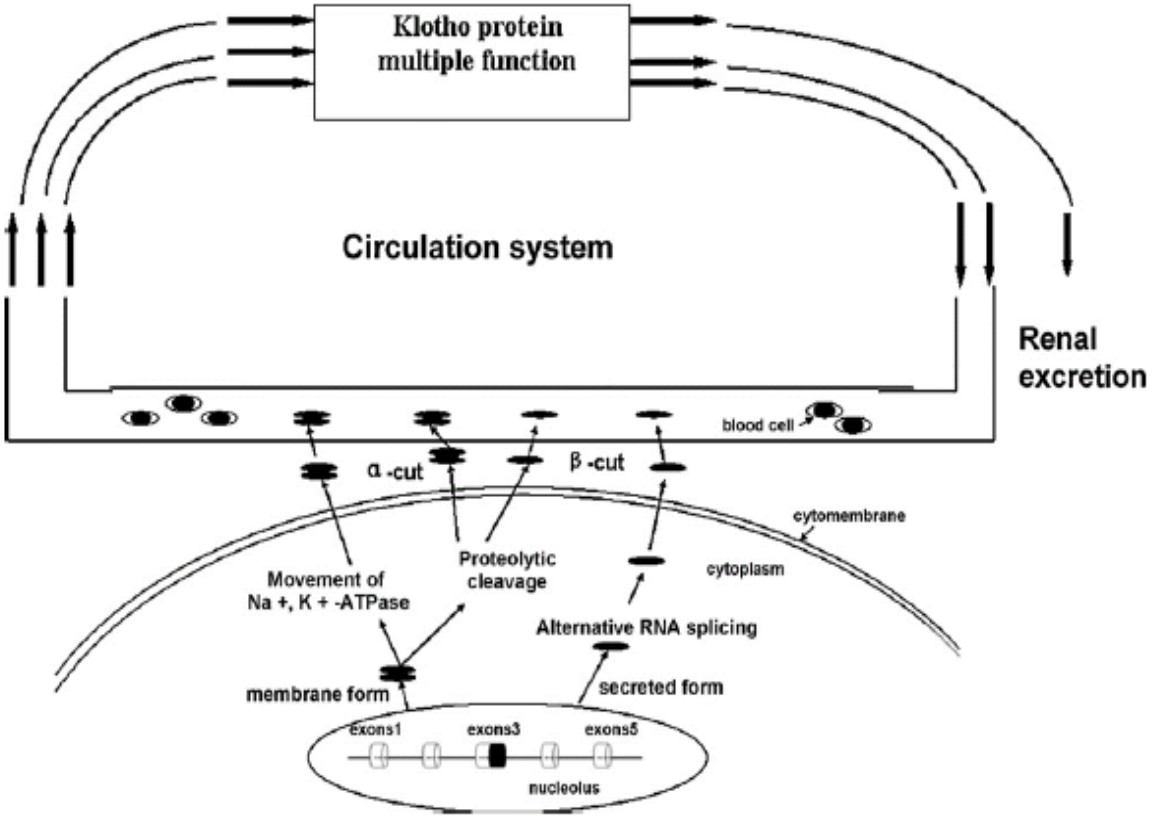


Fig. 2. The generation of secreted form klotho. The klotho protein can enter the circulation *via* three pathways: (1) *Alternative RNA splicing:* the klotho gene can directly generate secreted form klotho protein by an alternative RNA splicing. The secreted klotho protein is liberated into the extracellular space and subsequently the circulation. (2) *Proteolytic cleavage:* klotho protein could be cleaved and released to the circulation system. (3) *Na^+, K^+-ATPase:* klotho can bind to Na^+, K^+-ATPase, the complex of klotho and Na^+, K^+-ATPase could be going up to the cell surface where klotho is cleaved and secreted into extracellular space and hence the circulation system.

Ageing Research Reviews 2009;8:43–51

Nephrol Dial Transplant (2012) 27: 2650–2657
doi: 10.1093/ndt/gfs160

NDT
Nephrology Dialysis Transplantation

Editorial Review

The emerging role of Klotho in clinical nephrology

Ming Chang Hu[1,2,3], Makoto Kuro-o[1,4] and Orson W. Moe[1,2,5]

[1]Charles and Jane Pak Center for Mineral Metabolism and Clinical Research, University of Texas Southwestern Medical Center, Dallas, TX, USA, [2]Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX, USA, [3]Department of Pediatrics, University of Texas Southwestern Medical Center, Dallas, TX, USA, [4]Department of Pathology, University of Texas Southwestern Medical Center, Dallas, TX, USA and [5]Department of Physiology, University of Texas Southwestern Medical Center, Dallas, TX, USA

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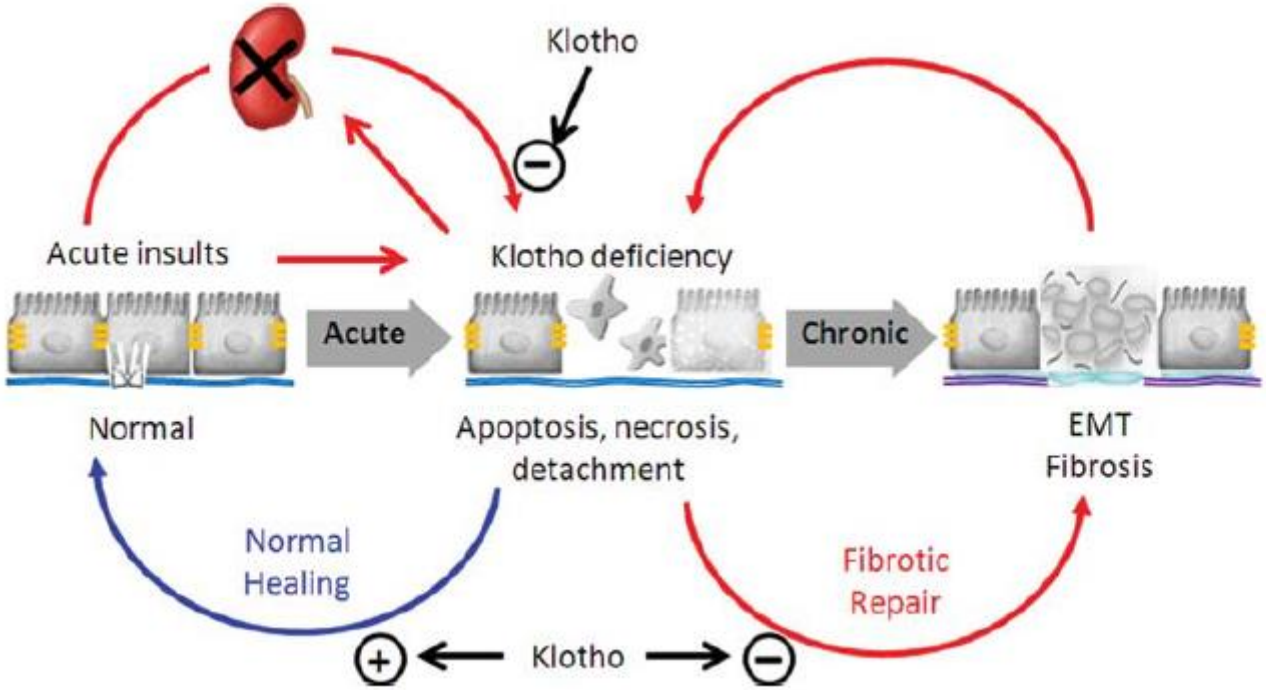


Fig. 2. Proposed model of beneficial roles of Klotho in AKI: after acute exposure to insults such as ischemia, nephrotoxin or obstruction, Klotho is reduced in the kidney, plasma and urine. Oxidative stress and cytokine production also directly suppress renal Klotho expression. The low Klotho level may be a pathological intermediate for exacerbation of kidney damage. If kidney damage is mild, the kidney undergoes normal tissue regeneration to restore normal kidney morphology. In more serious injury, reparative regeneration is replaced by fibrosis, which further worsens renal Klotho deficiency. Administrations of exogenous Klotho protein can effectively prevent and limit kidney injury, promote normal healing and prevent-fibrosis.

Klotho in AKI

- Patients with AKI were found to have drastic reductions in urinary Klotho.

- AKI is a state of acute reversible Klotho deficiency, low Klotho exacerbates kidney injury and its restoration attenuates renal damage and promotes recovery from AKI.

Kidney Int. 2010 Dec;78(12):1240-51.

Why is Klotho of particular interest for nephrology?

- Klotho is involved in the
  - Renal control of calcium, phosphate and vitamin D metabolism.
  - Suppresses phosphate re-absorption in renal proximal tubule, by directly binding to FGF receptors.
  - Regulates Ca^{2+} re-absorption in the distal convoluted tubule by
    - Stabilizing the TRPV5 Ca^{2+} channel in the plasma membrane

Nephrol Dial Transplant 2007; 22: 1524–1526

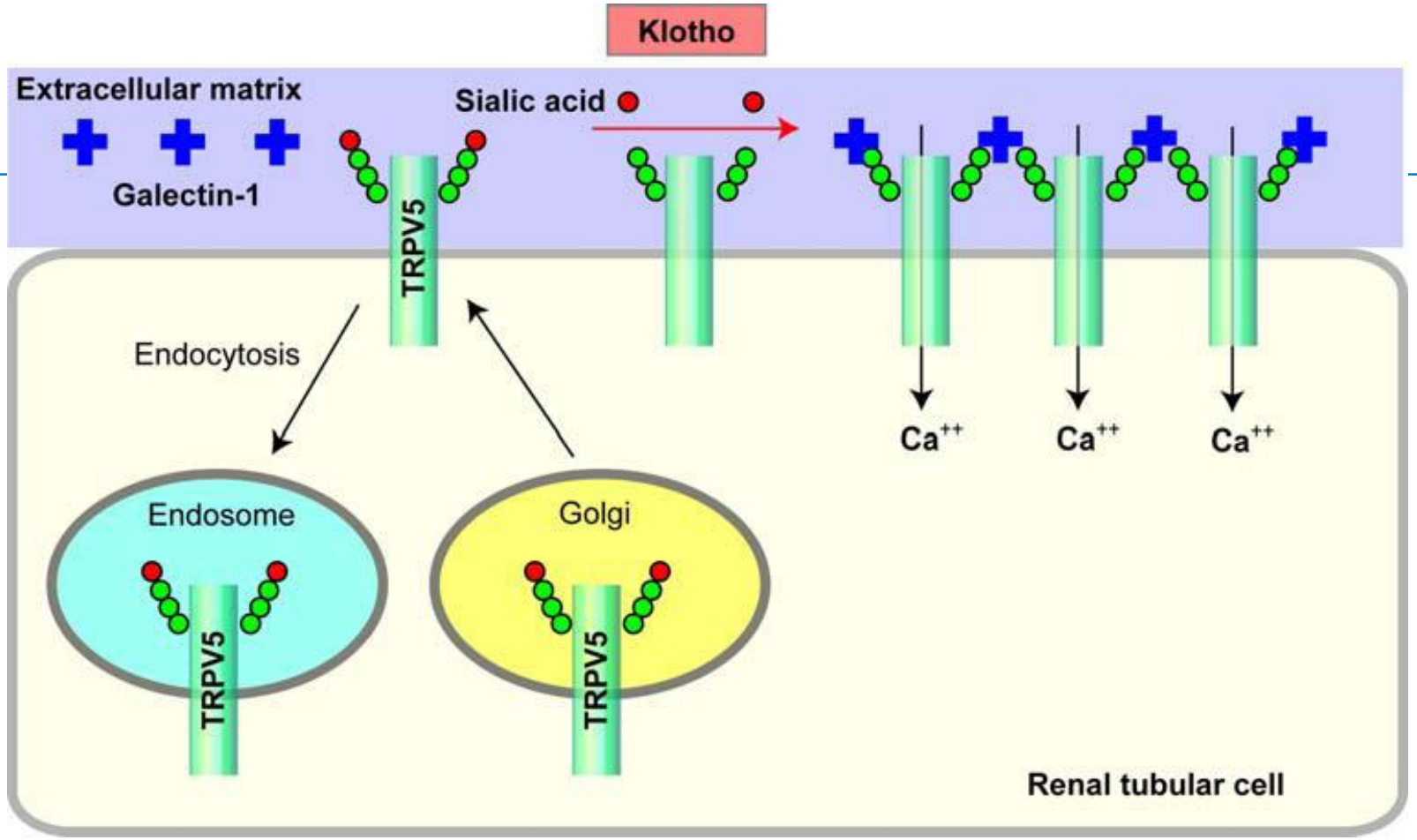


Fig. 3 Klotho inhibits internalization of cell-surface calcium channel TRPV5. The number of TRPV5 on the plasma membrane is determined by counterbalance between insertion by forward trafficking from Golgi and removal by endocytosis to endosomes. Terminals of sugar chains of many cell-surface glycoproteins are capped with sialic acids (*red*). Secreted Klotho protein removes these sialic acids through its putative α2→6 sialidase activity and exposes underlying galactose residues (*green*) in the glycans. The exposed galactose then binds to galectin-1 (*blue*) in the extracellular matrix. Galectin-1 traps TRPV5 on the cell surface and prevents its endocytosis, leading to accumulation of TRPV5 on the plasma membrane and increase in calcium influx

Pflugers Arch - Eur J Physiol 2010; 459:333–343

Why is Klotho of particular interest for nephrology?

- Klotho
  - Inhibits renal 1-alpha 25 hydroxylase activity and thereby
    - Decreases circulating calcitriol levels.
  - Therefore appears to
    - Synergize with the renal tubular effects of parathyroid hormone (PTH) on Ca^{2+} and phosphate transport, whereas
    - Antagonizes the stimulatory effect of PTH on calcitriol synthesis by the kidney.

Nephrol Dial Transplant 2007; 22: 1524–1526

Why is Klotho of particular interest for nephrology?

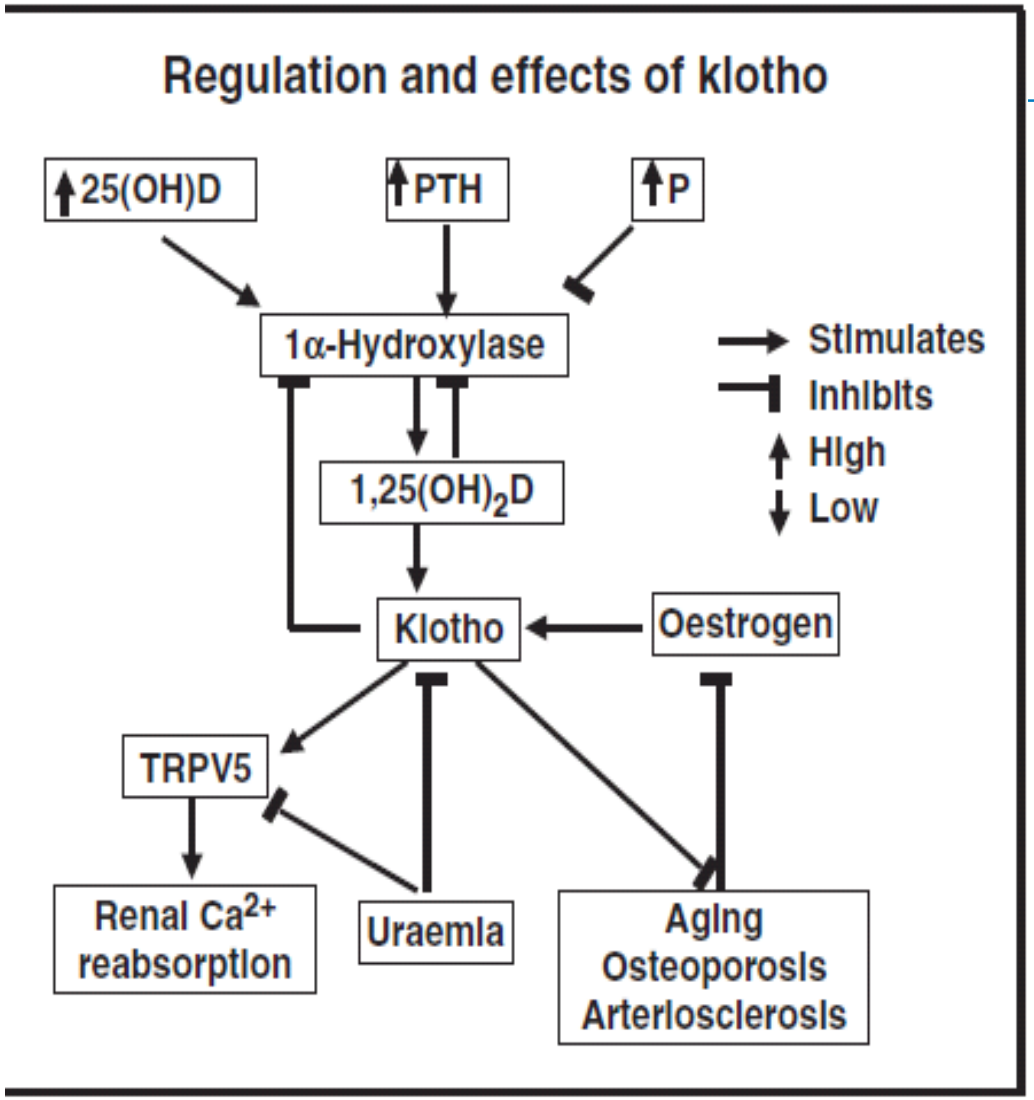
- Klotho-deficient mice and FGF23-deficient mice have an identical phenotype including
  - Hyperphosphataemia, hypercalcaemia, elevated plasma calcitriol and vascular calcification, in addition to premature ageing
- In contrast, over-expression of the *Klotho* gene
  - Extends the lifespan and increases resistance to oxidative stress

Nephrol Dial Transplant 2007; 22: 1524–1526

Why is Klotho of particular interest for nephrology?

- These observations were highly suggestive of a close cooperation between Klotho and FGF23 and/or its receptor(s).

Nephrol Dial Transplant 2007; 22: 1524–1526



1,25(OH)2D being a major stimulus, which is feedback regulated by klotho via an inhibition of the 1-hydroxylase activity.

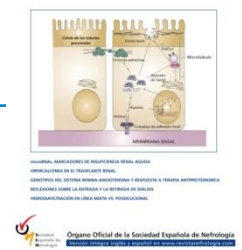
Klotho has a significant impact on the Ca $^{2+}$ reabsorption via the epithelial Ca $^{2+}$ channel, TRPV5, in the distal convoluted and connecting tubules

Klotho is produced in the kidneys and is reduced in chronic uraemia

Mutations in the klotho gene and klotho deficiency have been related to the process of ageing, osteoporosis, arteriosclerosis, ectopic calcifications and skin atrophy

Nephrol Dial Transplant 2006; 21: 1770–1772

http://www.revistanefrologia.com

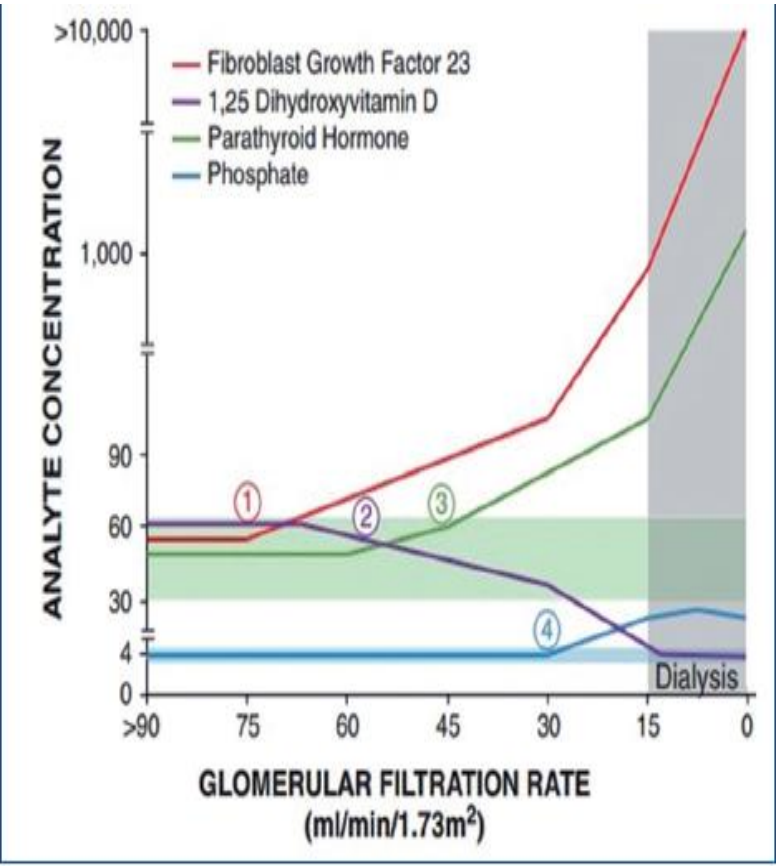


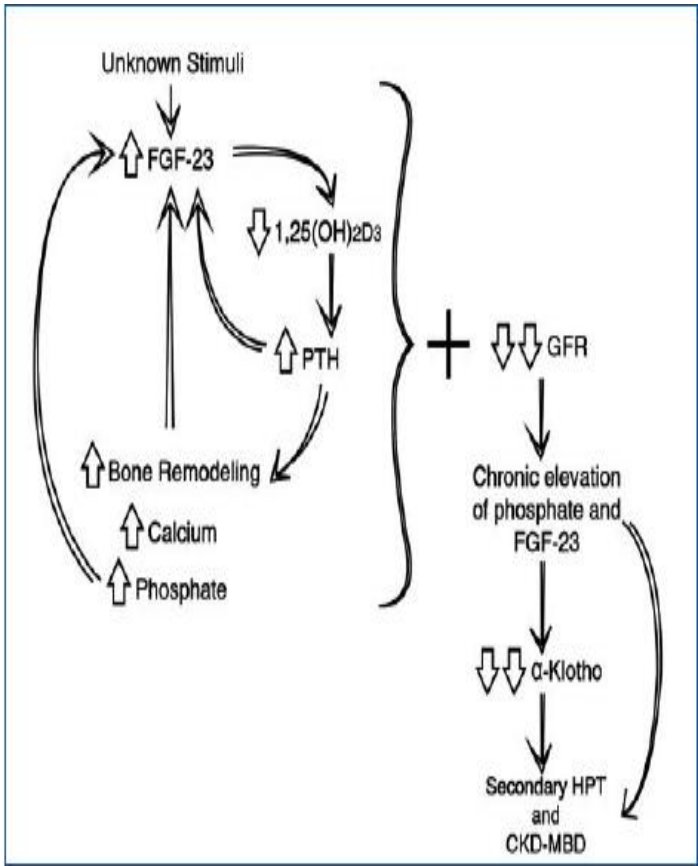
The role of Fibroblast Growth Factor 23 in chronic kidney disease-mineral and bone disorder

Hugo Diniz, João M. Frazão

Nephrology Department. Hospital S. João. Nephrology Research and Development Unit and School of Medicine. Porto University, Porto (Portugal)

Nefrologia 2013;33(6):835-44

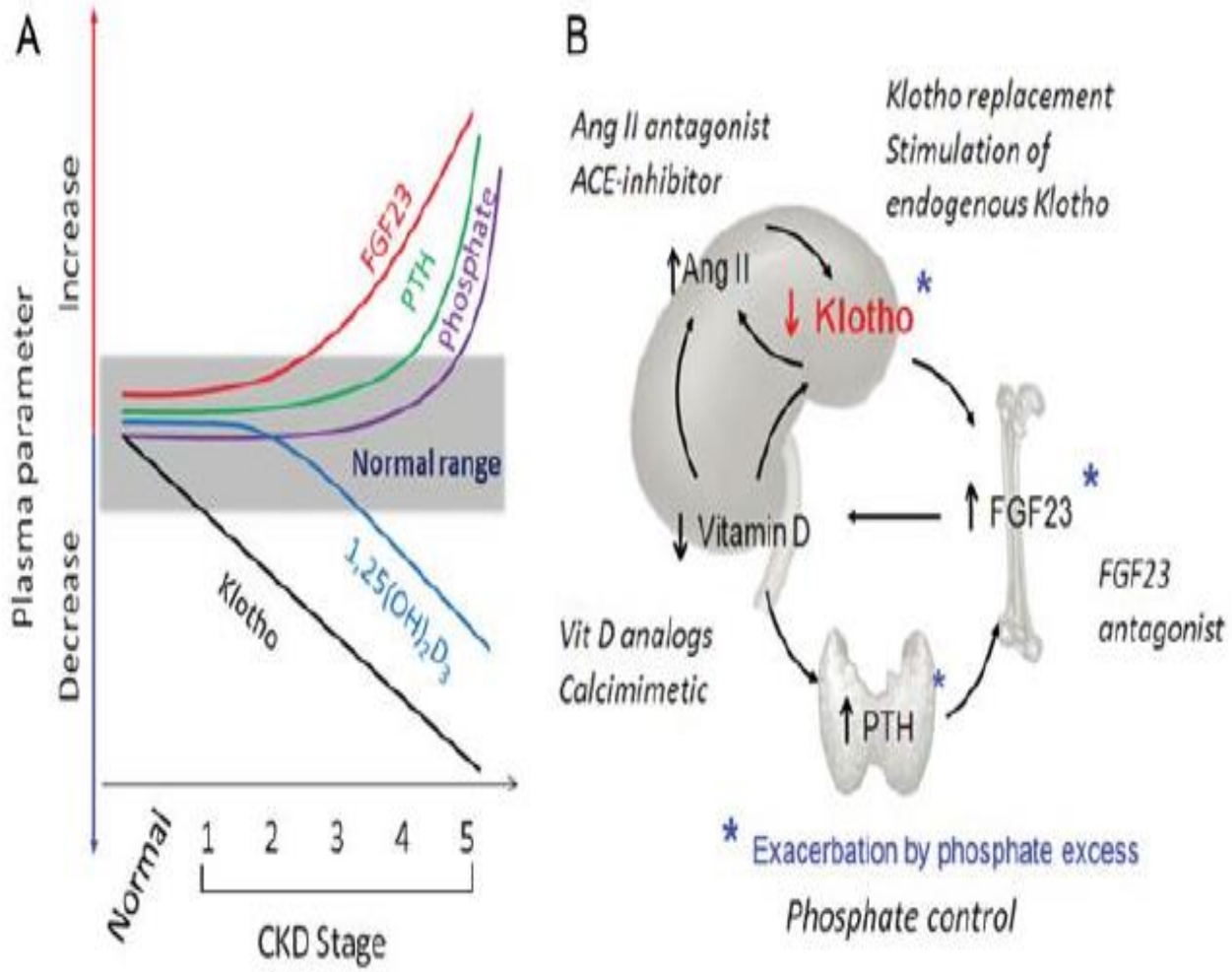




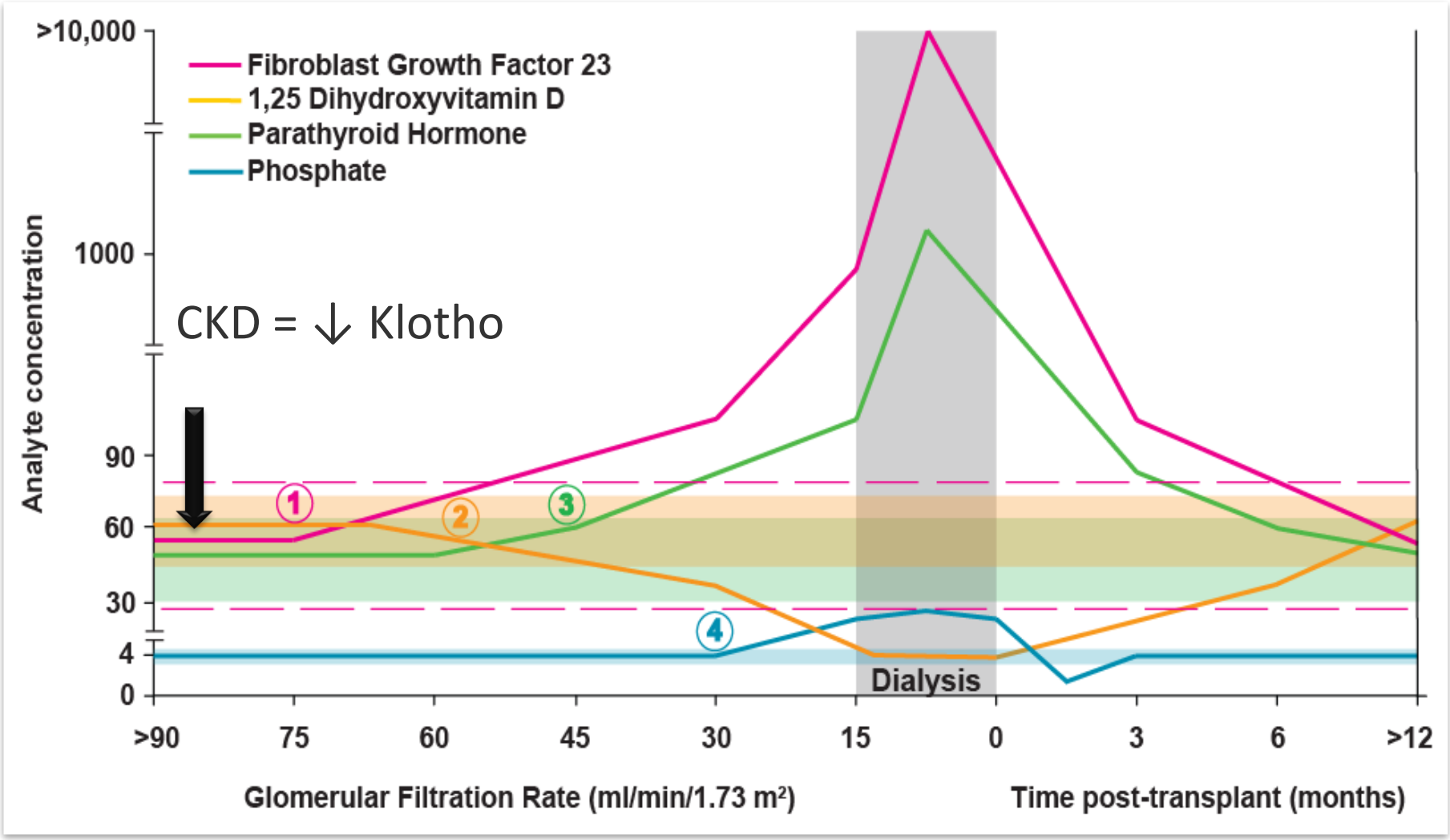
Klotho and FGF23 in CKD

- Epidemiological studies have identified high serum levels of phosphate and FGF23 as independent mortality risks in CKD patients
- Importantly, serum FGF23 levels increase before serum phosphate levels increase during the progression of CKD suggesting that
  - Resistance to FGF23 may be one of the earliest changes in phosphate metabolism in CKD
    - Although the mechanism of FGF23 resistance is yet to be determined, it can be caused by a decrease in renal Klotho expression

Nephrol Dial Transplant 2009;24: 1705–1708


A
Plasma parameter
Increase
Decrease
FGF23
PTH
Phosphate
Normal range
1,25(OH)2D3
Klotho
Normal
1
2
3
4
5
CKD Stage
B
Ang II antagonist
ACE-inhibitor
Klotho replacement
Stimulation of
endogenous Klotho
↑Ang II
↓ Klotho *
↓ Vitamin D
↑ FGF23 *
FGF23
antagonist
Vit D analogs
Calcimimetic
↑ PTH *
* Exacerbation by phosphate excess
Phosphate control

Klotho: Early decrease in CKD



Vascular Klotho Deficiency Potentiates the Development of Human Artery Calcification and Mediates Resistance to Fibroblast Growth Factor 23

Kenneth Lim, MD; Tzong-Shi Lu, PhD; Guerman Molostvov, MD, PhD; Christina Lee, BS; F.T. Lam, MD; Daniel Zehnder, MD, PhD; Li-Li Hsiao, MD, PhD

Background—Klotho is known to function as a cofactor for the phosphatonin, fibroblast growth factor (FGF)-23 at the kidney. FGF-23 levels rise in chronic kidney disease (CKD) despite progression of accelerated vascular calcification. There are currently conflicting data on whether FGF-23 may exhibit direct vasculoprotective effects in CKD.

Methods and Results—In this study, we describe for the first time endogenous Klotho expression in human arteries and human aortic smooth muscle cells. We show that CKD is a state of vascular Klotho deficiency promoted by chronic circulating stress factors, including proinflammatory, uremic, and disordered metabolic conditions. Mechanistic studies demonstrated that Klotho knockdown potentiated the development of accelerated calcification through a Runx2 and myocardin-serum response factor–dependent pathway. Klotho knockdown studies further revealed that vascular cells are a Klotho-dependent target tissue for FGF-23. FGF-23 mediated cellular activation of p-ERK, p-AKT, and cellular proliferative effects, which were abrogated following Klotho knockdown. We next showed that vascular Klotho deficiency driven by procalcific stressors could be restored by vitamin D receptor activators, in vitro and further confirmed using human arterial organ cultures from CKD patients, in vivo. Furthermore, restoration of suppressed Klotho expression by vitamin D receptor activators conferred human aortic smooth muscle cells responsive to FGF-23 signaling and unmasked potential anticalcific effects.

Conclusions—Chronic metabolic stress factors found in CKD promote vascular Klotho deficiency. Mechanistic studies revealed a bifunctional role for local vascular Klotho, first, as an endogenous inhibitor of vascular calcification and, second, as a cofactor required for vascular FGF-23 signaling. Furthermore, vitamin D receptor activators can restore Klotho expression and unmask FGF-23 anticalcific effects. (*Circulation*. 2012;125:2243-2255.)

Restoration of Klotho by VDR activation has been recently described in experimental animals

Lim K, et al. Circulation 2012;125:2243–55

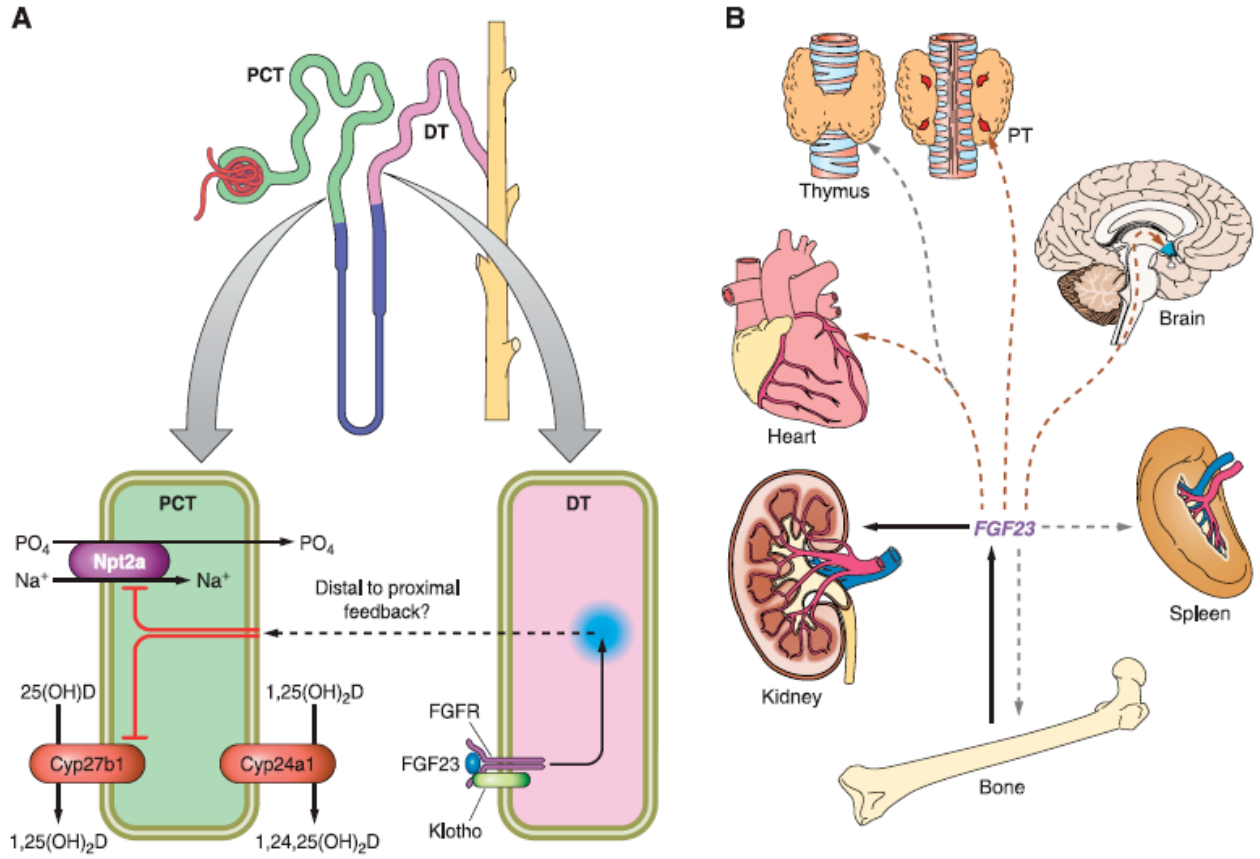
Physiol Rev 92: 131–155, 2012
doi:10.1152/physrev.00002.2011

REGULATION AND FUNCTION OF THE FGF23/KLOTHO ENDOCRINE PATHWAYS

Aline Martin, Valentin David, and L. Darryl Quarles

University of Tennessee Health Science Center, Memphis, Tennessee

REGULATION AND FUNCTION OF FGF23



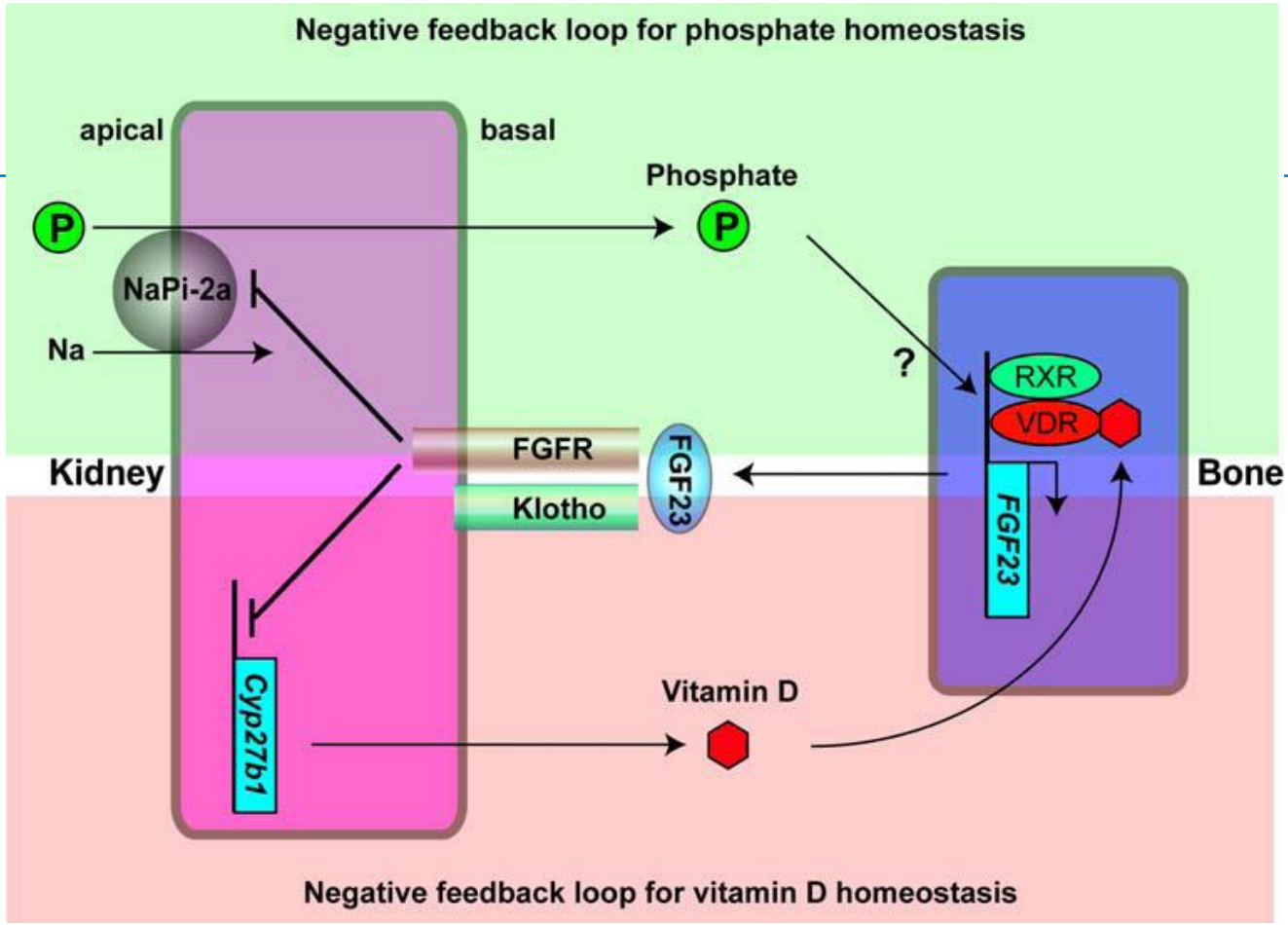


Fig. 1 The bone–kidney endocrine axis that regulates phosphate and vitamin D homeostasis. High serum phosphate increases FGF23 expression in the bone. FGF23 secreted from the bone binds to the Klotho–FGFR complex expressed on the kidney and suppresses phosphate reabsorption by inhibiting NaPi-2a (a negative feedback loop for phosphate homeostasis). High serum vitamin D increases FGF23 expression. Active vitamin D (1,25-dihydroxyvitamin D_3) binds to vitamin D receptor (*VDR*) in osteocytes, which forms a heterodimer with a nuclear receptor RXR and directly binds to a promoter region of the FGF23 gene to transactivate its expression. FGF23 acts on the Klotho–FGFR complex in the kidney and suppresses expression of *Cyp27b1* gene that encodes 1α-hydroxylase (a negative feedback loop for vitamin D homeostasis)

Pflugers Arch - Eur J Physiol 2010; 459:333–343

Regulation of FGF23 signaling by Klotho

- Klotho functions as an obligatory coreceptor for FGF23
  - Experimental studies have demonstrated convincingly that FGF23, in the absence of klotho, cannot exert its bioactivities
    - For instance, despite extremely high serum levels of FGF23 (about 2000-fold higher) in *klotho* ablated mice, FGF is unable to exert its phosphaturic effects in these mice

Kidney Int. 2008 ; 74(5): 566–570.

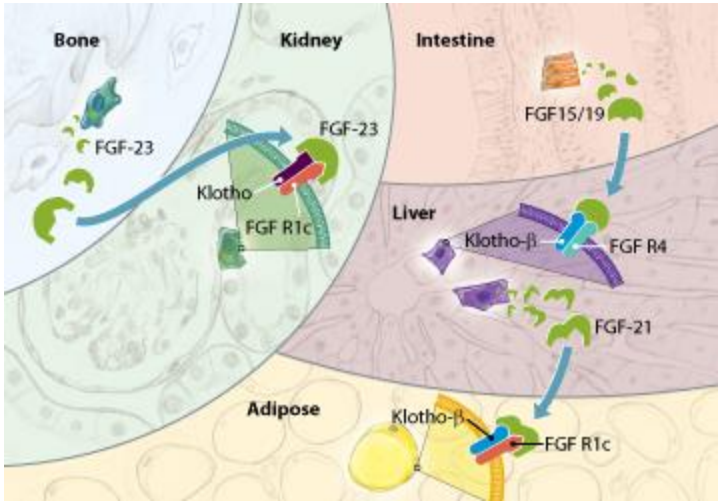
Regulation of FGF23 signaling by Klotho

- The fact that FGF23 requires Klotho as a co-receptor explains
  - Why Klotho-deficient mice develop phenotypes identical with those observed in FGF23-deficient mice and
  - Why Klotho-deficient mice had extremely high serum FGF23 levels

Nephrol Dial Transplant 2009;24: 1705–1708

Regulation of FGF23 signaling by Klotho

- Kidney-specific expression of Klotho explains why FGF23 can identify the kidney as its target organ among many other tissues that express multiple FGFR isoforms



Nephrol Dial Transplant 2009;24: 1705–1708

Klotho in Phosphate metabolism

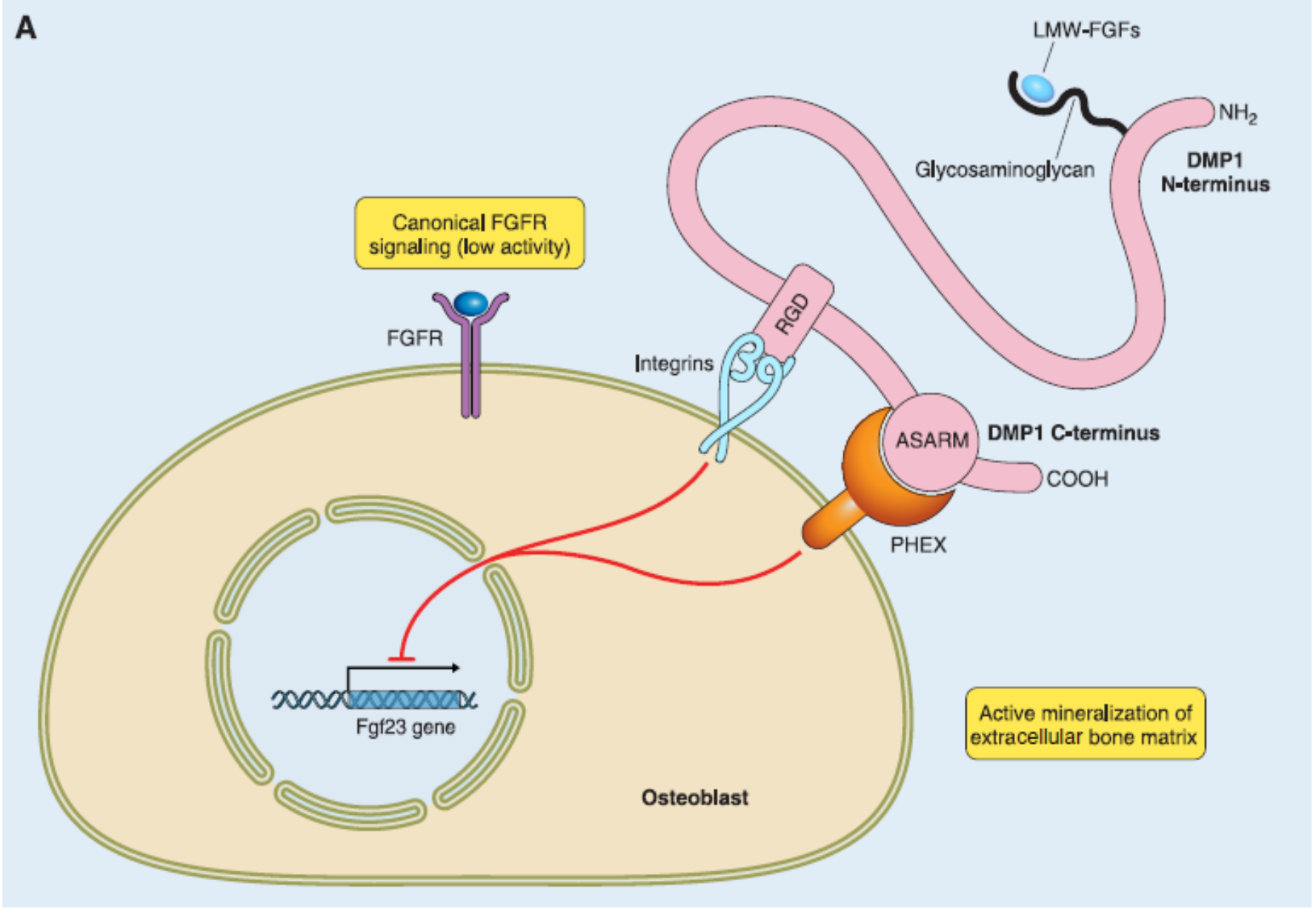
- The bone–kidney endocrine axis mediated by FGF23 and Klotho has emerged as an essential component in the regulation of phosphate homeostasis
  - When phosphate is in excess, FGF23 is secreted from bone and acts on the kidney where Klotho is expressed
  - As a phosphaturic hormone, FGF23 reduces the amount of sodium phosphate co-transporter type-2a (NaPi-2a) on the brush border membrane of proximal tubules, thereby promoting renal phosphate excretion

Nephrol Dial Transplant 2009;24: 1705–1708

Klotho in Phosphate metabolism

- As a counter-regulatory hormone for vitamin D, FGF23 suppresses synthesis and promotes inactivation of 1,25- dihydroxyvitamin D3 in proximal tubules.

Nephrol Dial Transplant 2009;24: 1705–1708



Physiol Rev 92: 131–155, 2012
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REGULATION AND FUNCTION OF THE FGF23/KLOTHO ENDOCRINE PATHWAYS

Aline Martin, Valentin David, and L. Darryl Quarles

University of Tennessee Health Science Center, Memphis, Tennessee

Regulation of FGF23 signaling by Klotho

- Conversion by Klotho of canonical FGF receptor into FGF23-specific receptor

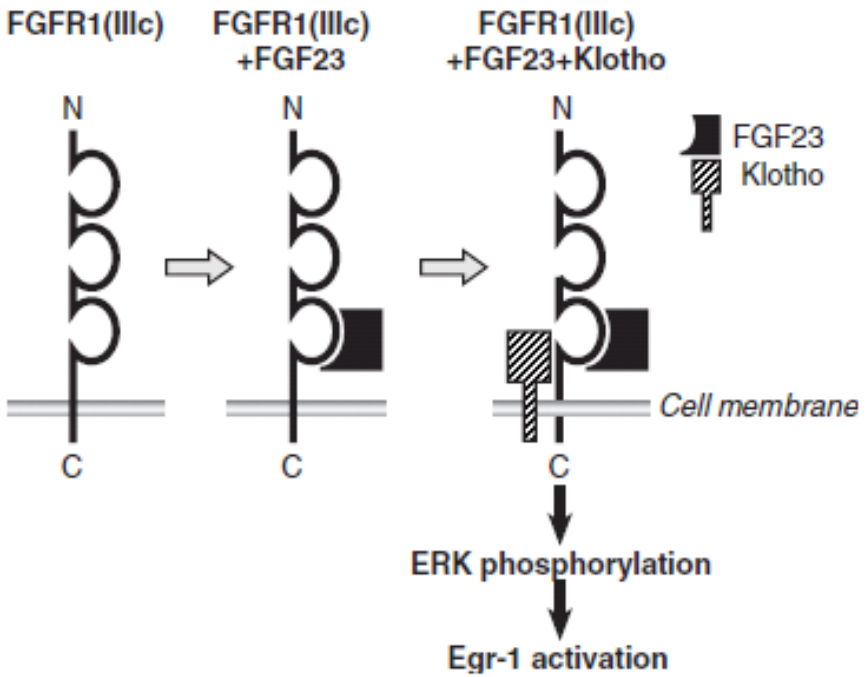
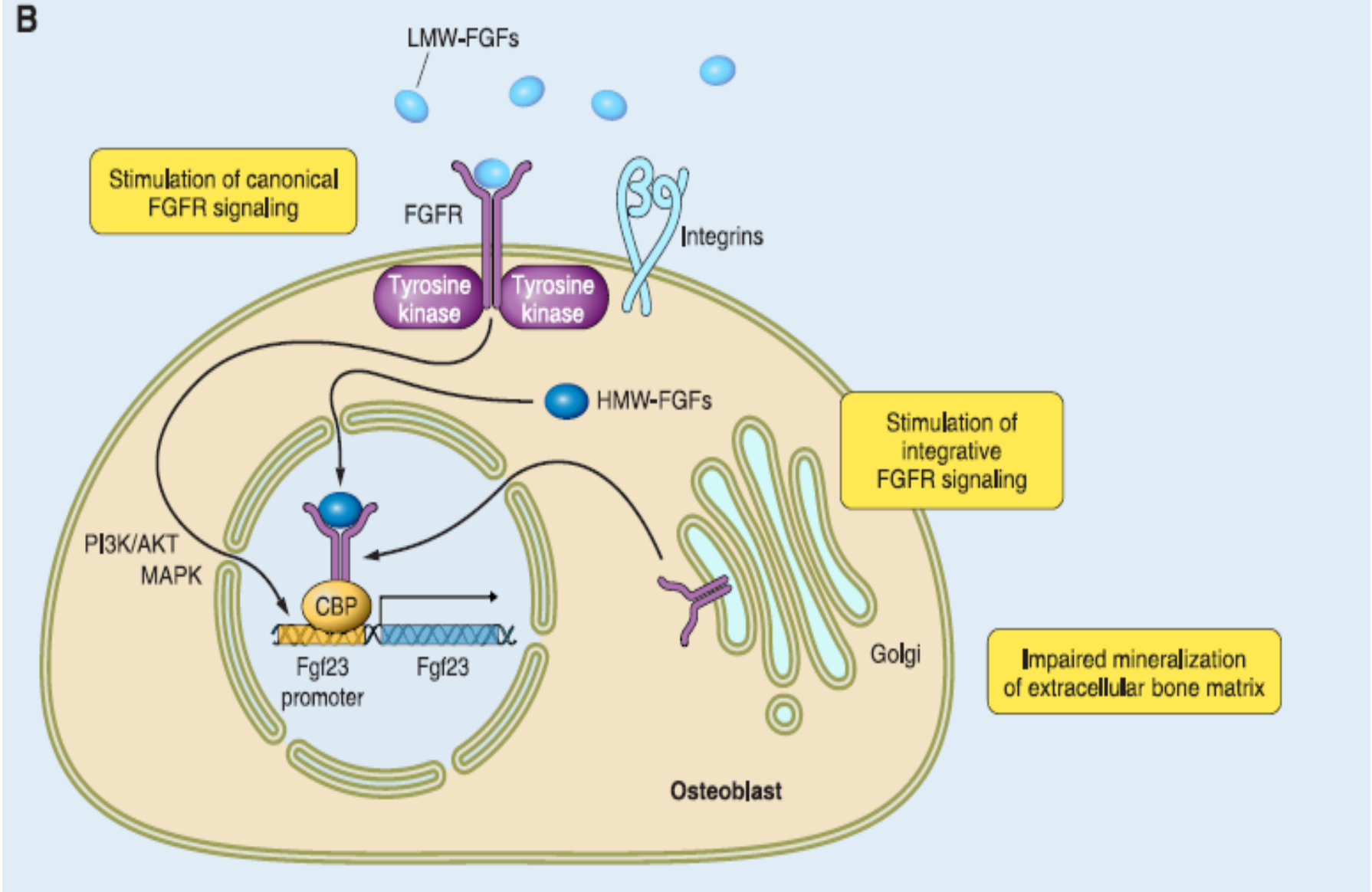


Fig. 2. Schematic view of the interaction between FGF23, Klotho and their receptor, FGFR1(IIIc).

Nephrol Dial Transplant 2007; 22: 1524–1526



Physiol Rev 92: 131–155, 2012
doi: 10.1152/physrev.00002.2011

REGULATION AND FUNCTION OF THE FGF23/KLOTHO ENDOCRINE PATHWAYS

Aline Martin, Valentin David, and L. Darryl Quarles

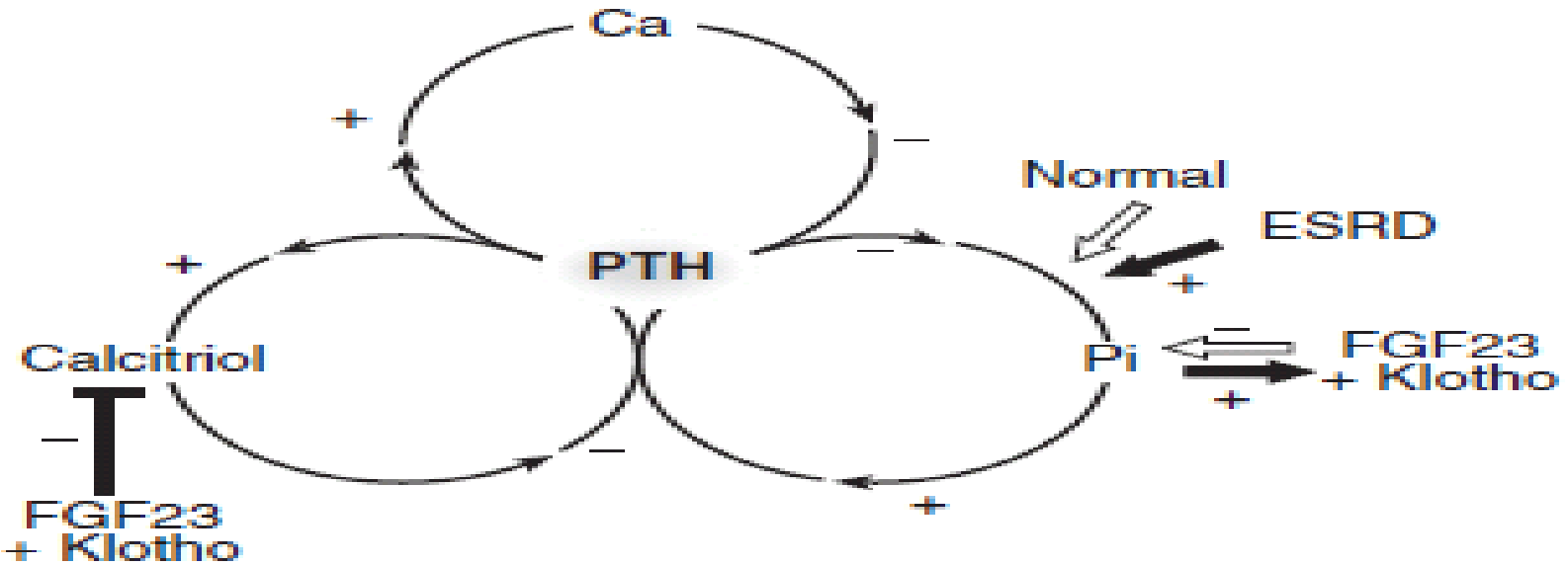
University of Tennessee Health Science Center, Memphis, Tennessee

There is growing evidence that another physiological function of FGF23 is to respond to changes in bone mineralization and turnover to adjust renal phosphate handling and balance the phosphate flux from bone. Bone is a buffer for minerals and can release calcium and phosphate into the circulation. Impaired mineralization would impair bone buffering capacity, leading to adaptive changes to excrete greater amounts of phosphate. Consistent with this possibility, there is an inverse relationship between FGF23 production by osteocytes and impaired mineralization.

free P_i for mineralization of cortical bone (6). These findings suggest that primary physiochemical-mediated impairment of mineralization can somehow stimulate FGF23 expression in bone. Alternatively, the finding of hypophos-

B) BONE TURNOVER CAN REGULATE FGF23 PRODUCTION. Low turnover bone disease, such as adynamic bone, leads to decreased phosphate buffering by bone, which could lead to increased production of FGF23, similar to defective mineralization. Consistent with this possibility, antiresorptive

Altogether, the concept that bone mineralization and turnover might regulate the local production of FGF23 is an interesting hypothesis that explains the existing observations but requires experimental validation.



Effect of FGF23 plus Klotho on renal tubular re-absorption of inorganic phosphate (iP) and synthesis of 1,25 dihydroxy vitamin D (calcitriol).

- **FGF23 + Klotho act synergistically with PTH to reduce tubular iP reabsorption. However, FGF23 + Klotho inhibit tubular calcitriol synthesis, in contrast to PTH which stimulates it**
- **In end-stage renal disease (ESRD), the physiological inhibition of tubular iP re-absorption by both PTH and FGF23 + Klotho becomes ineffective. The concomitant increase in PTH secretion leads to excessive iP release from bone into the extracellular space. The clinical consequence is hyperphosphataemia.**

Nephrol Dial Transplant 2007; 22: 1524–1526

Fibroblast Growth Factor 23

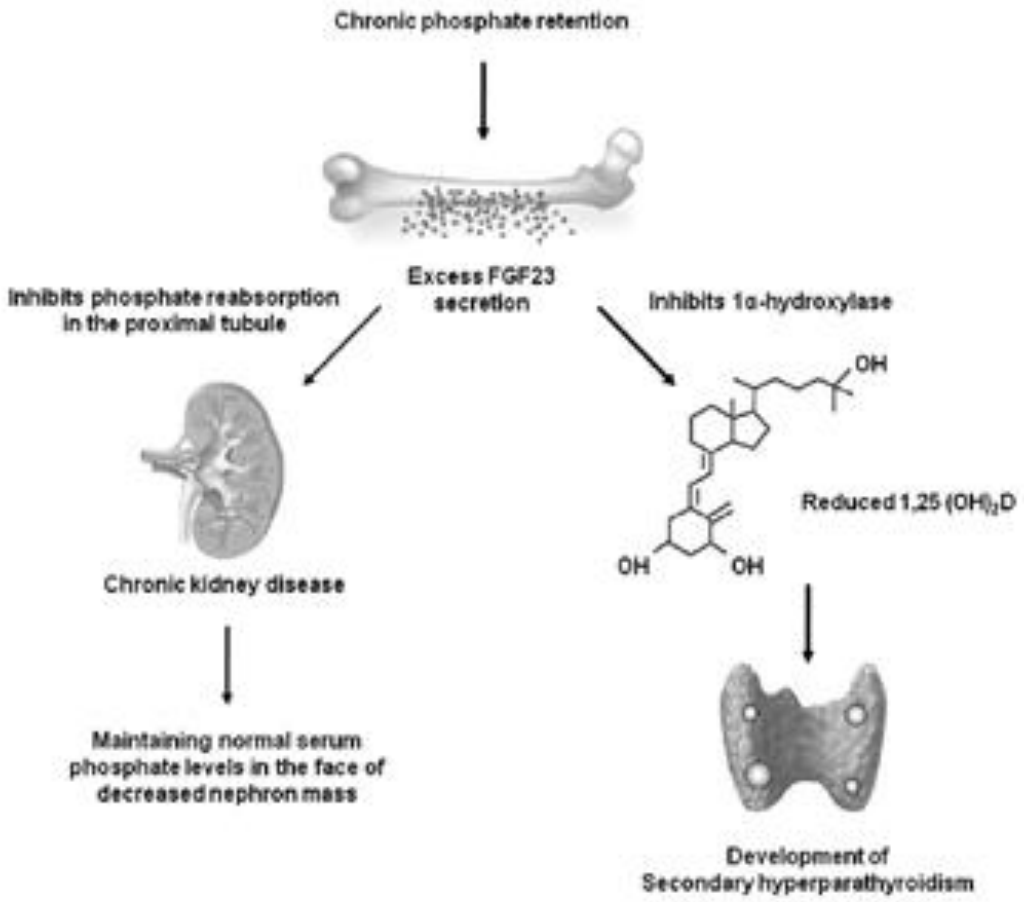
- Is a **phosphatonin**, i.e. hormone regulating P excretion
- FGF-23 produced exclusively in osteocytes and bone-lining cells
- Synthesis ↑ by P, PTH and calcitriol
- Most rapid inducer of expression is calcitriol, with the factor going up within 3-4 hrs of IV administration
- The physiologic stimulus is thought to be high dietary phoshorus intake but it is not clear whether the serum P level or the net intake (?intestinal sensor) provides the proximal signal
- FGF-23 receptor in the kidney requires the coreceptor klotho (also found in the parathyroids), which ↓ in aging and CKD

Actions of FGF-23

- Downregulates luminal sodium/phosphate cotransporters in the proximal tubule, decreasing phosphorus reabsorption and therefore increasing its excretion
- Inhibits 1α-hydroxylase (*CYP27B1*), decreasing the conversion of 25-hydroxyvitamin D (25[OH]D) to 1,25-dihydoxyvitamin D (1,25$[OH]_2D_3$; calcitriol).
- **It is thought that FGF-23 rather than decreased renal mass is the major factor downregulating calcitriol in CKD**
- Stimulates 24-hydroxylase (*CYP24*), leading to vitamin D degradation
- Inhibits PTH secretion in the short term but probably not in the long term (↓ VDRA signaling leading to parathyroid resistance)

Klotho and FGF23 in CKD

- It remains to be determined whether renal Klotho expression levels reflect functional nephron mass that can respond to FGF23



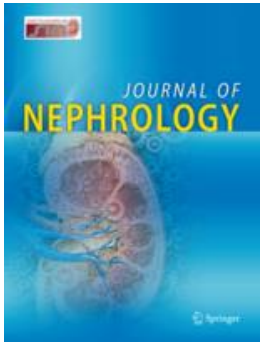


Fig. 1 - Role of FGF23 in patients with early-stage chronic kidney disease (CKD). In patients with early-stage CKD, FGF23 secretion is increased to maintain a neutral phosphate balance, but this results in suppression of renal $1,25(OH)_2D$ production and thereby triggers the early development of secondary hyperparathyroidism. $1,25(OH)_2D$ = 1,25-dihydroxyvitamin D; FGF23 = fibroblast growth factor 23.

J Nephrol 2010; 23(06): 619-625

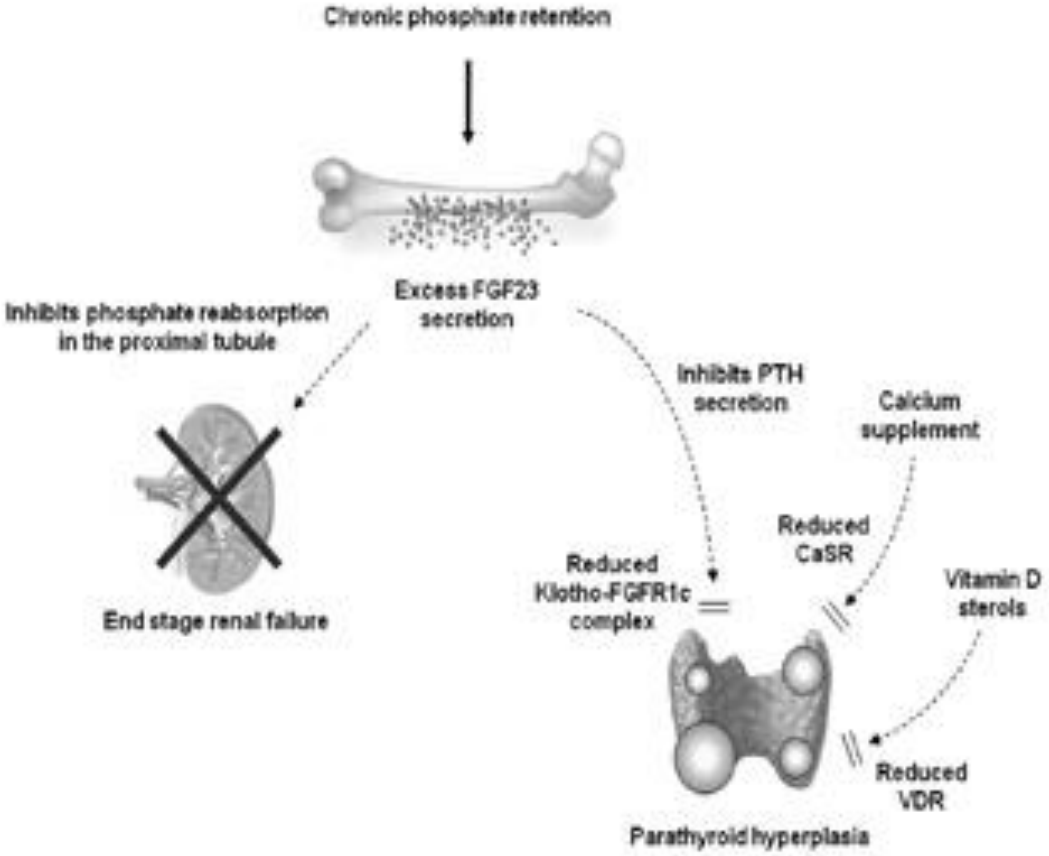
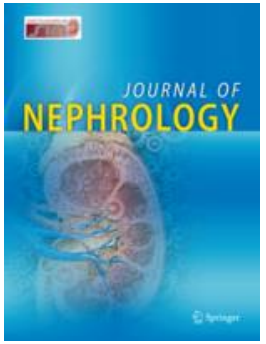


Fig. 2 - Role of FGF23 in dialysis patients. In dialysis patients without functioning kidneys, FGF23 secretion is markedly elevated in response to chronic phosphate retention and active vitamin D therapy. In this setting, increased FGF23 should act on the parathyroid as a negative regulator, but fails to suppress PTH secretion. This parathyroid resistance to FGF23 may be due to down-regulation of the Klotho-FGFR1c complex. CaSR = calcium-sensing receptor; FGF23 = fibroblast growth factor 23; FGFR1c = fibroblast growth factor receptor 1c; PTH = parathyroid hormone; VDR = vitamin D receptor.



J Nephrol 2010; 23(06): 619-625

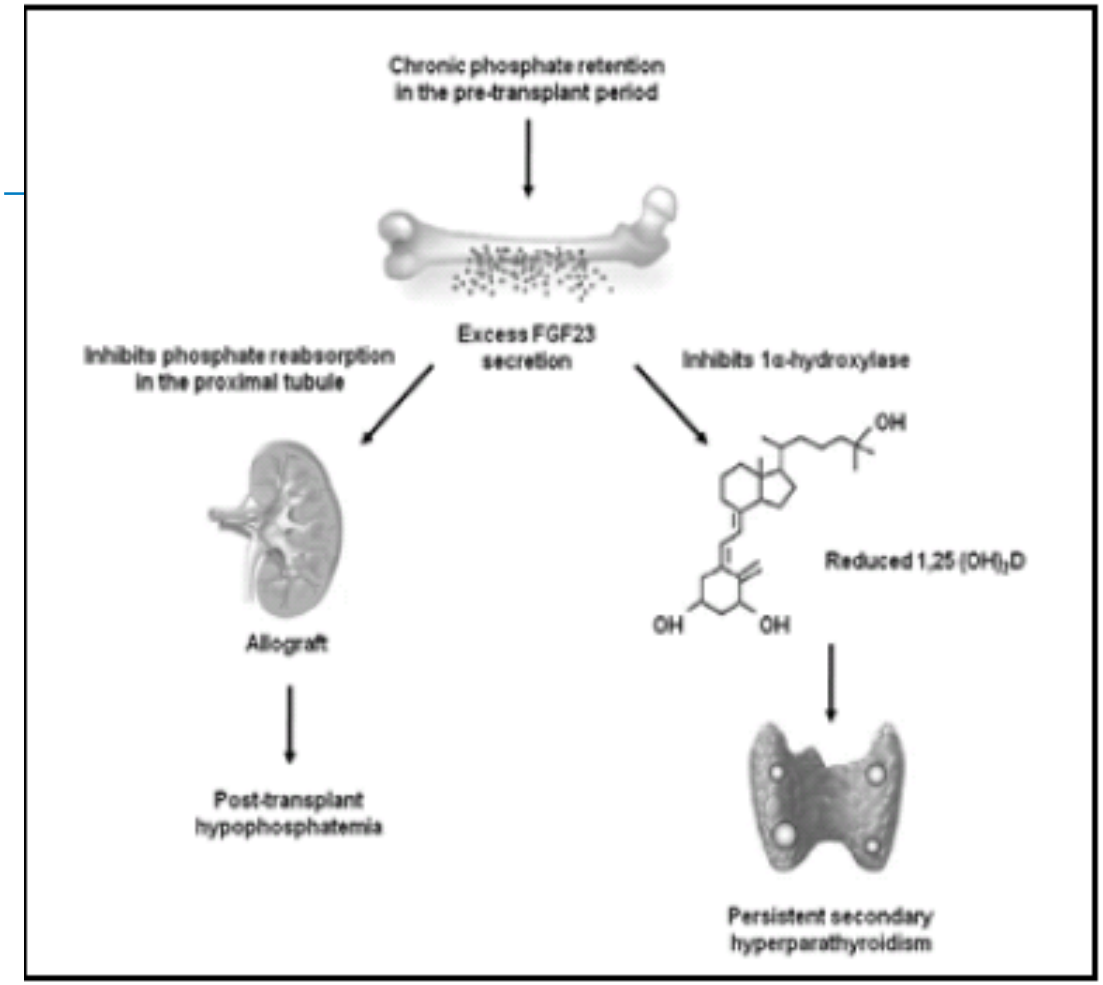
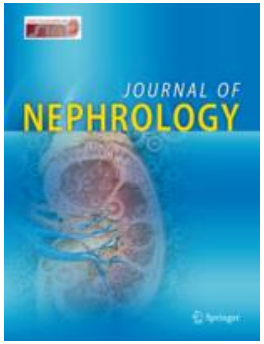
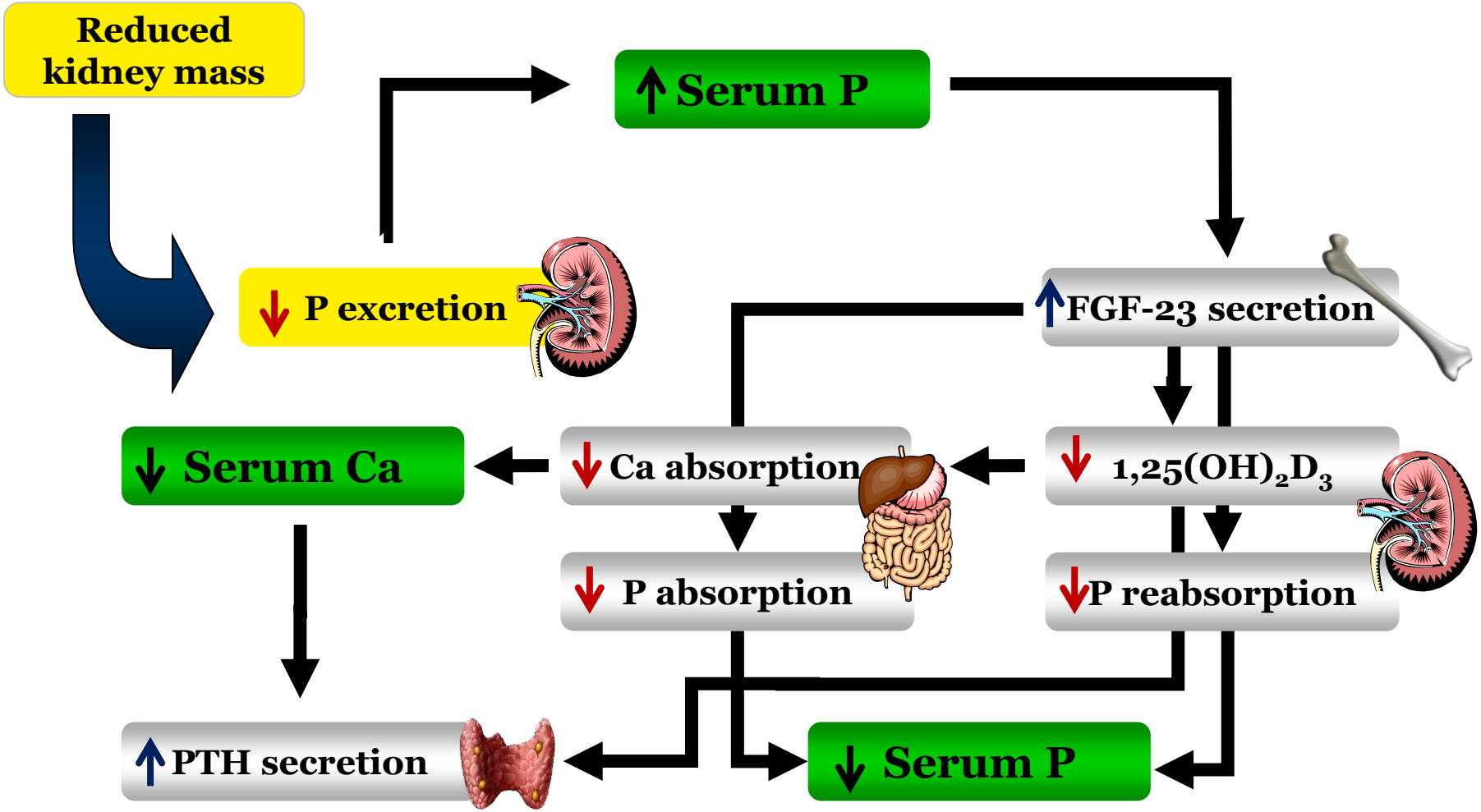


Fig. 3 - Role of FGF23 in renal transplant patients. In patients undergoing kidney transplantation, elevated FGF23 due to chronic phosphate retention in the pretransplant period acts on the allograft to promote phosphaturia and suppress $1,25(OH)_2D$ production. This results in post-transplant hypophosphatemia and persistent secondary hyperparathyroidism. $1,25(OH)_2D$ = 1,25-dihydroxy vitamin D; FGF23 = fibroblast growth factor 23.



J Nephrol 2010; 23(06): 619-625


Reduced kidney mass
↑ Serum P
↓ P excretion
↑ FGF-23 secretion
↓ Serum Ca
↓ Ca absorption
↓ 1,25(OH)2D3
↓ P absorption
↓P reabsorption
↑ PTH secretion
↓ Serum P

Conclusions

regulation and function. We have advanced the hypothesis that the principal physiological functions of FGF23 and the reason for its predominant expression in bone is that this hormone functions to protect the organism from the toxic effects of excess phosphate and $1,25(OH)_2D$. We propose that $1,25(OH)_2D$ directly regulates FGF23 transcription in osteoblasts/osteocytes, but that phosphate, rather than directly modulating FGF23, has indirect effects that are mediated through the effects of phosphate on extracellular matrix mineralization. We also propose that bone formation and the bone buffering capacity of bone provides the mechanistic link between phosphate and regulation of FGF23 through the regulation of extracellular matrix factors involving the endopeptidase PHEX and the SIBLING protein dentin matrix protein 1 (DMP1). Finally, regulation of FGF23 is integrated with mineral and energy metabolism through cross-talk between PTH, $1,25(OH)_2D$, leptin, and secreted Klotho.

Klotho in CKD

- Klotho is an early biomarker for CKD.

- Replacement of Klotho may have therapeutic potential for CKD.

J Am Soc Nephrol. 2011 Jan;22(1):124-36.

Conclusions

- It has become increasingly clear that phosphate metabolism plays a critical role in the pathophysiology in CKD and that

- Hyperphosphataemia should be aggressively treated to improve life expectancy of CKD patients
  - In this context, the bone–kidney–parathyroid endocrine axis mediated by Klotho and FGF23 is expected to be a novel target of therapeutic intervention

Conclusions

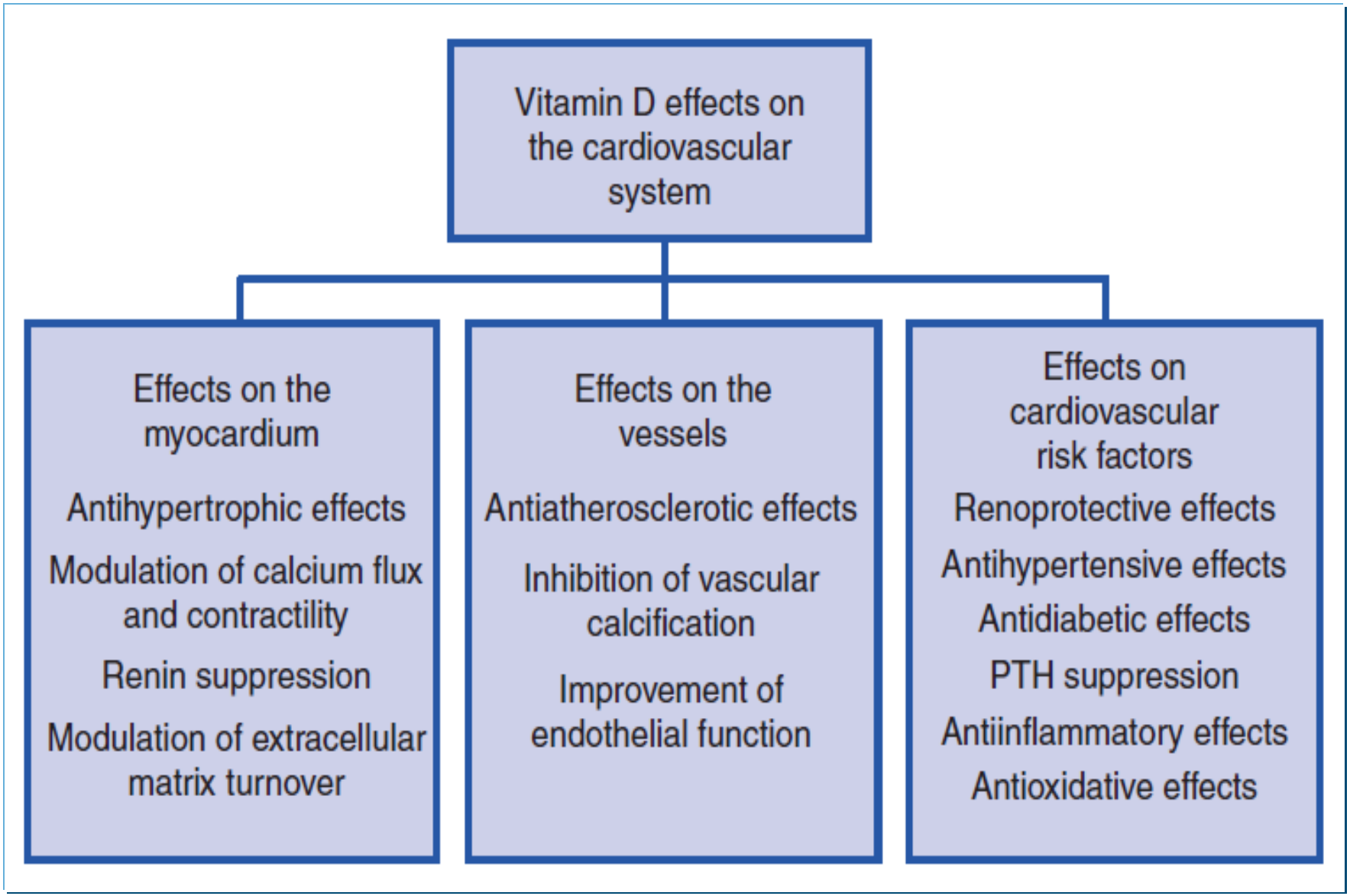
- FGF23 - Klotho plays a central role in the pathogenesis of altered mineral metabolism and secondary hyperparathyroidism in CKD patients
- FGF23 - Klotho can be used not only as a biomarker for assessing phosphate retention but also as a predictor of mortality and future development of refractory hyperparathyroidism
  - Further elucidation of FGF23 function and regulation will help to establish a more rational approach for the management of the mineral and bone disorders that are associated with high burden of morbidity and mortality in CKD patients

Fibroblast growth factor 23: friend or foe in uremia?

Orson W. Moe

Department of Internal Medicine, Department of Physiology, and Charles and Jane Pak Center of Mineral Metabolism, University of Texas Southwestern Medical Center, Dallas, Texas, USA.

therapeutics in CKD. Instead, it should alert the field of the need to abandon the overly simplistic FGF23 toxicity model. Efforts should be directed toward defining the roles of FGF23 in physiology and CKD. What is actually driving up the FGF23 levels in CKD? What are the optimal levels in various stages of CKD for its different roles? What are the ways to enhance endogenous on-target signaling as adaptive reactions and decrease the off-target effects? Instead



Pilz et al, Kidney Int Suppl 2011

Mizobuchi M et al J Steroid BMBiol 2010

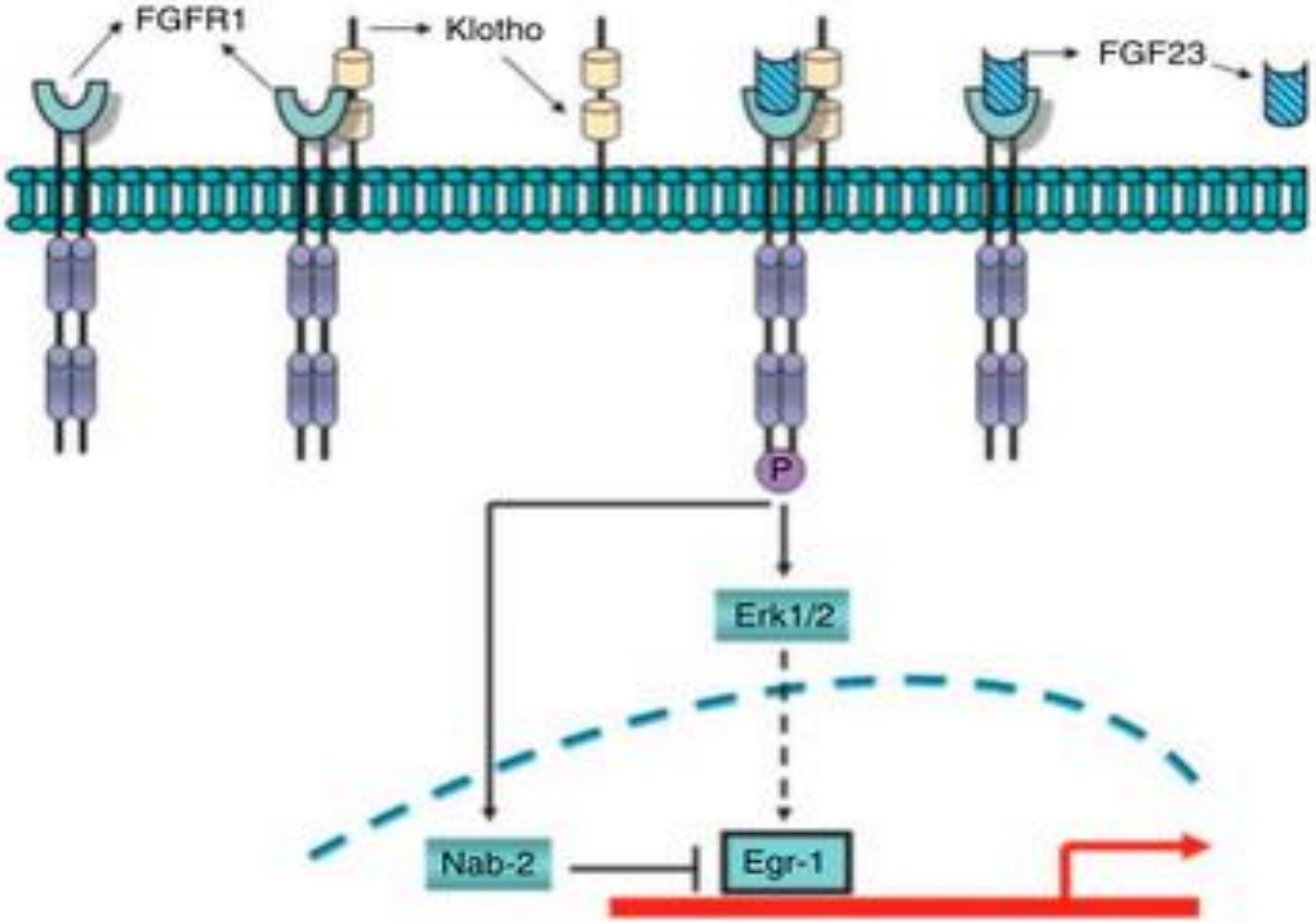
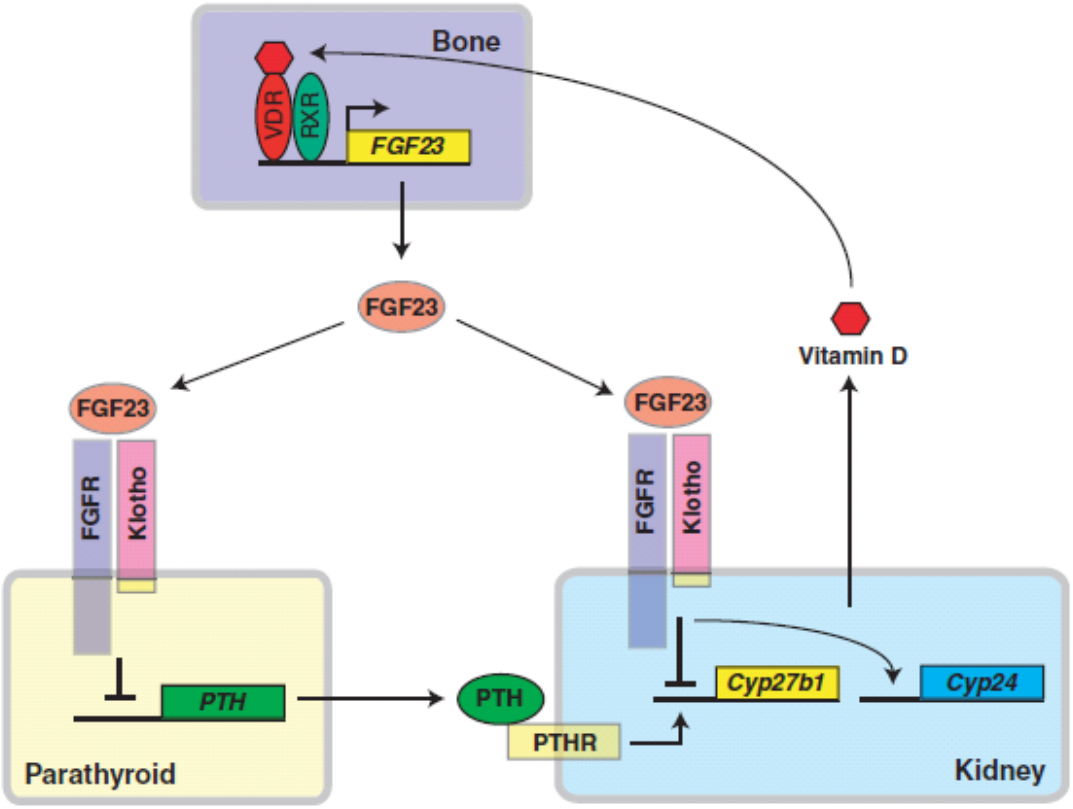


Figure 3 Schematic outline of FGF23–FGFR interactions. Signaling of FGF23–FGFR involves klotho as a cofactor to induce such downstream signaling molecules as Erk1/2, which could influence the activation of Egr-1. FGF23 also induces Nab-2 (Fukuda *et al.* 2007), specific corepressor of Egr-1 that could suppress the transcriptional activity of Egr-1, and thereby establish a negative feedback loop to regulate physiological activities of FGF23.

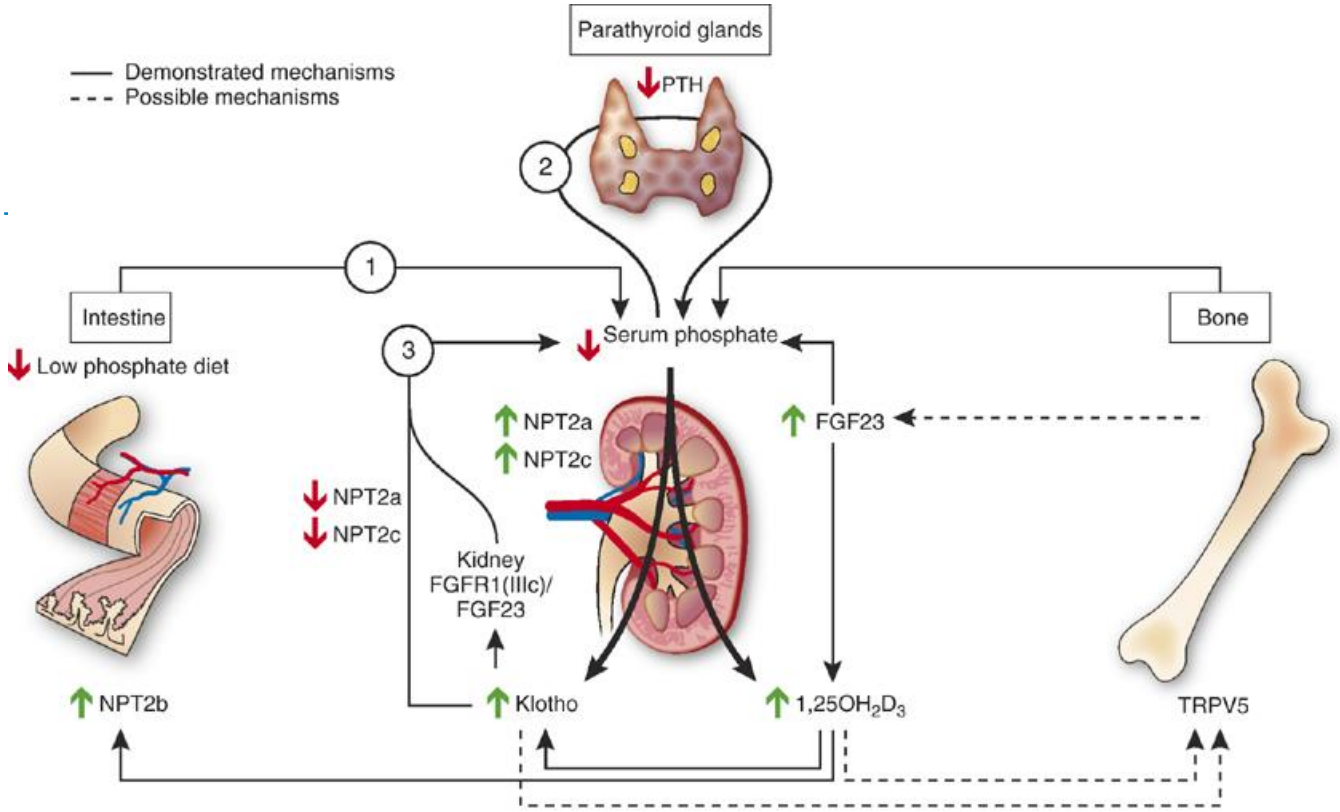
The bone–kidney–parathyroid endocrine axes mediated by FGF23 and Klotho



Active vitaminD(1,25-dihydroxyvitaminD3) binds to the vitamin Dreceptor (VDR) in osteocytes. The ligand-boundVDRforms a heterodimerwith a nuclear receptor RXRand transactivates the FGF23 gene expression.
FGF23 secreted from bone acts on the Klotho–FGFR complex in kidney (the bone–kidney axis) and parathyroid gland (the bone–parathyroid axis).
In kidney, FGF23 suppresses synthesis of active vitamin D by down-regulating expression of the *Cyp27b1* gene and promotes its inactivation by up-regulating expression of the *Cyp24* gene, thereby closing a negative feedback loop for vitamin D homeostasis. In the parathyroid gland, FGF23 suppresses production and secretion of PTH. Since PTH is a potent inducer of *Cyp27b1* gene expression, suppression of PTH by FGF23 reduces expression of the *Cyp27b1* gene as well as serum levels of 1,25-dihydroxyvitamin D3, which closes another long negative feedback loop for vitamin D homeostasis. Klotho and FGF23 are indispensable for the regulation of vitamin D metabolism because defects in either Klotho or FGF23 cause hypervitaminosis D.

Nephrol Dial Transplant 2009;24: 1705–1708

regulation by klotho: Hypotheses.

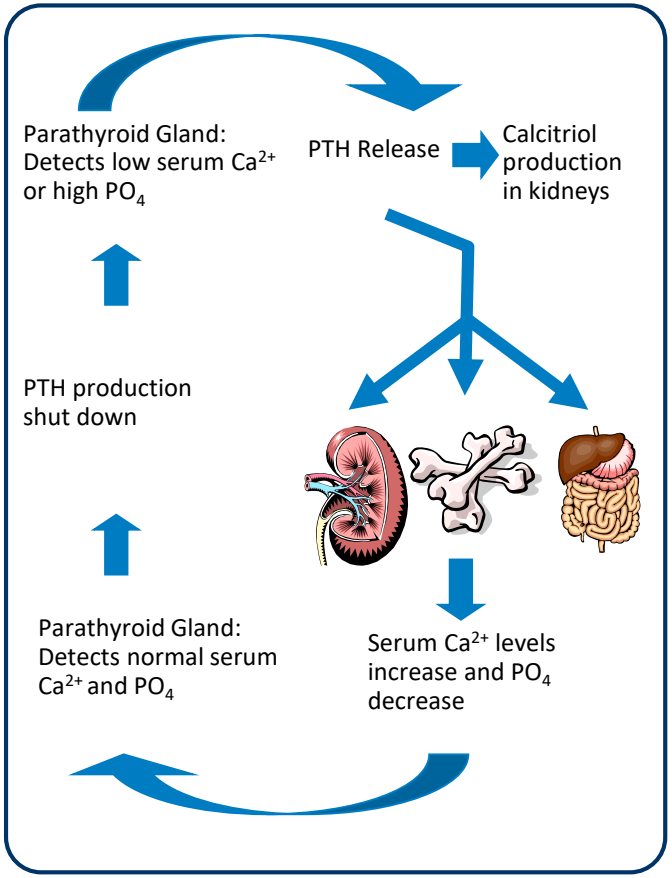


The first arrow starts from the intestine, where a reduced dietary phosphate intake diminishes serum phosphate concentration and leads to a decrease in PTH secretion, which physiologically reduces urinary phosphate excretion. In addition, to save phosphate, the renal action of FGF23 will decrease facilitating tubular phosphate reabsorption by the stimulation of sodium-dependent phosphate cotransporters (NPT2a and NPT2c). It will also facilitate the synthesis of 1,25(OH)2D3 in spite of low PTH levels. The increase in calcitriol levels stimulates sodium-dependent phosphate cotransporter type IIb expression and intestinal phosphate absorption. Then, to counteract the activation of these three phosphate-saving mechanisms and to avoid hyperphosphatemia, the renal synthesis of klotho is increased. This increase in renal klotho will facilitate the phosphaturic action of FGF23. Klotho binds to FGFR1(IIIc) and forms the specific FGF23 receptor. Furthermore, klotho negatively regulates the synthesis of 1,25(OH)2D3 by enabling FGF23 binding to its receptor and thereby its inhibitory effect on 1--hydroxylase activity. At the bone level, klotho could stimulate bone resorption and phosphate release by acting on TRPV5, which is a recently identified osteoclast function modulator. The increased levels of 1,25(OH)2D3 could also stimulate osteoclast differentiation and bone resorption and thereby phosphate release. It could also stimulate skeletal FGF23 synthesis to control further, at the renal level, any excessive increase in serum phosphate resulting from the activation of the prophosphatemic mechanisms. Abbreviations: PTH, parathyroid hormone; FGF23, fibroblast growth factor-23; TRPV5, epithelial calcium channel TRPV5 (transient receptor potential vallinoid-5).

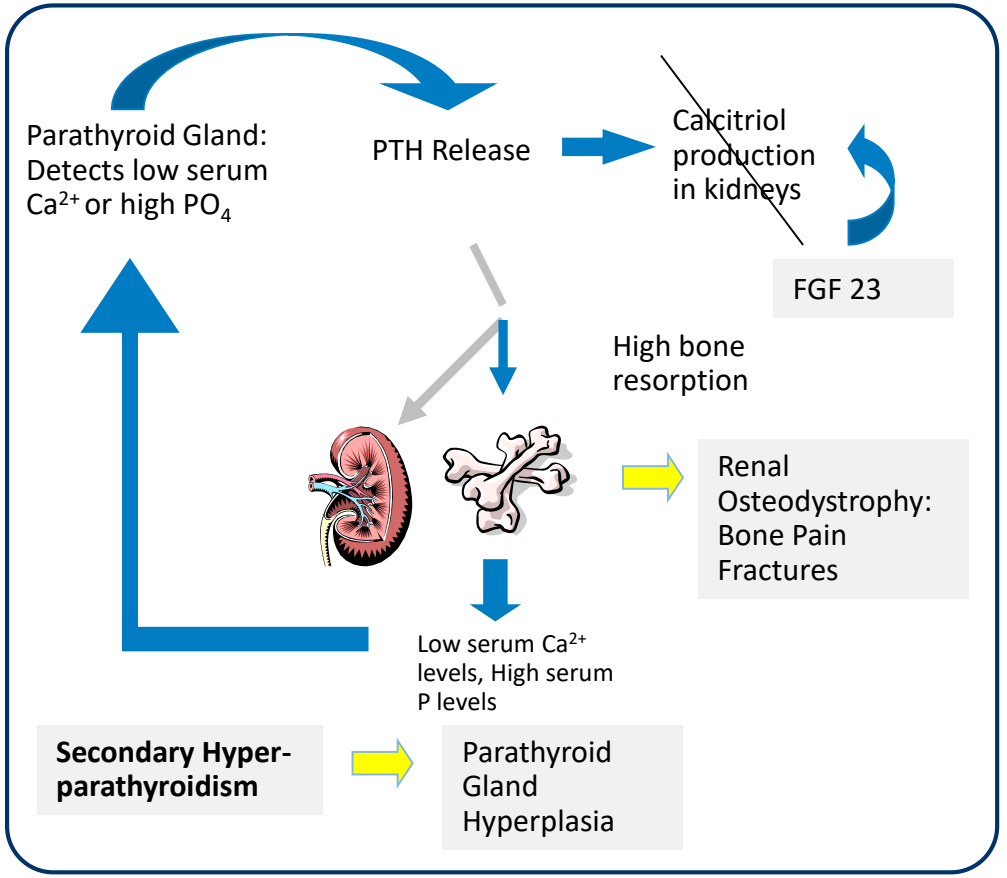
Kidney Int. 2007 Apr;71(8):730-7.

SHPT

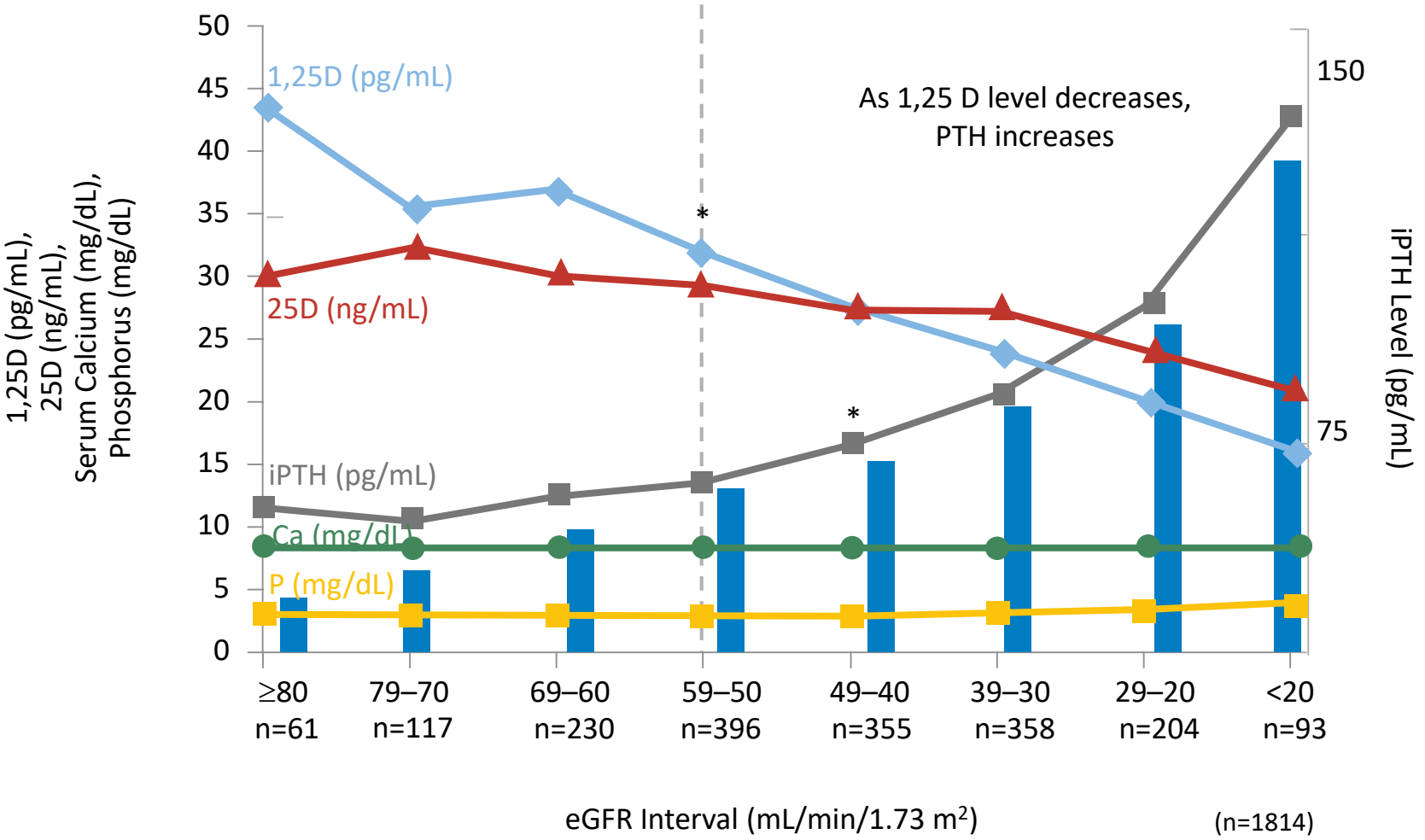
HEALTHY PERSON: MAINTAINING THE CA^{2+} – PO_4 BALANCE



ESRD PATIENT: DEVELOPMENT OF SECONDARY HYPERPARATHYROIDISM



Reduced VDR Activation in SHPT

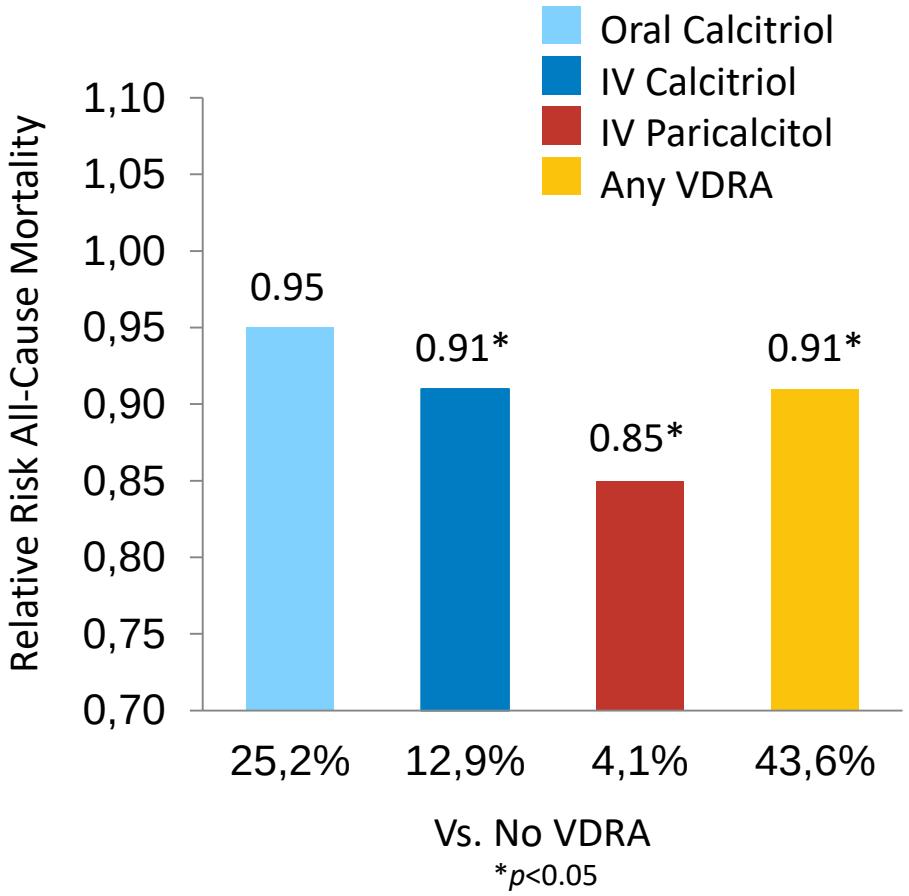


*P<.001.

Levin, et al. *Kidney Int*. 2007;71:31-38.

VDRAs on Survival

Association Between Different VDRAs in HD



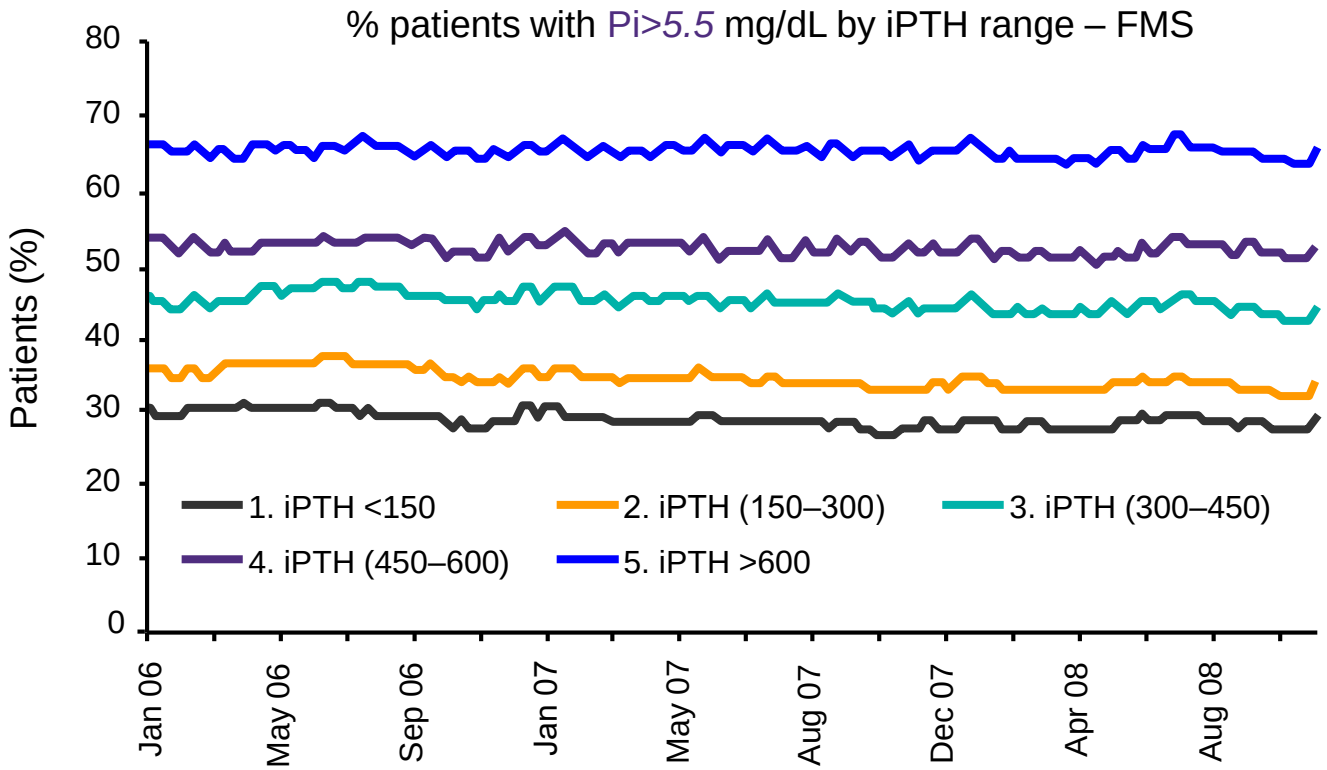
- Prospective cohort study
- 1996–2004 (n=29,696) at 627 facilities
- Adjusted for comorbidities and lab results
- Results of time-dependent analysis:
  - Paricalcitol's relative risk (RR) for all-cause time-adjusted mortality was 0.85 compared to 0.91 for IV calcitriol ($p<0.05$) and 0.95 for oral calcitriol
  - Paricalcitol associated with a 15% lower mortality rate compared with no VDR activator therapy

DOPPS
Dialysis Outcomes and Practice Patterns Study

Tentori F, et al. *Nephrol Dial Transplant.* 2009;24:963-972.

Link between phosphate and PTH control:

In patients with high iPTH levels uncontrolled P levels are more common



Data on file Fresenius Medical Care US, data as of Nov 2008, n=221,000

THANK YOU