Phosphate regulation revisited – New genes, new molecules

Gérard Friedlander, M.D., Ph.D.
Phosphate Homeostasis

46 mmoles (1400mg) → 1,25(OH)₂D₃ → 36 mmoles

NPT2b

16 mmoles (500mg) → PTH 1,25(OH)₂D₃ → 30 mmoles (900mg)

NPT2a/c

14% → PiT1/2 → Extracellular medium → 6 mmoles

85% → PiT1/2 → Extracellular medium → 6 mmoles → PTH 1,25(OH)₂D₃
Renal Pi cotransporters

Lumen → Proximal convoluted tubule → Blood

- **Npt1**
- **Npt2a**
- **PiT2**
- **Npt2c**

- **2 Na⁺**, **HPO₄²⁻**
- **2 Na⁺**, **HPO₄²⁻**
- **3 Na⁺**, **HPO₄²⁻**
- **2 Na⁺**, **HPO₄²⁻**

**Regulators:**
- PTH
- PO₄²⁻
- Vit D
- FGF23

**PiT1**
Regulation of tubular phosphate reabsorption
Serum Phosphate and longevity

Serum Phosphate and mortality

Tonelli Circulation 2005
Serum Phosphate and mortality

Tonelli Circulation 2009
Diet restriction and longevity

Fontana Science 2010
Diet restriction and longevity

Fontana Science 2010
Yeast

Dietary restriction

Glucose
Amino acids

Gpr1
TOR
Sch9
RIM15
Nucleus

Cytoplasm

Acetyl coenzyme A (CoA)

RAS

Activation of anti-aging transcription factors

HIF-1
DAF-16
FOXO

Protective and metabolic activities that increase life span

Glycogen accumulation (except flies and mammals), glycerol accumulation (only yeast), fat accumulation (except yeast), antioxidant enzyme SOD, catalase (except flies), HSPs (except mammals), autophagy, translation, ER stress, other?

Anti-aging

Worms

Dietary restriction

Ins/IGF-1–like

DAF-2

Inhibition of nutrient-sensing pathways

CHICO

Flies

Dietary restriction

Ins/IGF-1–like

INR

Activation of anti-aging transcription factors

S6K

Protective and metabolic activities that increase life span

Anti-aging

Mammals

Dietary restriction

IGF-1

GHR

Production of anti-aging factors

S6K

Protective and metabolic activities that increase life span

Anti-aging

Fontana Science 2010
Yeast

Dietary restriction

Glucose
Amino acids

Gpr1
RAS
Sch9 (S6K)
TOR
AC
PKA
RIM15
MSN2/4
Cytosol

Nucleus

GIS1
HIF-1
DAF-16
FOXO
4E-BP
Anti-aging

Protective and metabolic activities that increase life span

Glycogen accumulation (except flies and mammals), glycerol accumulation (only yeast), fat accumulation (except yeast), antioxidant enzyme SOD, catalase (except flies), HSPs (except mammals), autophagy, translation, ER stress, other?

Anti-aging

Worms

Dietary restriction

Ins/IGF-1-like
DAF-2
INR
CHICO
RAS
PI3K
AKT
S6K
FOXO

Flies

Dietary restriction

Ins/IGF-1-like
DAF-16
FOXO

Mammals

Dietary restriction

IGF-1
GH
IGF-1R
GHR
RAS
PI3K
AKT
S6K
FOXO

Inhibition of nutrient-sensing pathways

Activation of anti-aging transcription factors
The Bone-Kidney axis

Kuro-o Mech Ageing Dev 2010
Fibroblast Growth Factor 23

- Identified in patients with Autosomal Dominant hypophosphatemic Rickets (ADHR), or tumoral osteomalacia
- Plasma concentration in healthy subjects < 50 pg/ml.
- 32 kd circulating glycosylated peptide, 251 amino acids.

Site of synthesis: Bone, brain, parathyroid gland, thymus, liver
Physiological role of FGF23

**FGF23 regulates serum phosphate concentration**

1- Reduces renal phosphate reabsorption by decreasing the expression of phosphate transporters

2- Decreases calcitriol concentration
   Inhibits $1\alpha$ hydroxylase activity
   Stimulates 24 hydroxylase activity

3- Inhibits phosphate intestinal absorption via calcitriol decrease

4- Decreases PTH mRNA expression and PTH secretion
FGF23 Overexpression

Renal phosphate leak with low calcitriol concentration and marked bone demineralization

Shimada, PNAS 2001; 98, 6500
Control of FGF23 concentration

Dietary Pi

Serum Pi

Serum calcitriol

Ferrari 2005 JCEM 90, 1519.

Burnett 2006 JBMR 21, 1187

Liu 2006 JASN 17, 1305
Klotho deficient or KO mice exhibit a phenotype similar to that of FGF23-/- mice but serum FGF23 concentration is increased. Can Klotho be the FGF23 receptor?

Klotho is a 1014-AA single pass transmembrane protein

- KL1
- KL2

Secreted form

Shedding by ADAMs 10, 17

Stimulated by insulin

Alternative splicing

FGF23

Klotho

FGFR

ERK
Klotho expression

Kidney: distal convoluted tubule

Kuro-o, Nature 1997, 390:45

Parathyroid

DCT and PT glands express FGFRs

Li Cell Struct Funct 2004, 29: 91

Ben-Dov, JCI 2007; 117, 12: 4003
Autosomal Dominant Hypophosphatemic Rickets: ADHR
Mutations in FGF23: resistant to proteolytic cleavage

X-Linked Hypophosphatemic Rickets: XLH. Mutations in PHEX

Autosomal Recessive Hypophosphatemia: ARH. Mutations in DMP1

Fibrous dysplasia, McCune Albright syndrome: post zygotic
mutations in GNAS

Phenotype of patients
- Severe bone demineralization, rickets
- Hypophosphatemia, low TmP/GFR
- Serum calcium and urinary calcium excretion: normal or low
- Serum PTH concentration: normal or increased
- Inappropriate serum 1,25(OH) vitamin D concentration (normal or low)
- No renal lithiasis except when patients are treated with calcitriol
Pathophysiology of renal phosphate loss

- NPT2a, NPT2c, NHERF1
- FGF23, Klotho?

- Low TmP/GFR
- Hypophosphatemia

Depending on the cause of the renal phosphate loss and patient characteristics

- Absence or weak stimulation of calcitriol synthesis
- Bone demineralization

- Stimulation of calcitriol synthesis
- Increased intestinal Ca and P absorption
- Increased urinary Ca and P excretion
- Renal lithiasis

FGF23, Klotho?
Importance of calcitriol in renal Pi leak consequences

Human

- Renal lithiasis
- Bone demineralization

Prié JASN 1998:1264

Mouse

NPT2a−/− 1αOHase+/+

NPT2a−/− 1αOHase−/-

Tenenhouse AJP 2004:286;F675
FGF23 and CRF

D Prié personal data

Larsson 2003 KI,64:2272
Adaptation to the decrease of GFR

- Stimulation of FGF23 secretion by hyperphosphatemia
- Increased renal Pi excretion (NPT2a, c)
- Decreased calcitriol synthesis
- Decreased intestinal Pi absorption (NPT2b)
- Decrease of phosphatemia
- Decreased intestinal Ca absorption
- Stimulation of PTH secretion
- Increased excretion of Pi

- BUT decreased renal synthesis of Klotho in CRF
FGF23 is an independent predictive factor of GFR decline in CRF

Fliser JASN 2007, 18: 2601
Plasma FGF23 and calcifications in HD patients

- Plasma FGF23 is correlated with vascular calcifications (hand and arm) in diabetic and non-diabetic HD patients (Inaba Osteoporos Int 2006, 17: 1506)

- No correlation between plasma FGF23 and bone mineralization in HD patients (Urena Torres, KI 2008, 73: 102)
Association between FGF23 & mortality during the first year of HD

<table>
<thead>
<tr>
<th>Phosphate Level</th>
<th>Median cFGF-23 Level (interquartile range)</th>
<th>P Value</th>
<th>Odds Ratio for Death (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients Who Died (N = 200)</td>
<td>Patients Who Survived (N = 200)</td>
<td></td>
</tr>
<tr>
<td>All levels</td>
<td>2260 (1196–5296)</td>
<td>1406 (989–2741)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&lt;3.5 mg/dl</td>
<td>1790 (1175–3941)</td>
<td>1148 (927–2169)</td>
<td>0.008</td>
</tr>
<tr>
<td>3.5–4.4 mg/dl</td>
<td>2049 (1109–4865)</td>
<td>1131 (893–1629)</td>
<td>0.003</td>
</tr>
<tr>
<td>4.5–5.5 mg/dl</td>
<td>2207 (1186–5238)</td>
<td>1499 (1044–2262)</td>
<td>0.02</td>
</tr>
<tr>
<td>&gt;5.5 mg/dl</td>
<td>3541 (1871–10,491)</td>
<td>2686 (1527–6210)</td>
<td>0.29</td>
</tr>
</tbody>
</table>

FGF23 blunts PTH secretion

Komaba et al, KI 2010
The Bone-Parathyroid-Kidney axis

Intact Kidney

Komaba et al, KI 2010
The Bone-Parathyroid-Kidney axis
Chronic Kidney Disease

Komaba et al, KI 2010
Therapeutic interventions and FGF23
FGF23 & phosphate binders

Nagano N 2006 Kidney Int 69: 531
FGF23 antibodies: a new treatment?

Aono Y 2009 JBMR 24:1879
Several new causes of renal phosphate leak have been identified during the last few years:

- Mutations in renal phosphate transporters (NPT2a, NPT2c)
- Mutations in regulatory proteins (NHERF1)
- New phosphaturic hormones (FGF23-Klotho)

Renal phosphate loss increases the risk of renal stones and bone demineralization.

Serum calcitriol and FGF23 levels can modulate the consequences of increased urinary phosphate excretion.
Conclusion 2

- FGF23 is elevated in CRF and is an independent marker of progression and of mortality.
- FGF23 is a promising therapeutic target in CKD.
- The mechanisms by which FGF23 induces renal phosphate loss remain unclear at present.
- Whether Klotho affects phosphate balance independently of FGF23 remains to be established.
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