Fibroblast Growth Factor 23 (FGF23), Klotho and calcium/phosphate metabolism

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Control of renal phosphate excretion

- Parathyroid hormone
- Other hormone(s) ?

Control of renal phosphate excretion

- Parathyroid hormone
- **Phosphatonins ?**

Fibroblast Growth Factor 23 (FGF23)

ADHR Consortium.

Autosomal dominant hypophosphataemic rickets is associated with mutations in FGF23.

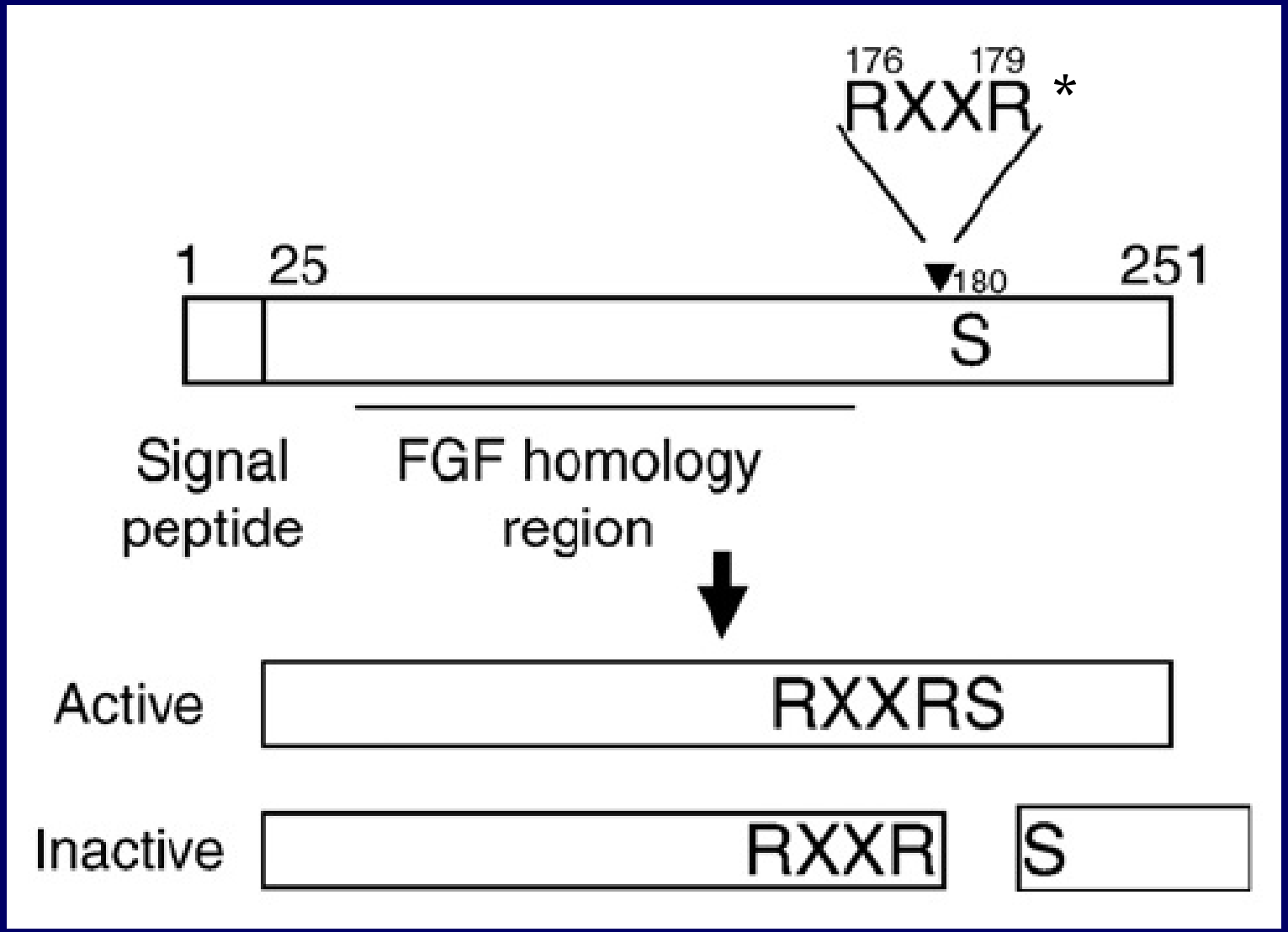
Nat Genet. 2000 Nov;26(3):345-8.

Shimada T, Mizutani S, Muto T, Yoneya T, Hino R, Takeda S, Takeuchi Y, Fujita T, Fukumoto S, Yamashita T.

Cloning and characterization of FGF23 as a causative factor of tumor-induced osteomalacia.

Proc Natl Acad Sci USA. 2001 May 22;98(11):6500-5.

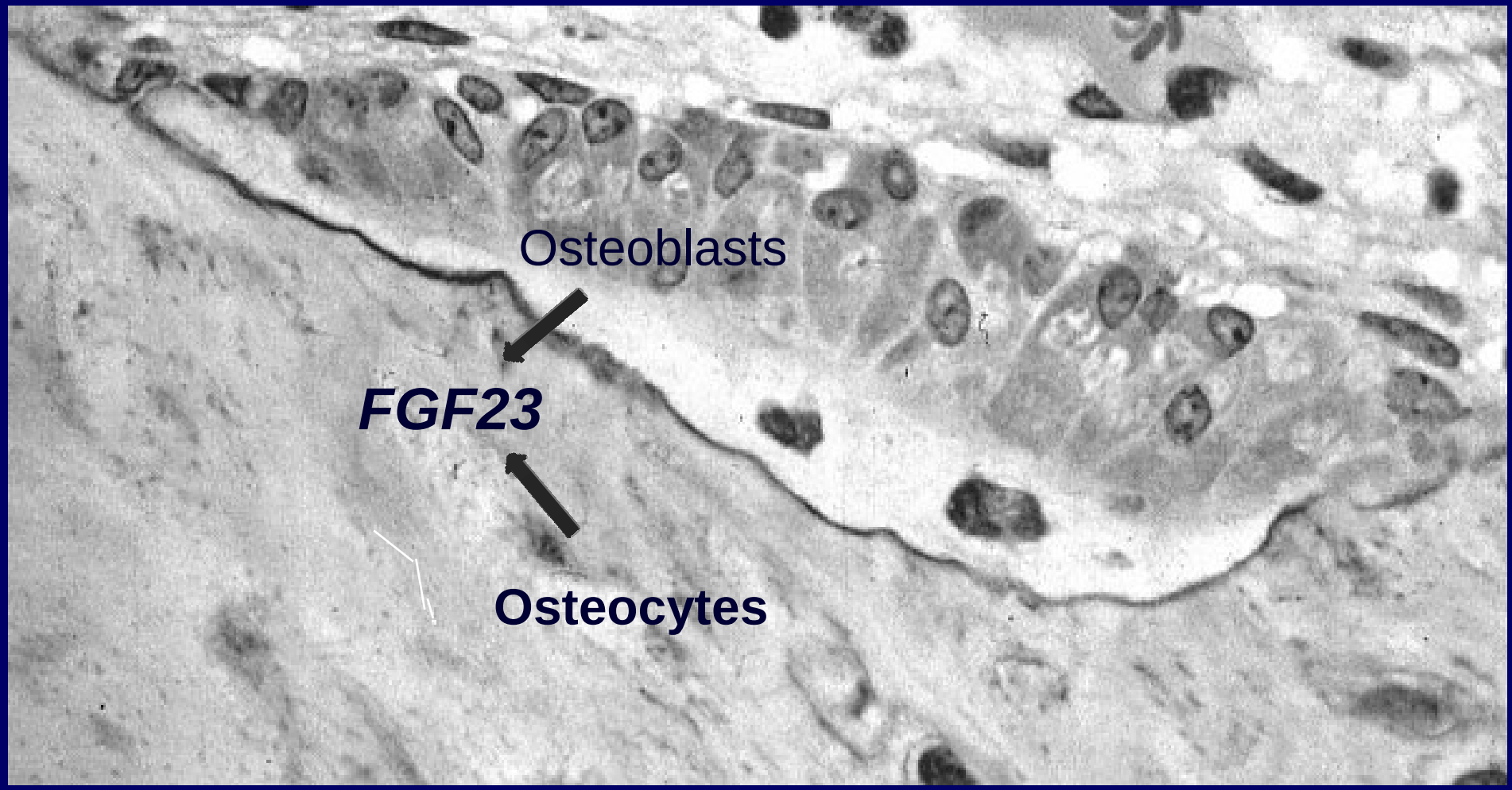
Structure of FGF23 protein *(251 amino acids)*



* R–X–X–R cleavage motif, cleavage by a yet unknown enzyme

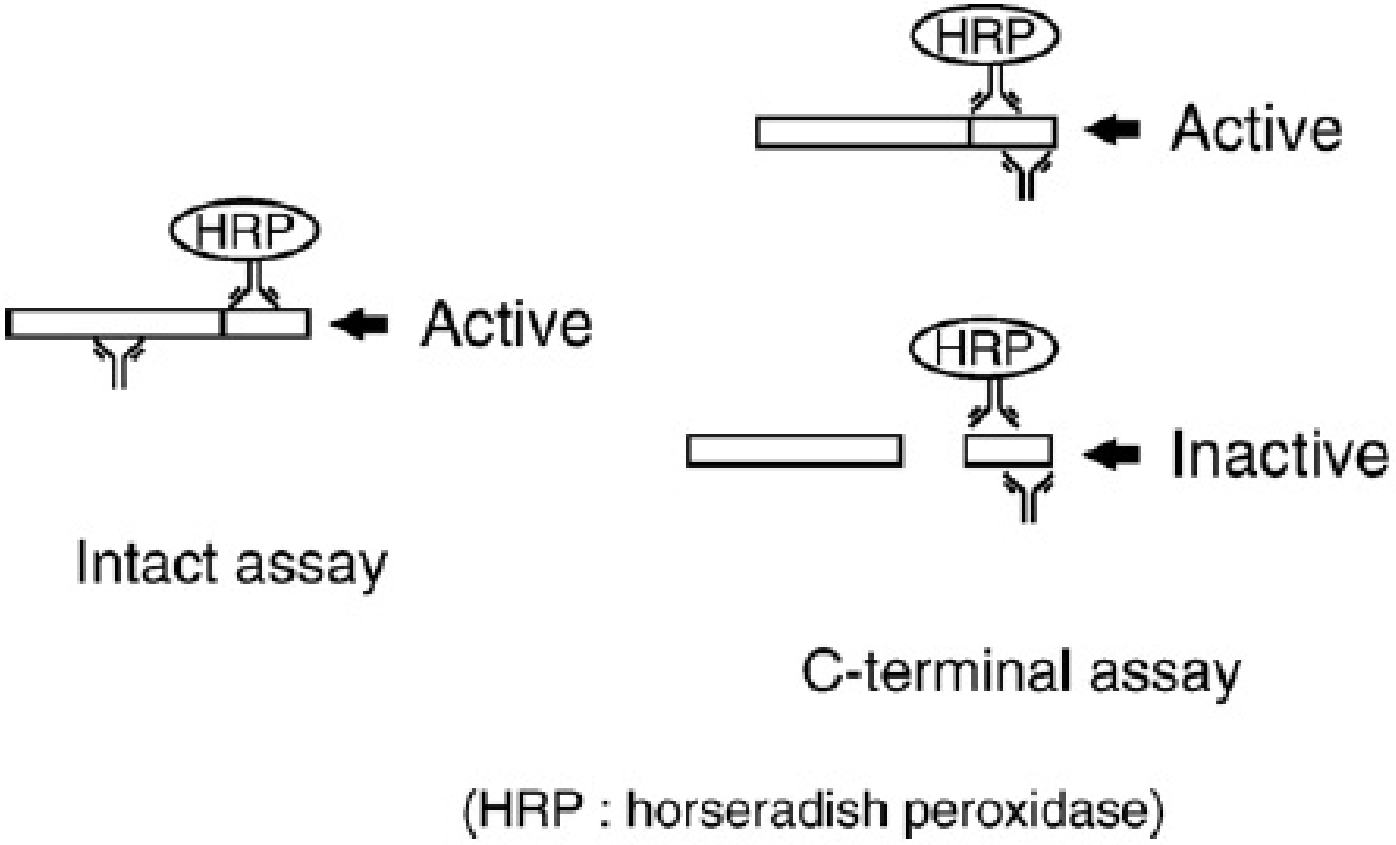
Fukumoto & Yamashita, Bone 2007;40:1190-95

FGF23 production sites in bone



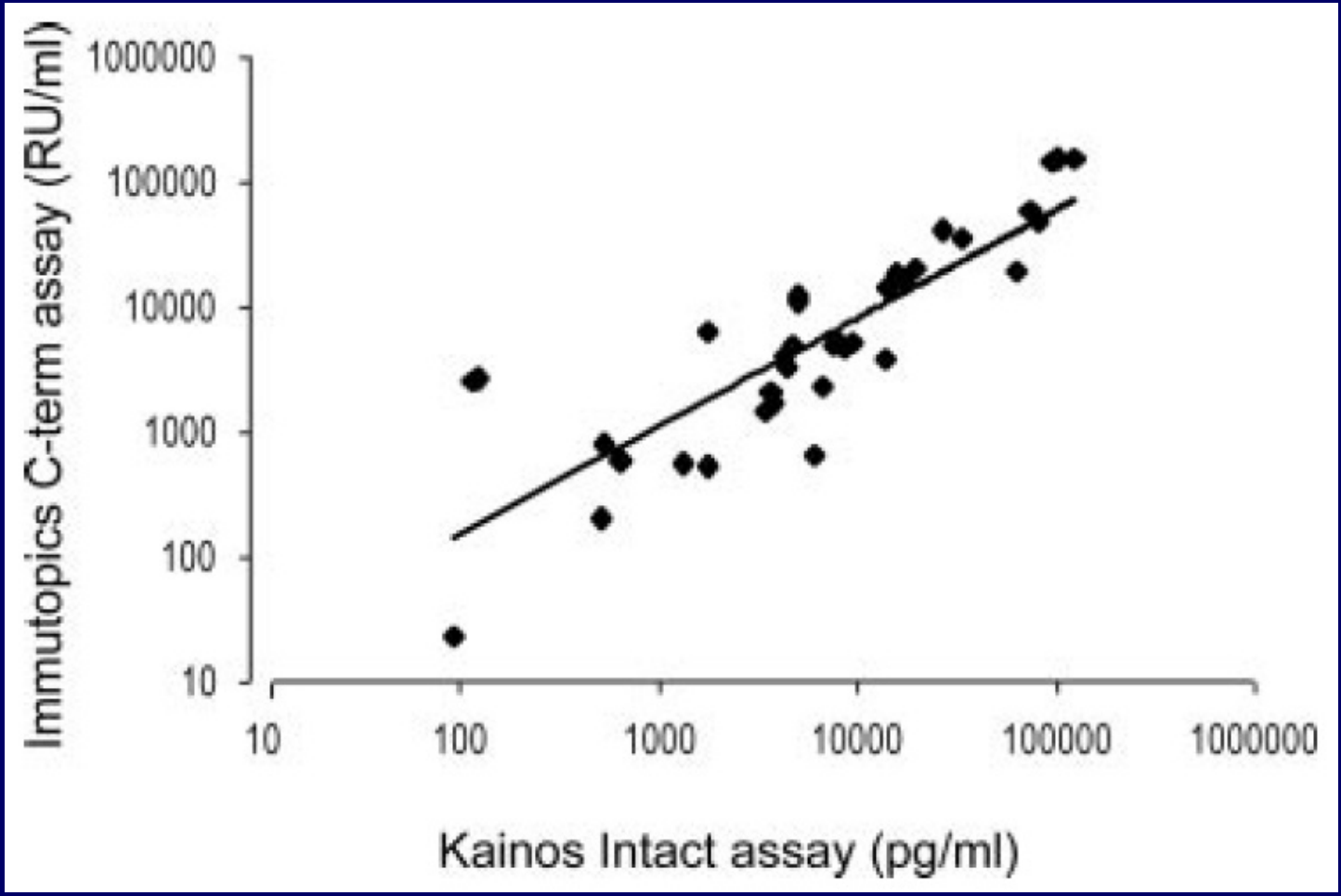
Bone microscopy picture from Wikipedia

Circulating FGF23 and measurement assays



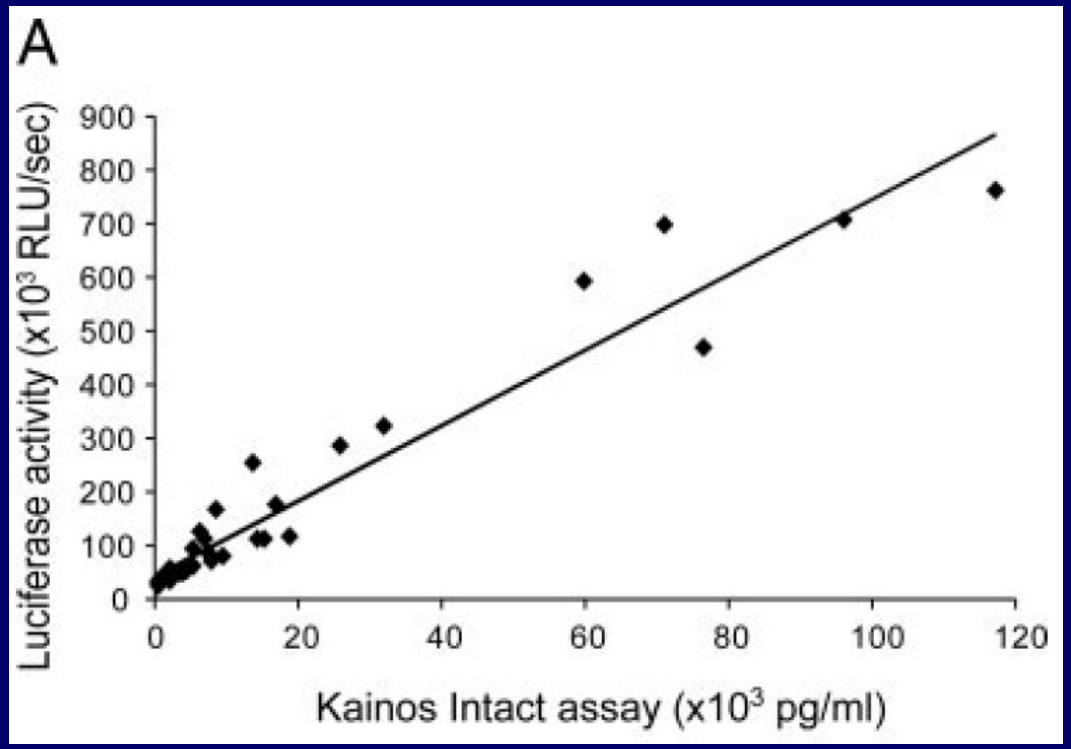
Fukumoto & Yamashita, Bone 2007;40:1190-95

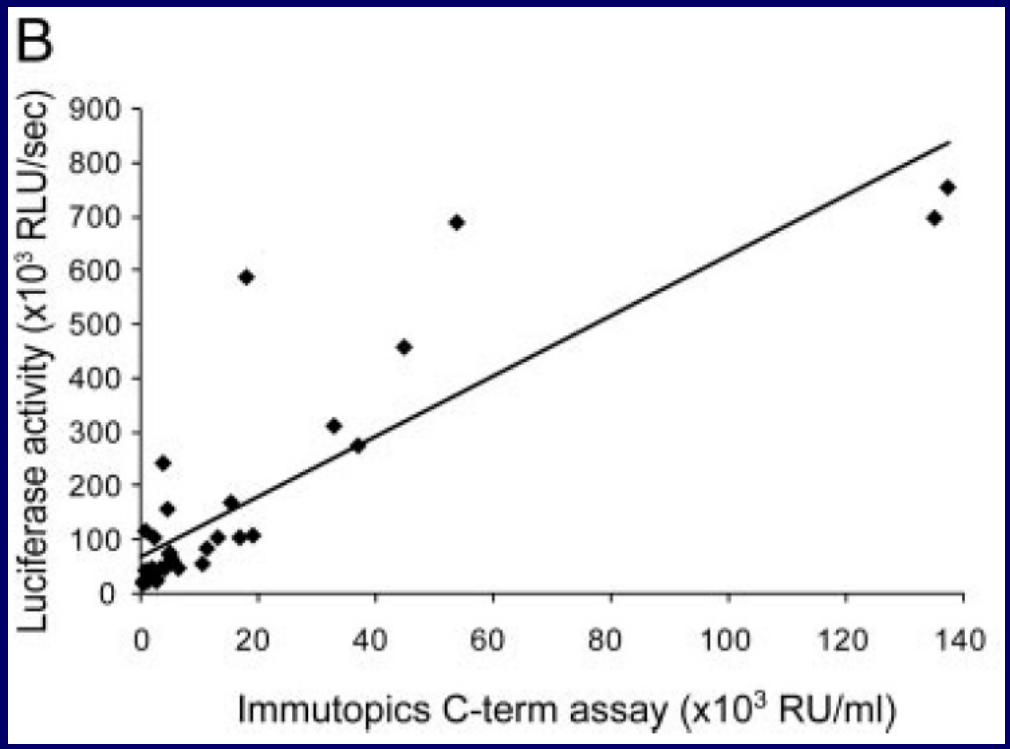
Correlation between plasma cFGF-23 and intact FGF23 in ESRD patients *(CAPD pts)*



Shimada T et al, JCEM 2010;95:578-85

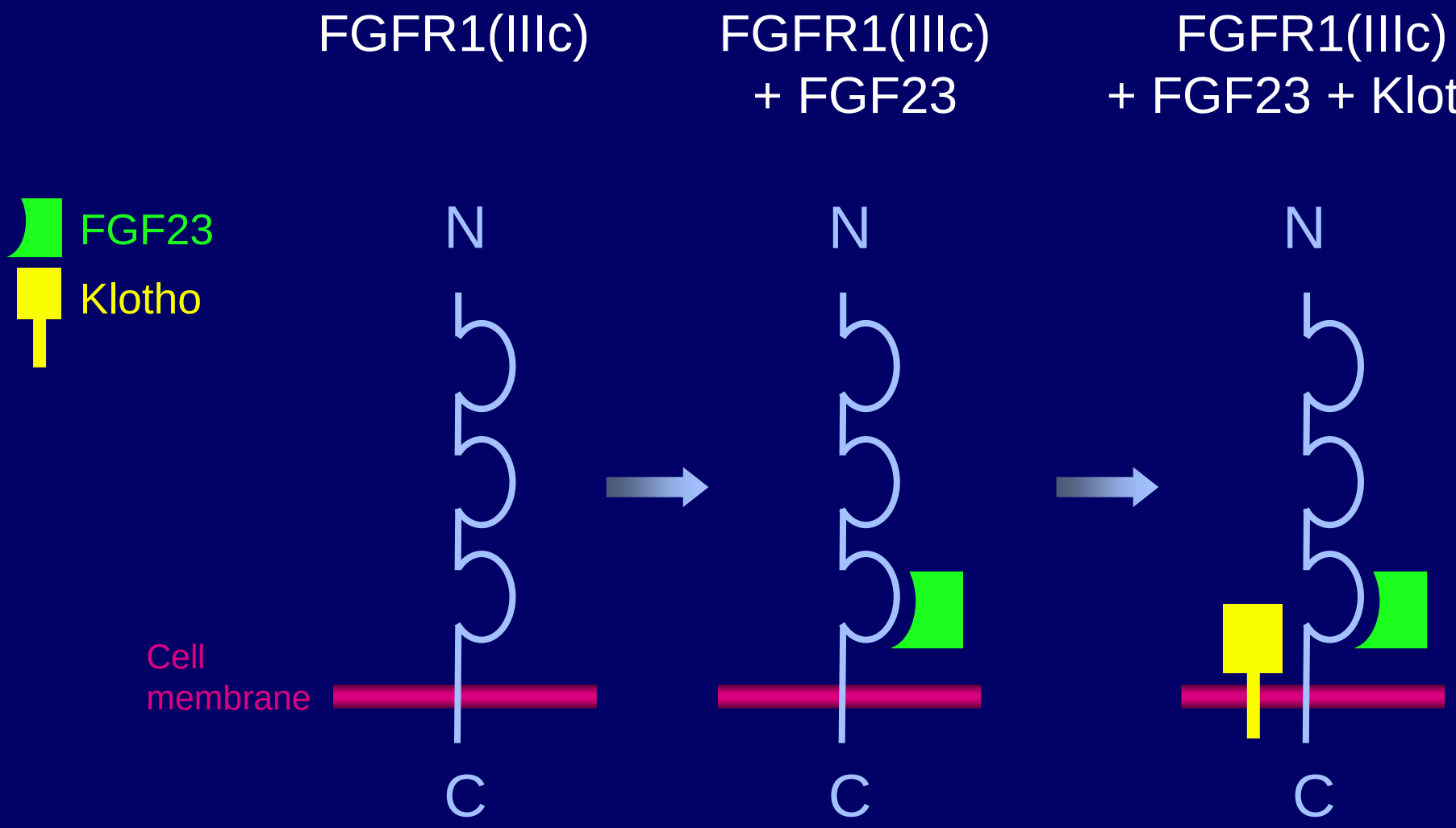
Correlation of plasma iFGF23 (A) and cFGF23 (B) with bioactive FGF23 *(CAPD pts)*





Shimada T et al, JCEM 2010;95:578-85

FGF receptor 1 activation by FGF23 and Klotho



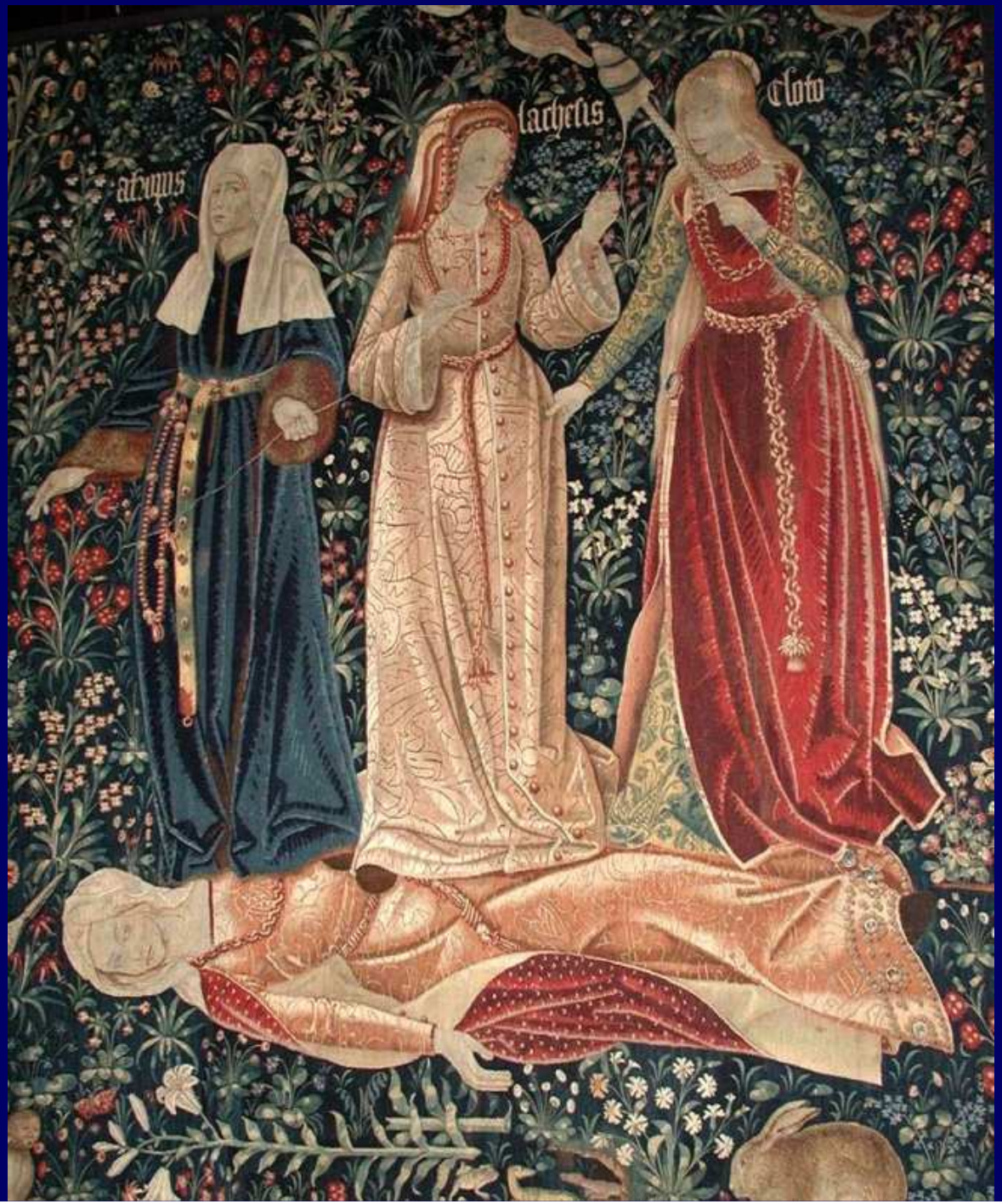
Mutation of the mouse *klotho* gene leads to a syndrome resembling ageing

Makoto Kuro-o*, Yutaka Matsumura*†, Hiroki Aizawa*†, Hiroshi Kawaguchi‡, Tatsuo Suga†, Toshihiro Utsugi†, Yoshio Ohyama†, Masahiko Kurabayashi†, Tadashi Kaname§, Eisuke Kume‖, Hitoshi Iwasaki‖, Akihiro Iida¶, Takako Shiraki-Iida*¶, Satoshi Nishikawa#, Ryozo Nagai†* & Yo-ichi Nabeshima*★††

* *Division of Molecular Genetics, National Institute of Neuroscience, NCNP, 4-1-1 Ogawahigashi, Kodaira, Tokyo 187, Japan*
† *The 2nd Department of Internal Medicine, University of Gunma School of Medicine, 3-39-22, Showa, Maebashi, Gunma 371, Japan*
‡ *Department of Orthopaedic Surgery, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo, Tokyo 113, Japan*
§ *Institute of Molecular Embryology and Genetics, Kumamoto University School of Medicine, Kumakoto 862, Japan*
‖ *Lead Optimization Research Laboratory, Tanabe Seiyaku Co. Ltd, 2-2-50 Kawagishi, Toda, Saitama 335, Japan*
¶ *Tokyo Research Laboratories, Kyowa Hakko Kogyo Co. Ltd, 3-6-6 Asahimachi, Machidashi, Tokyo 194, Japan*
# *Pharmaceutical Research Laboratories, Kyowa Hakko Kogyo Co. Ltd, 118 Shimotagari, Nagaizumi, Sunto, Shizuoka 411, Japan*
★ *Core Research for Evolutional Science & Technology (CREST), JRDC and* †† *Institute for Molecular and Cellular Biology, Osaka University, 1-3 Yamada-oka, Suita, Osaka 565, Japan*

A new gene, termed *klotho*, has been identified that is involved in the suppression of several ageing phenotypes. A defect in *klotho* gene expression in the mouse results in a syndrome that resembles human ageing, including a short lifespan, infertility, arteriosclerosis, skin atrophy, osteoporosis and emphysema. The gene encodes a membrane protein that shares sequence similarity with the β-glucosidase enzymes. The *klotho* gene product may function as part of a signalling pathway that regulates ageing *in vivo* and morbidity in age-related diseases.

Kuro-o M et al, Nature 1997;390:45-51



The Moirae (weavers of fate):

Κλωθώ

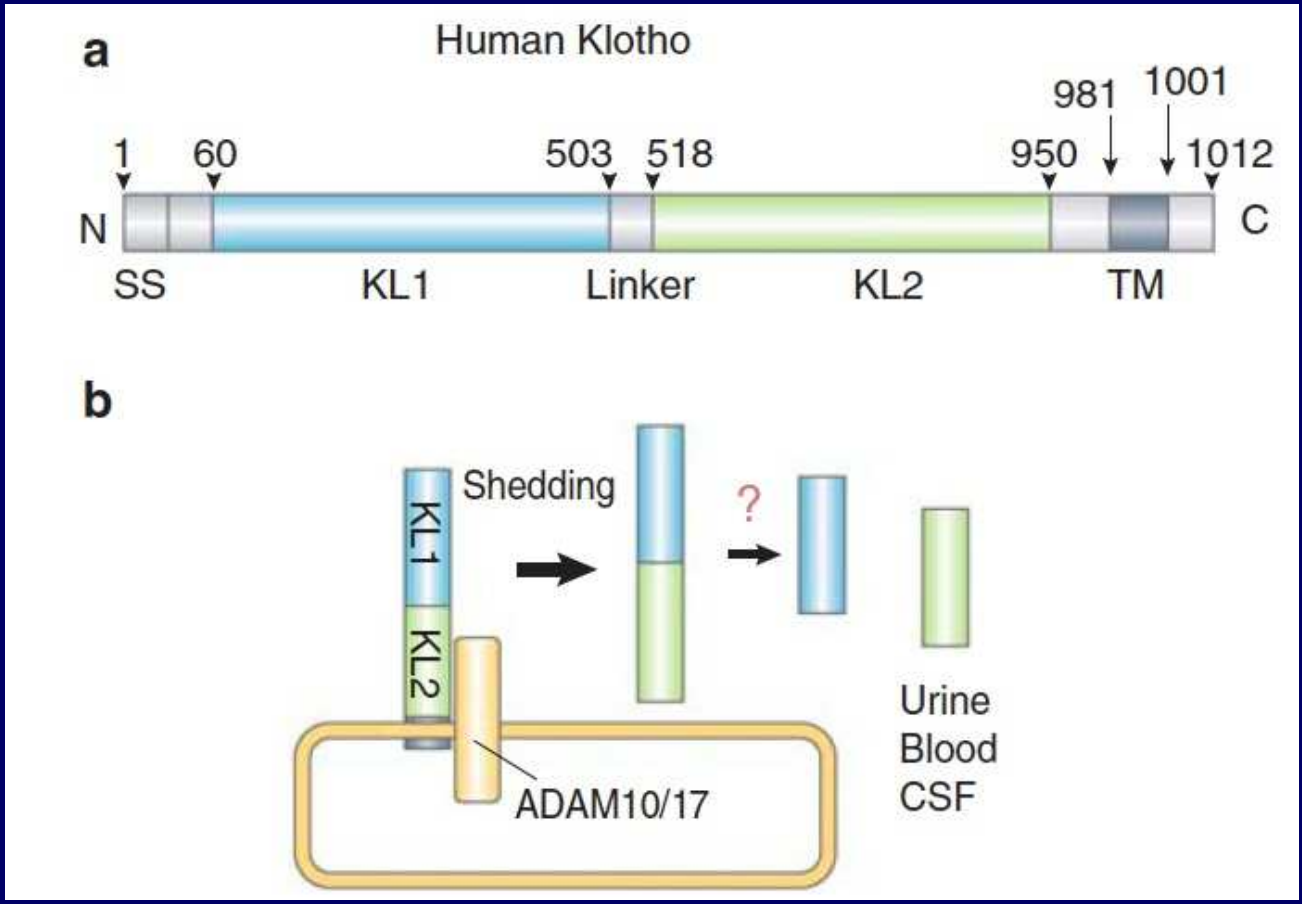
Λάχεσις

Άτροπος

16th century tapestry

http://en.wikipedia.org/wiki/Image:
The_Triumph_of_Death,_or_The_Three_Fates.jpg

Domain structure and topology of Klotho (α-Klotho)

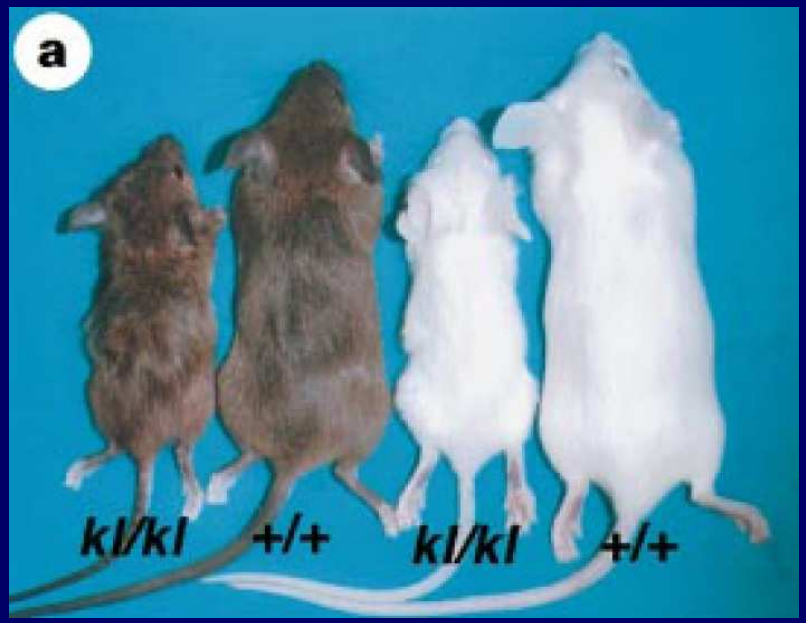


Functions of secreted Klotho:
- Interaction with FGF23
- Regulation of ion channels
- Regulation of transporters
- Regulation of insulin & IGF1

SS, signal sequence
KL1 & KL2, 1st & 2nd Klotho repeat
TM, transmembrane domain
Cleavage by ADAM10 or ADAM117

Huang CL, KI 2010

Mutation of klotho gene in the mouse



- reduced life expectancy
- decrease of spontaneous activity
- infertility
- skin thinning and atrophy

Mean survival

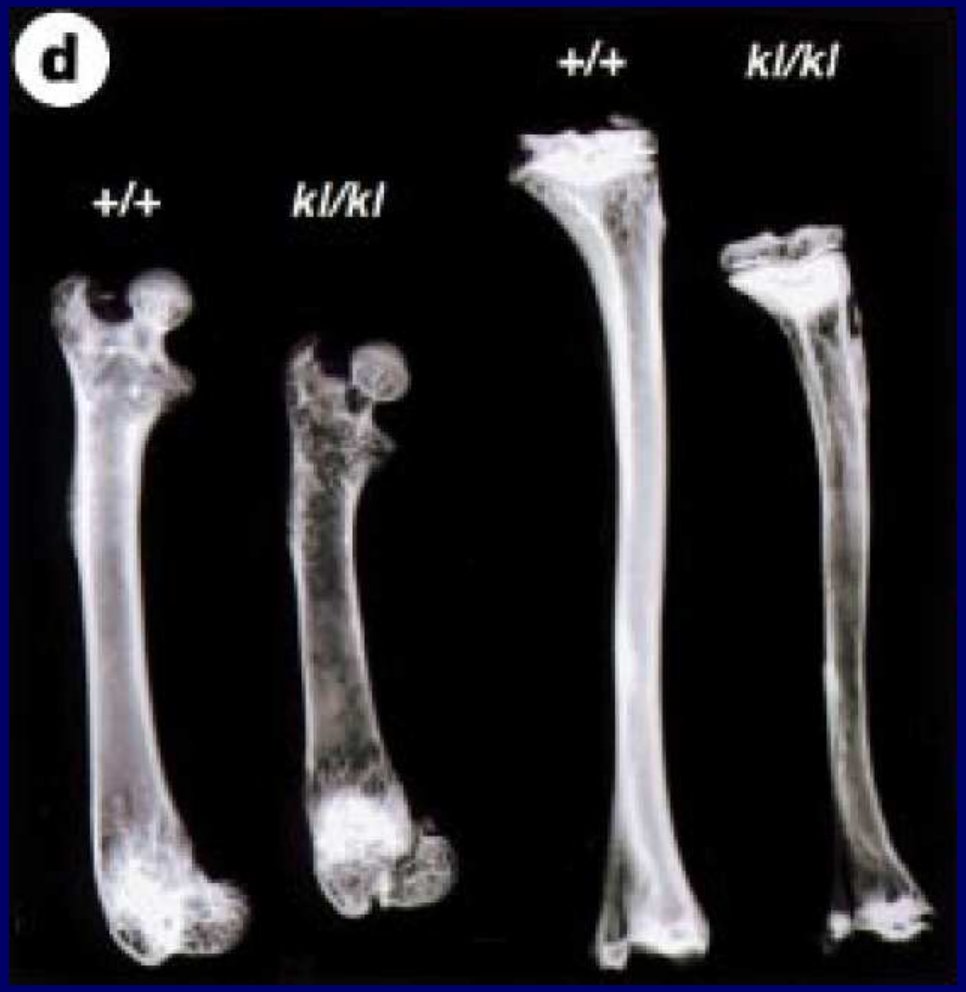
Control : 600 days

Klotho -/- : 380 days

- high serum phosphorus, calcitriol
- soft tissue calcifications
- osteopenia (low bone turnover)
- atherosclerosis/arteriosclerosis

Kuro-o M et al, Nature 1997;390:45-51

Skeletal phenotypes of klotho mutant mouse



Femur **Tibia**

Two phenotypes

Osteopetrosis

Elongation of bone trabeculae in epiphyses of long bones and vertebrae

Osteoporosis

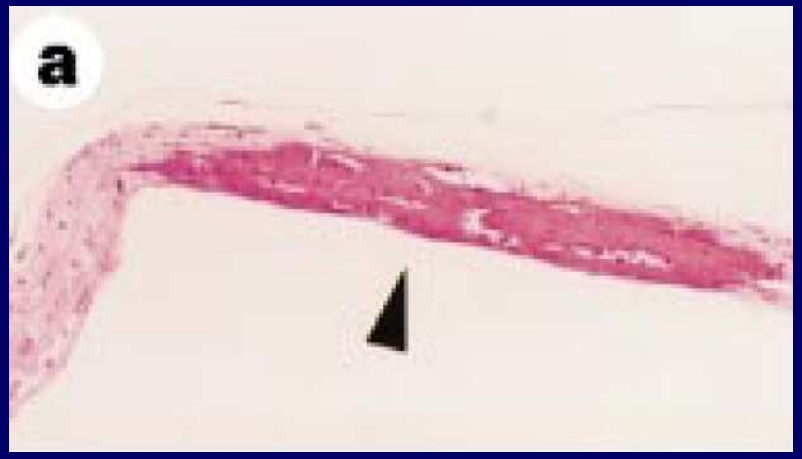
Cortical thinning of diaphyses of long bones

Possible mechanism

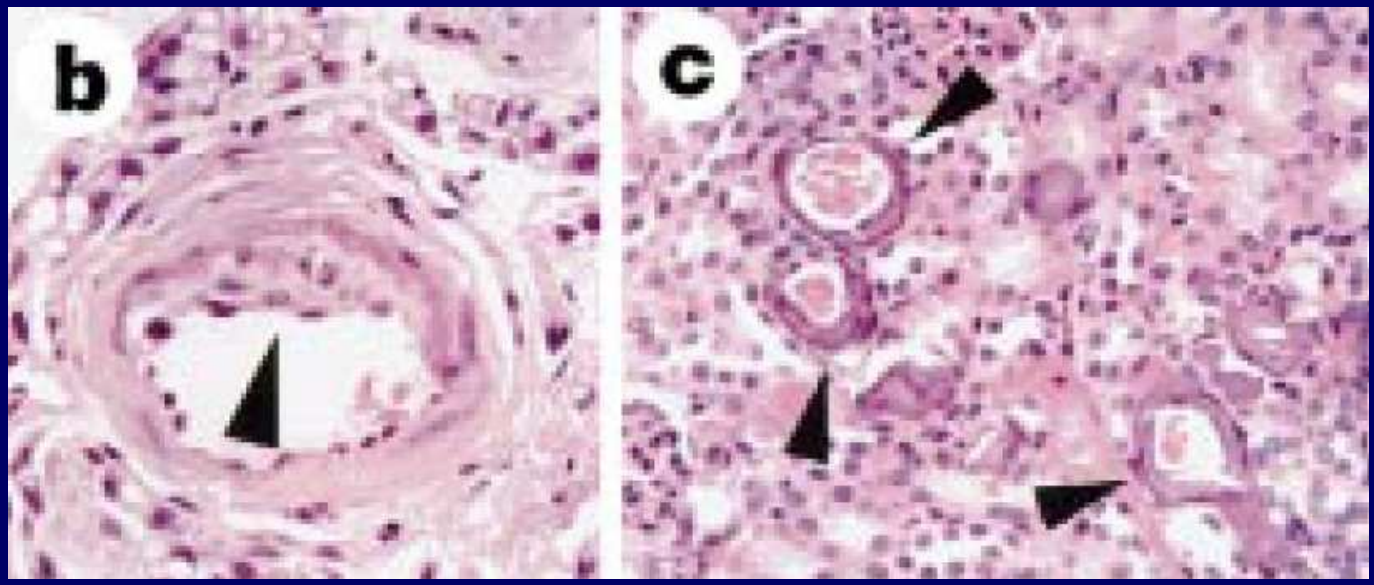
Site-specific changes of osteoclast activity with increase in OPG expression

Kuro-o M et al, Nature 1997;390:45-51

Vascular phenotypes in klotho mutant mice



Aorta calcification



Atherosclerosis (muscle)

Arteriolosclerosis (kidney)

Kuro-o M et al, Nature 1997;390:45-51

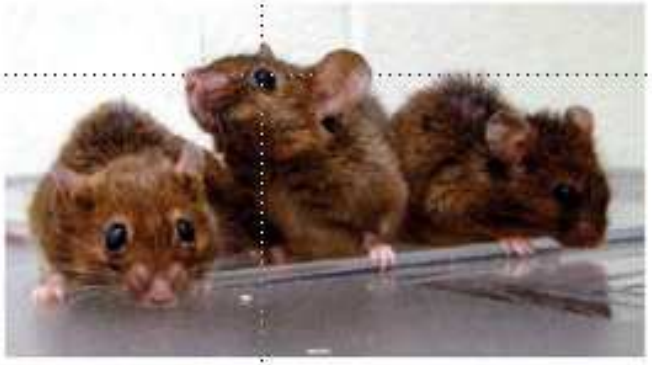
Klotho gene overexpression

NEWS OF THE WEEK

PHYSIOLOGY

Boosting Gene Extends Mouse Life Span

A protein named after the Greek goddess who spins life's thread has joined the short list of ways to extend a mouse's natural life span. Whereas lab mice can live about 2 years, mice engineered to overproduce this protein, called Klotho, have celebrated third birthdays, Makoto Kuro-o of the University of Texas Southwestern Medical Center in Dallas and his colleagues report online in this week's *Science* Express (www.sciencemag.org/cgi/content/abstract/1112766). The mutant rodents represent a rare case of a single gene substantially influencing life span in mammals.



Rare milestone. These mice, which overexpress the gene for Klotho, have celebrated their third birthdays.

into the blood and may act like a hormone. Males making extra Klotho lived up to 30% longer than normal males, and the mutant females survived 20% longer than normal counterparts. As with lab animals coaxed to have lengthy life spans, the altered rodents had fertility problems. They produced about half the expected number of offspring.

Males appeared more affected by Klotho than females did. Their blood, unlike that of females, contained more insulin than normal mice. This suggested that the male mutants were somewhat resistant to insulin—a symptom, in extreme

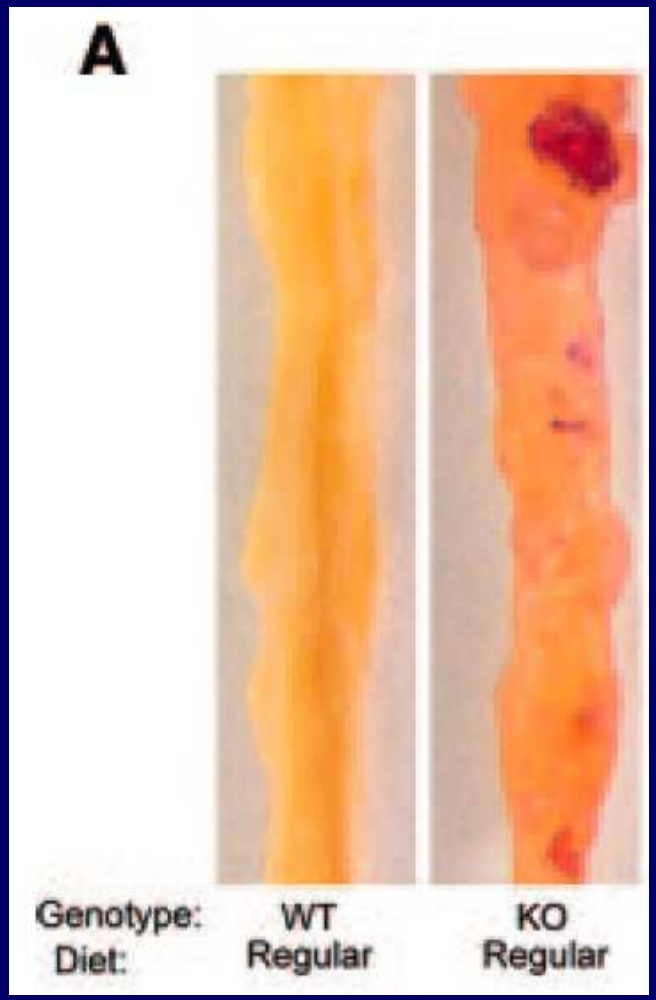
Science 26 August 2005, Vol 309

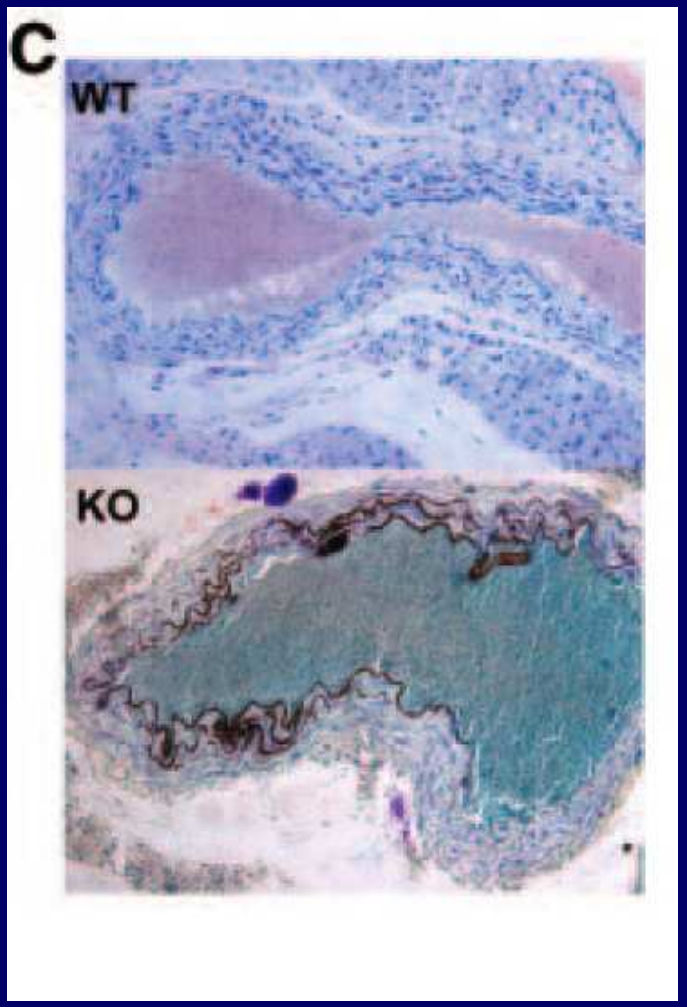
Klotho & fibroblast growth factor-23 (FGF23)

FGF-23 gene deletion : klotho-like phenotype

FGF23-/- mice : similar phenotype as kl-/- mice

Aorta calcification in FGF23-/- vs wild-type mice on regular diet



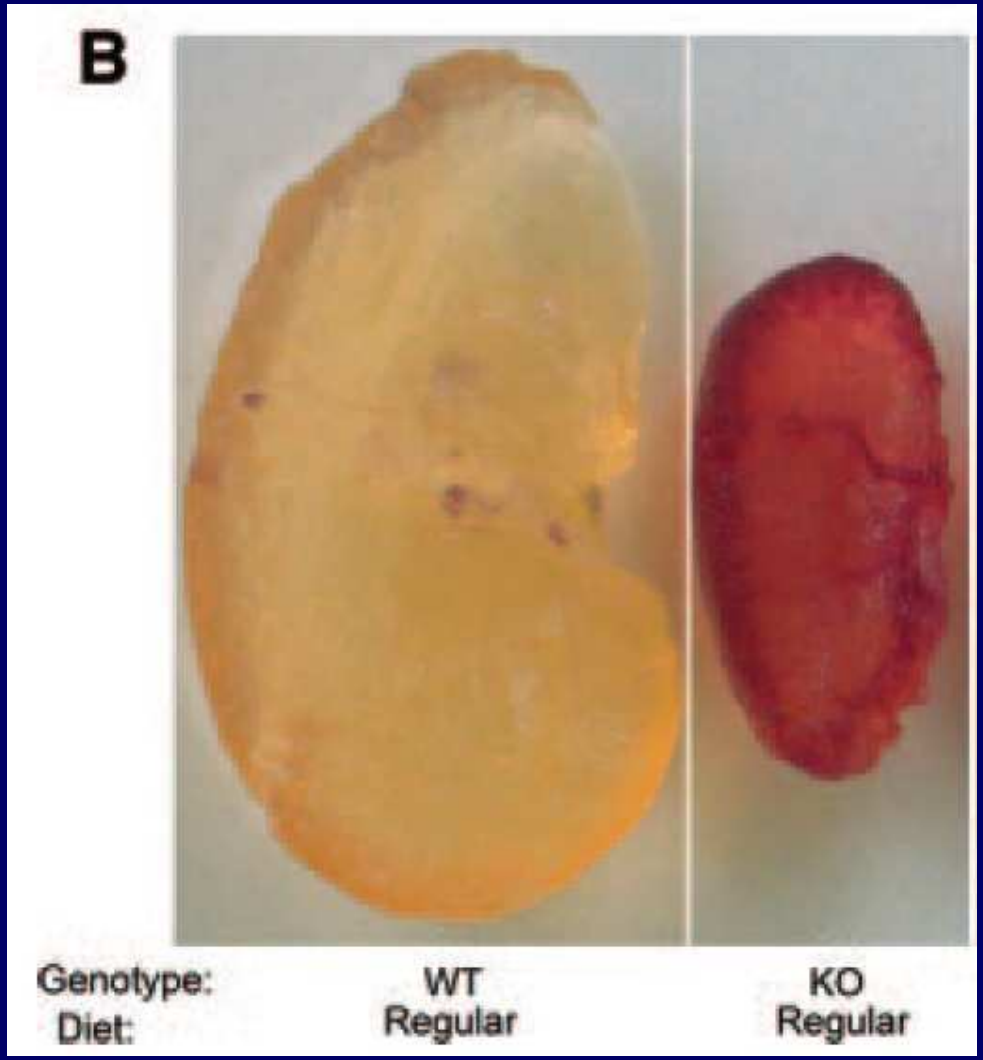


A, Alizarin Red-S stain
C, von Kossa stain

Stubbs JR et al, JASN 2007;18:2116-24

FGF23-/- mice : similar phenotype as kl-/- mice

Nephrocalcinosis in FGF23-/- vs wild-type mice on regular diet



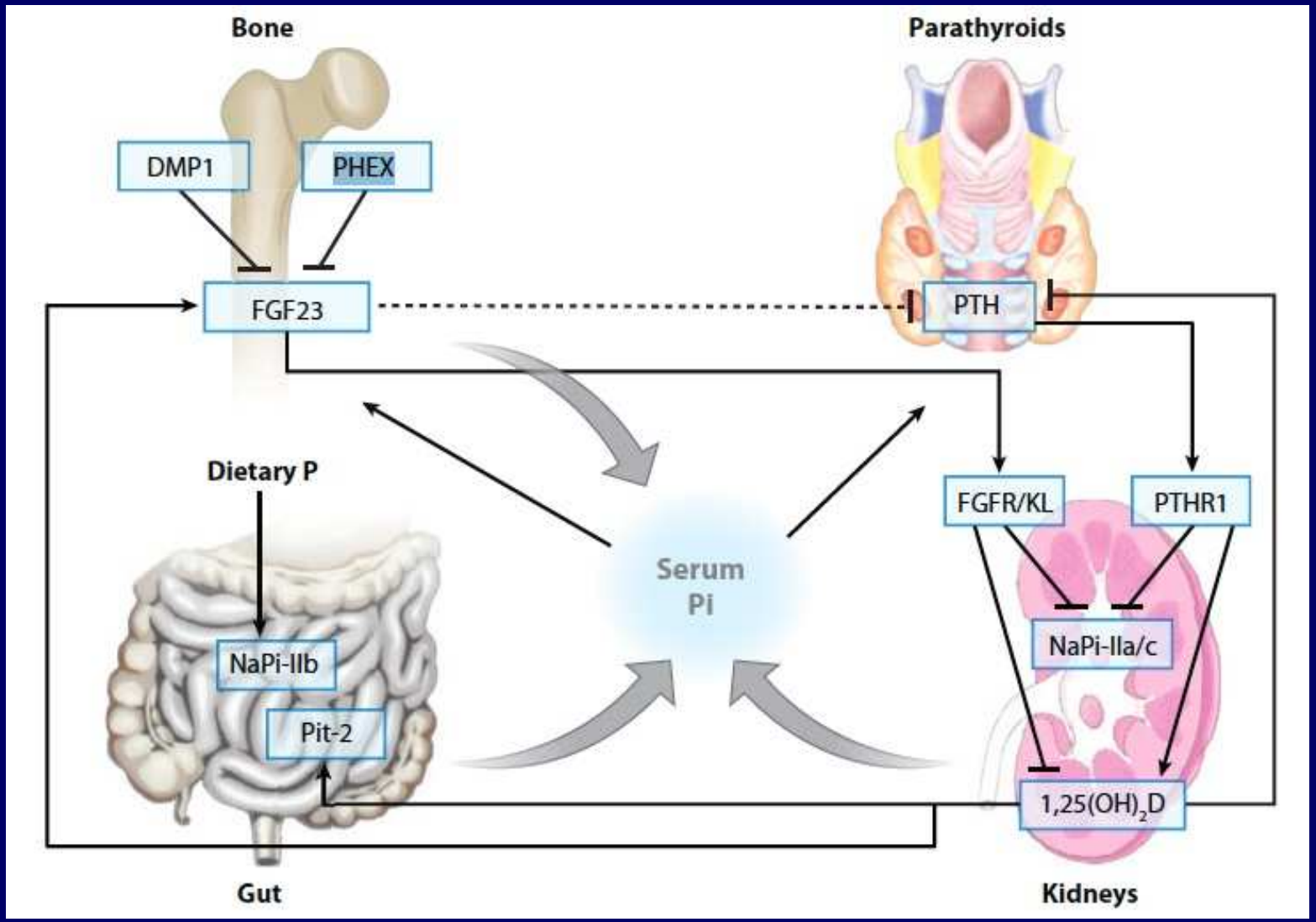
B, Alizarin Red-S stain

Stubbs JR et al, JASN 2007;18:2116-24

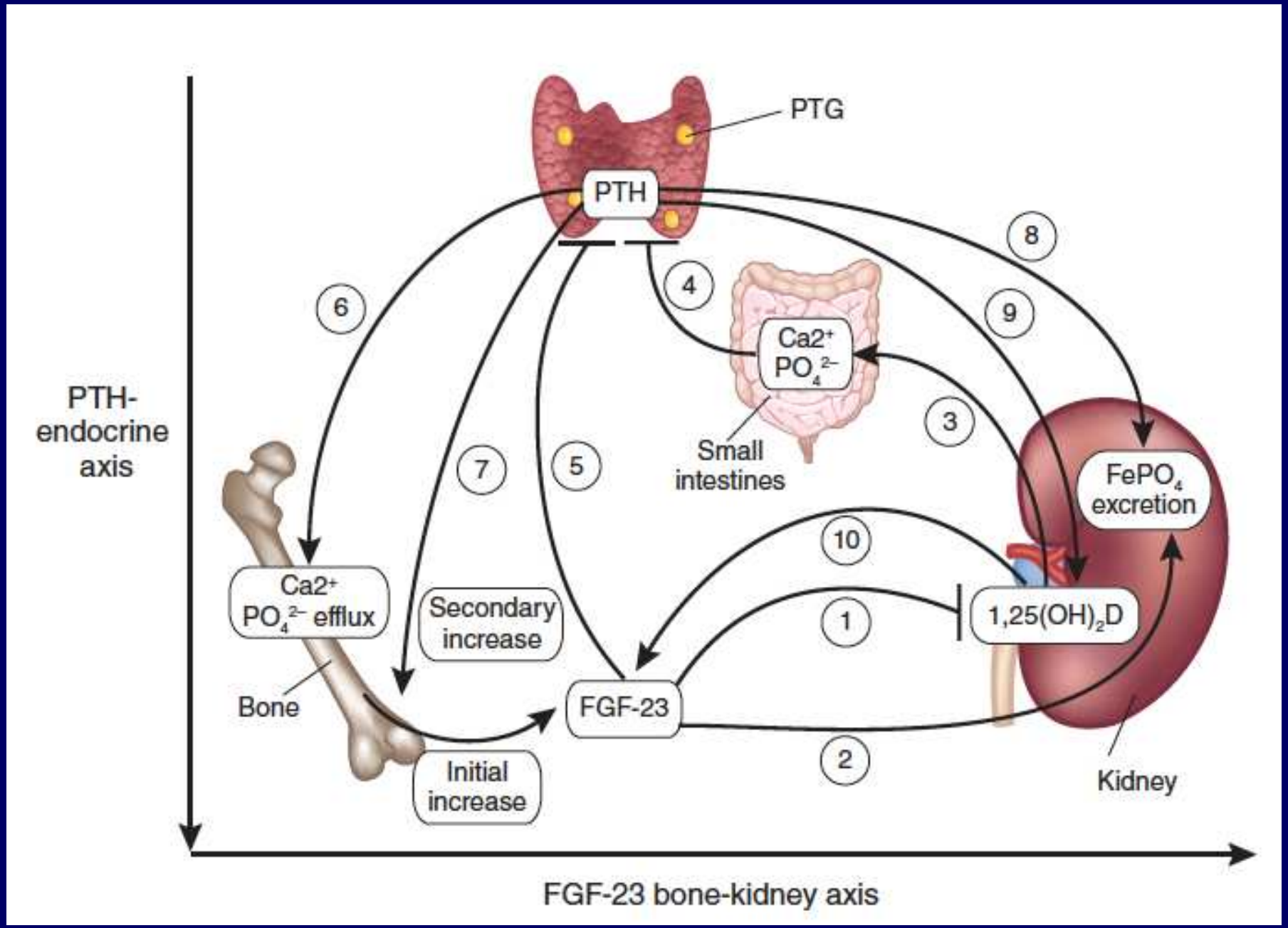
Physiological roles of FGF23 and Klotho

- FGF23 + Klotho : obligatory partners
- Main target organs, actions and interactions

Endocrine regulation of Pi homeostasis

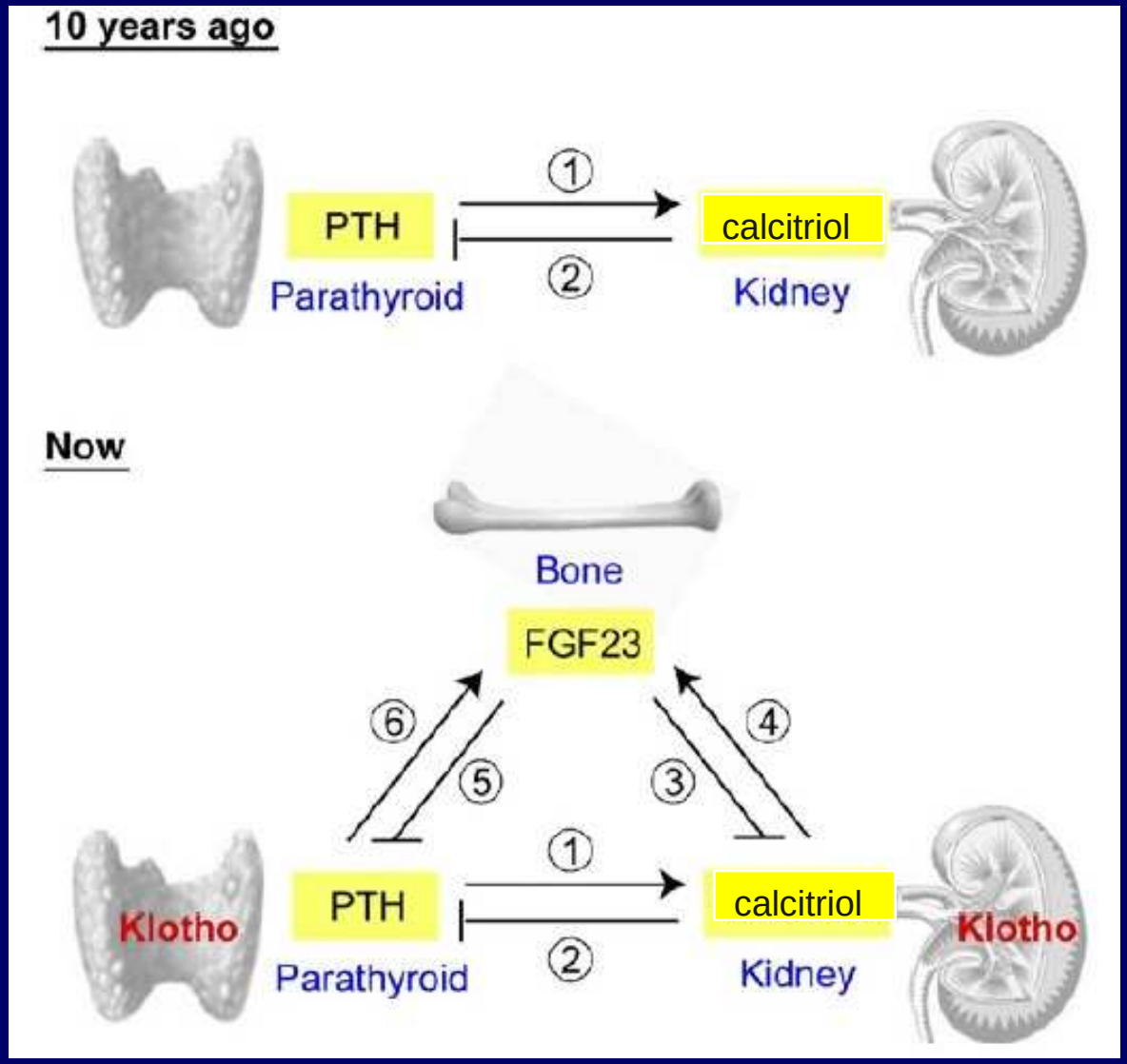


Bergwitz & Jüppner, Annu Rev Med 2010;61:91-104



Quarles LD, Nature Med 2011;17:428-29

Endocrine regulation of Pi homeostasis



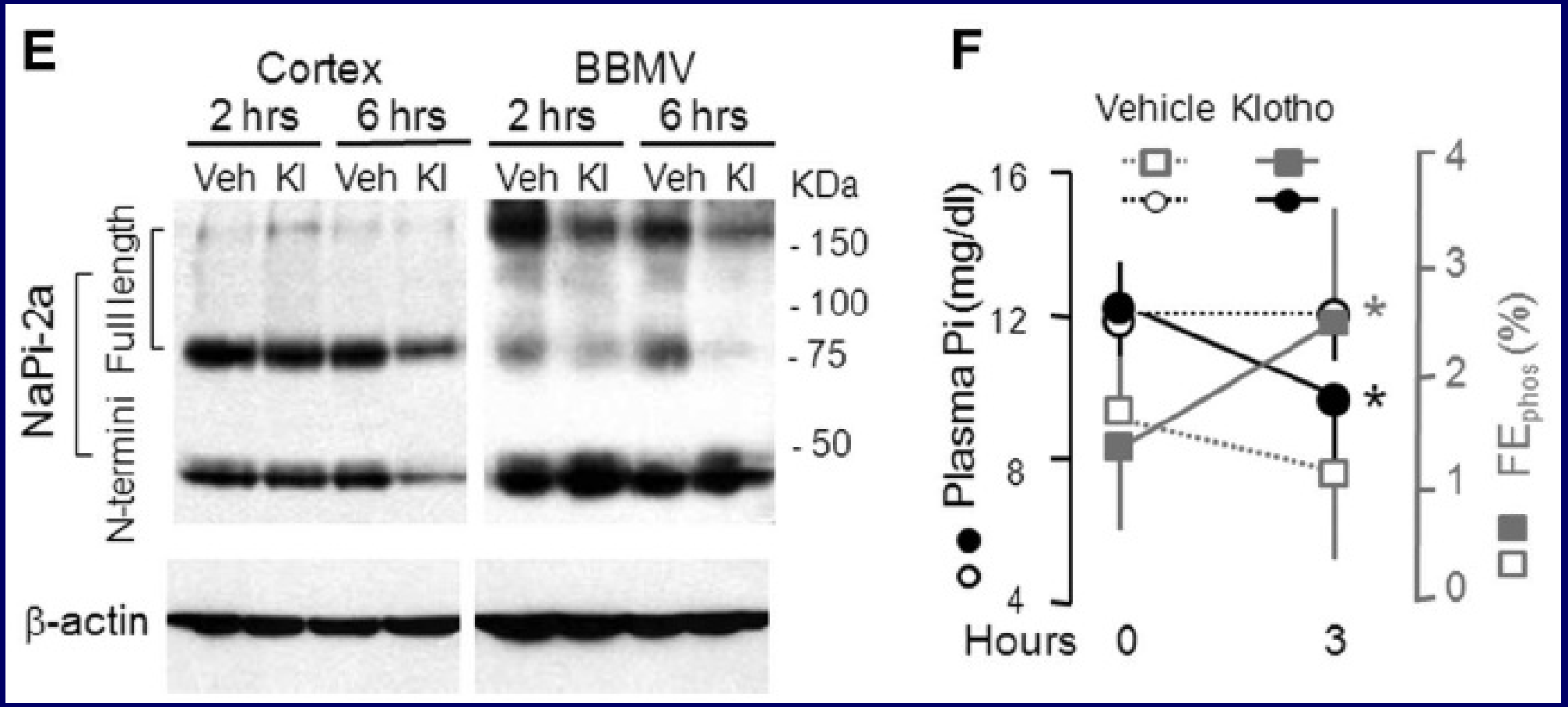
John GB et al, AJKD 2011;58:127-34

Klotho & fibroblast growth factor 23 (FGF23)

- Klotho, an obligatory partner of FGF23 ?
- Does Klotho exert FGF23 independent actions ?

Direct regulation of Pi homeostasis by Klotho independent of FGF23

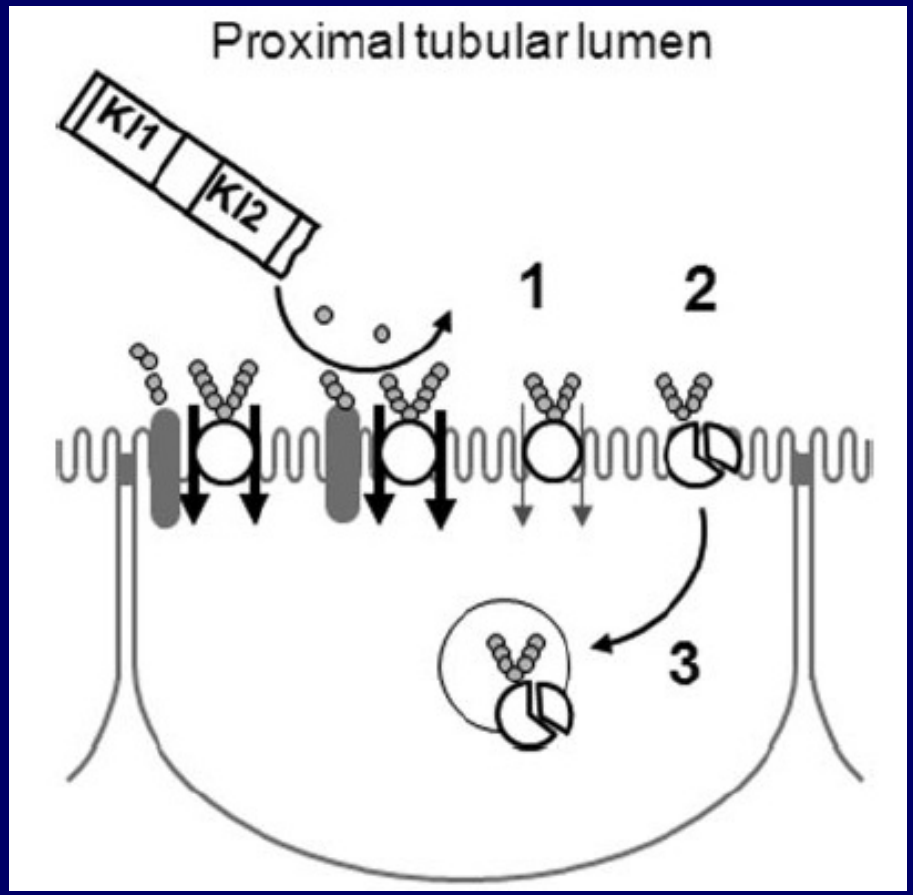
E, effect of recombinant mouse klotho vs vehicle on renal NaPi-2a *in vitro*
F, effect of klotho vs vehicle on plasma and urinary Pi *in FGF23-/- mice*

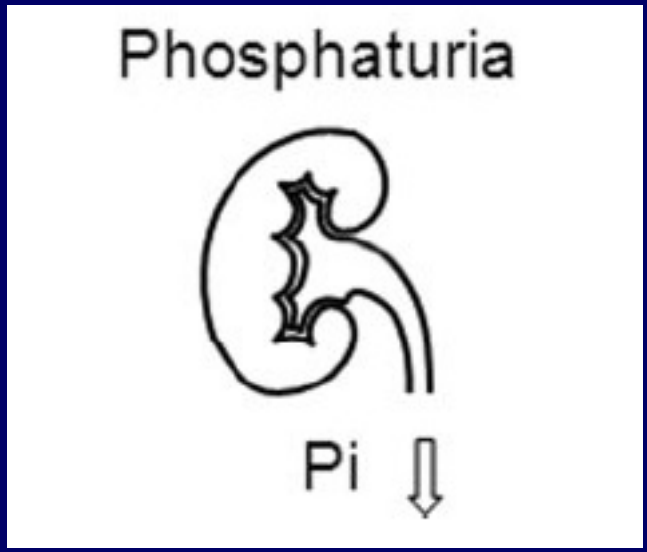


Hu MC et al, FASEB J 2010;24:3438-50

Direct Klotho effect on proximal tubule NaPi-2a

1) Klotho enzyme deglycosylates NaPi-2a protein in BBM
2) NaPi-2a protein is degraded by resident proteases in BBM
3) NaPi-2a protein is internalized from BBM to intracellular pool





Hu MC et al, FASEB J 2010;24:3438-50

FGF23 and Klotho : roles in disease states

- States of FGF23 dysregulation
- Increase in FGF23 and decrease in Klotho in CKD

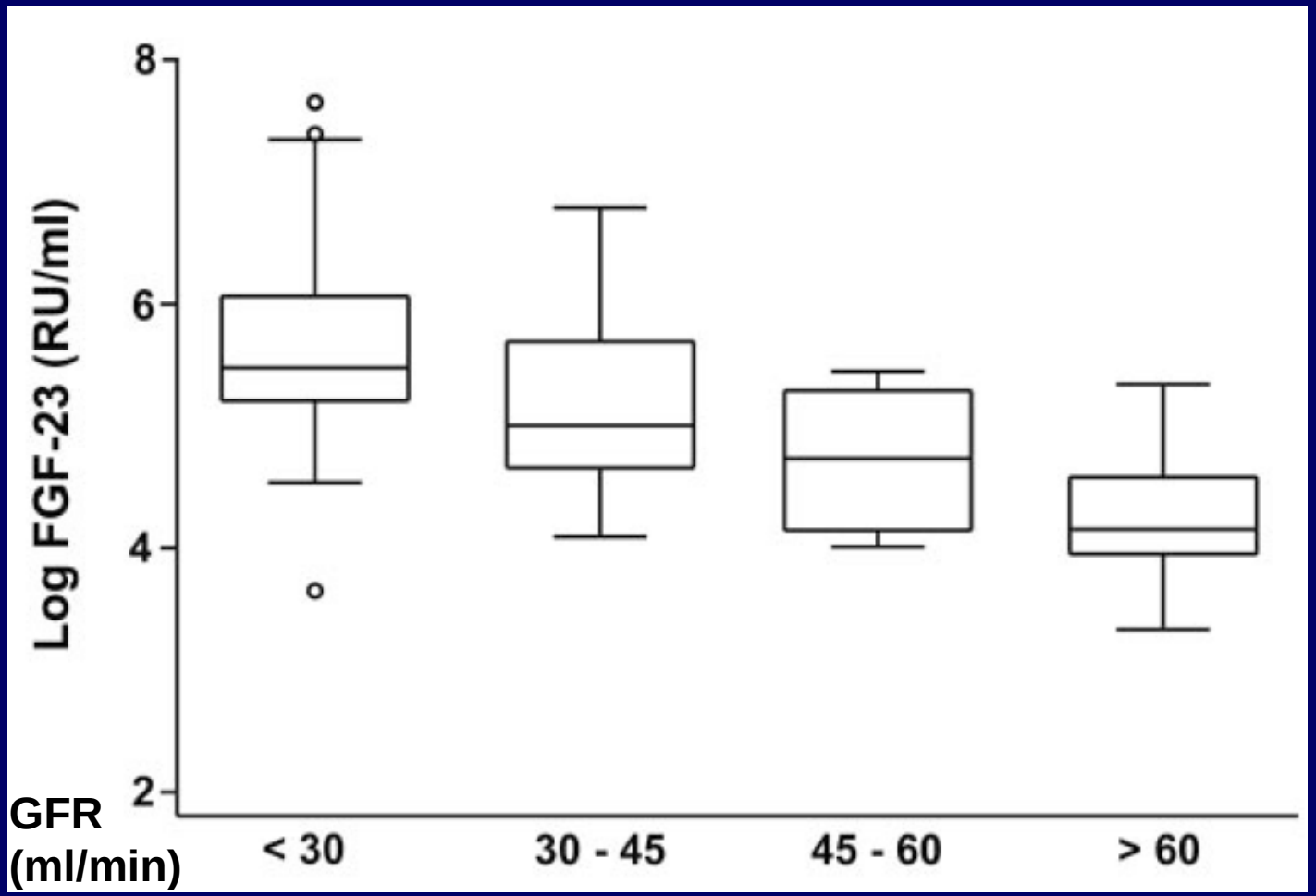
FGF23 dysregulation and disease states

Diseases owing to dysregulation of FGF23

Human diseases	Cause
Increased FGF23 activity	
X-linked hypophosphatemia	*PHEX* mutation
Autosomal dominant hypophosphatemic rickets	*FGF23* mutation
Autosomal recessive hypophosphatemic rickets/osteomalacia	*DMP1* mutation
McCune-Albright syndrome	*GNAS1* mutation
Osteoglophonic dysplasia	*FGFR1* mutation
Epidermal nevus syndrome	*FGFR3* mutation
Tumor-induced osteomalacia	FGF23-producing tumor
Decreased FGF23 activity	
Familial tumoral calcinosis	*GALNT3* or *FGF23* or *KL* mutation

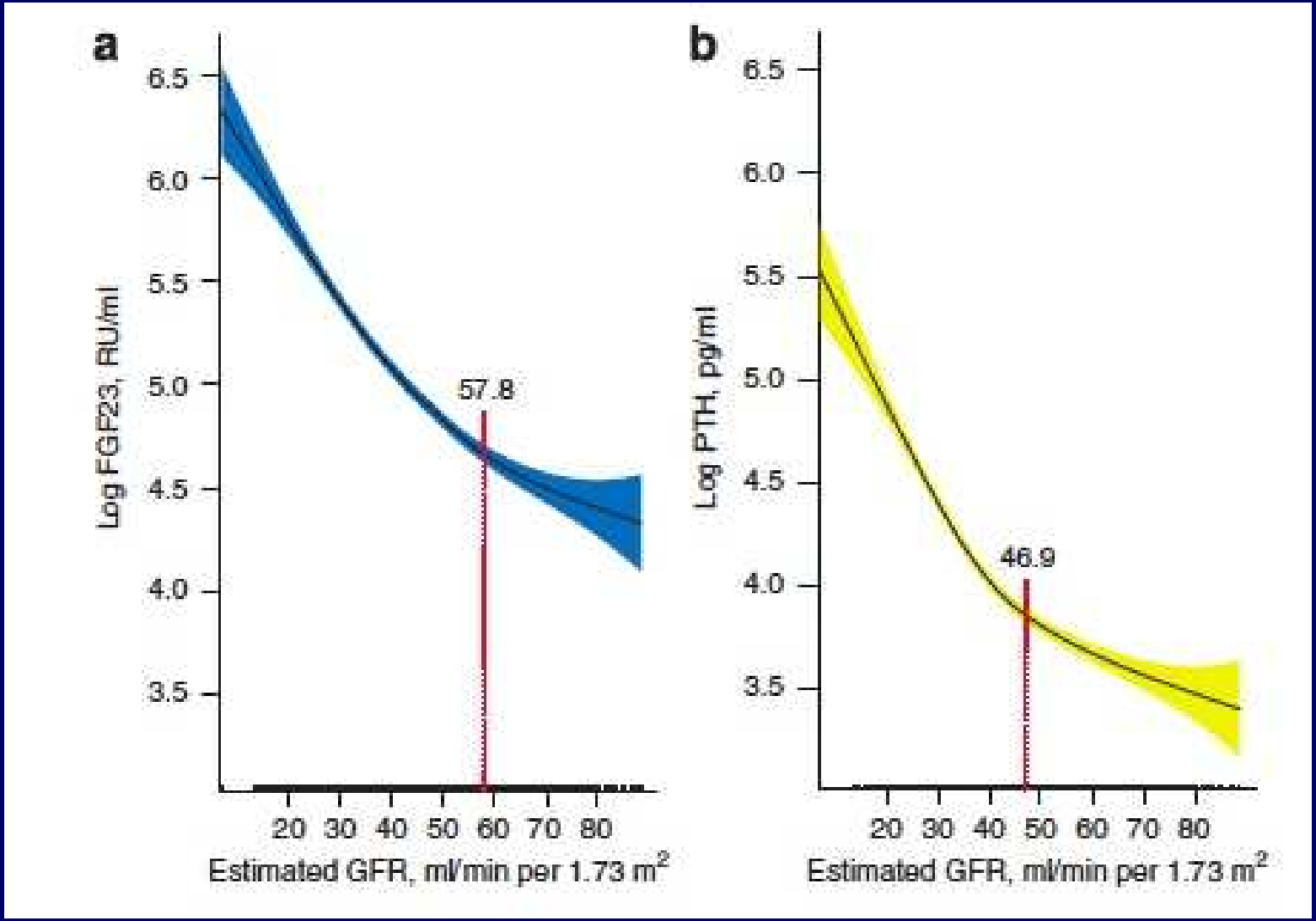
Razzaque M, Nat Rev Endocrinol 2009;5:611-619

Increase in serum FGF-23 with reduction of kidney function



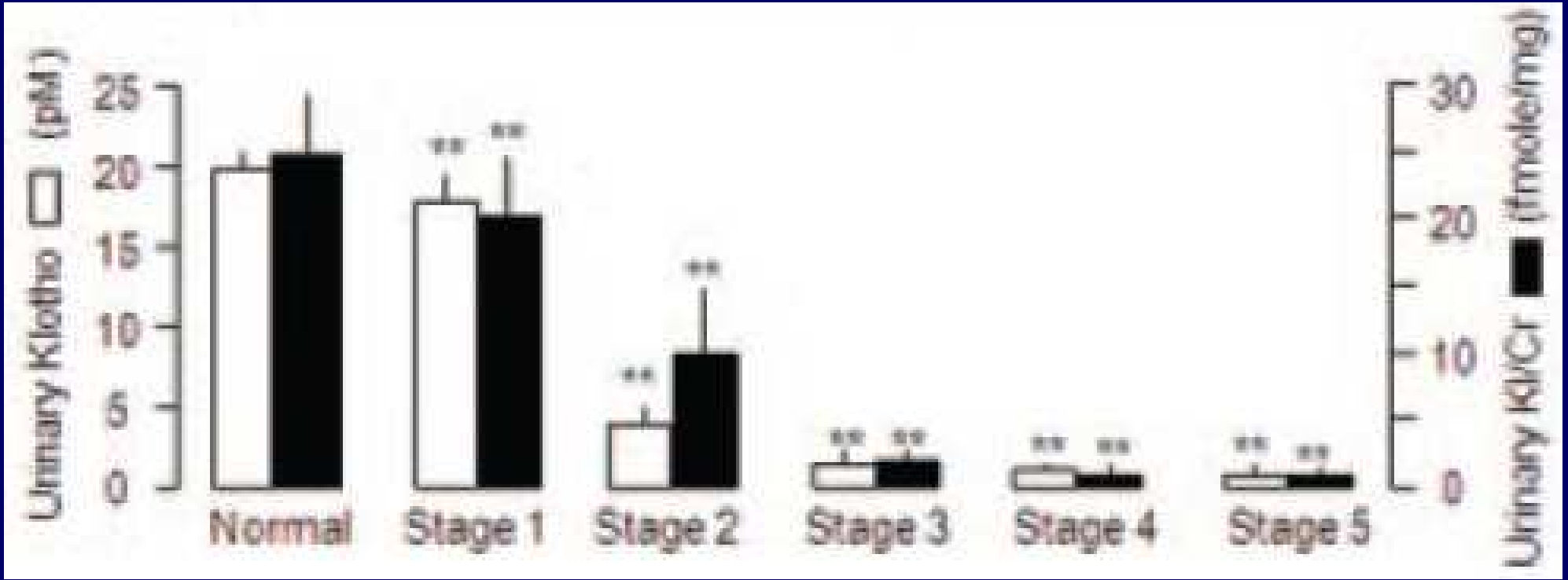
Gutierrez et al, JASN 2005;16:2205-15

Cubic spline functions of the associations of eGFR with log FGF23 and log PTH



Isakova T et al, KI 2011;79:1370-8

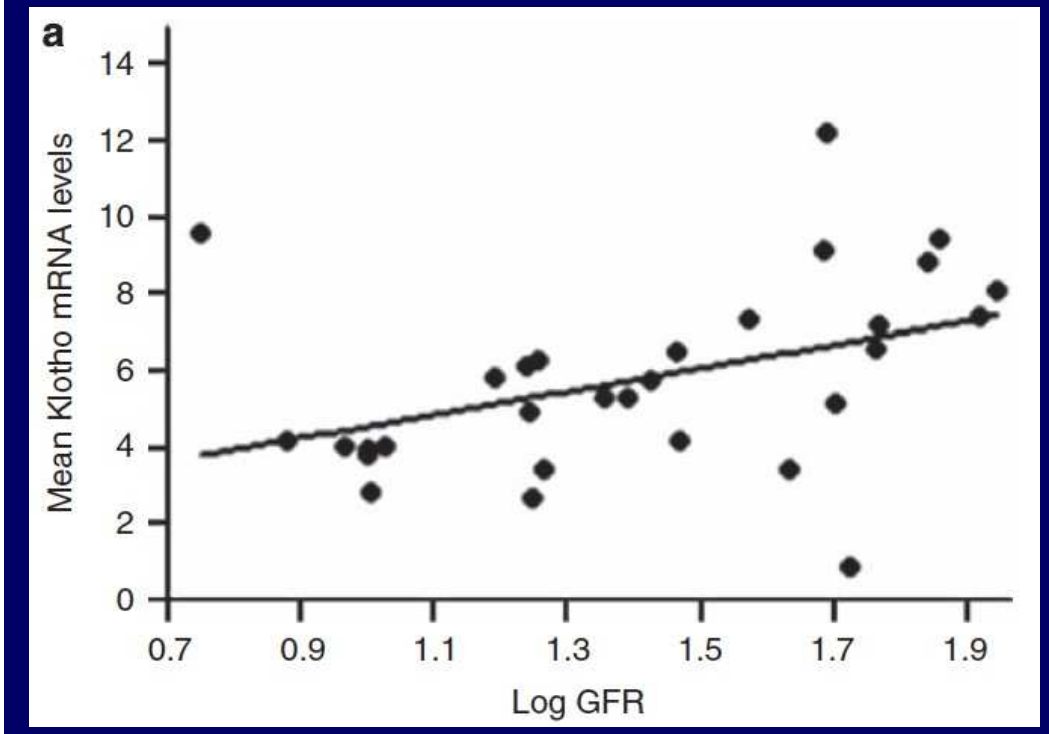
Decrease in urinary Klotho excretion with reduction of kidney function

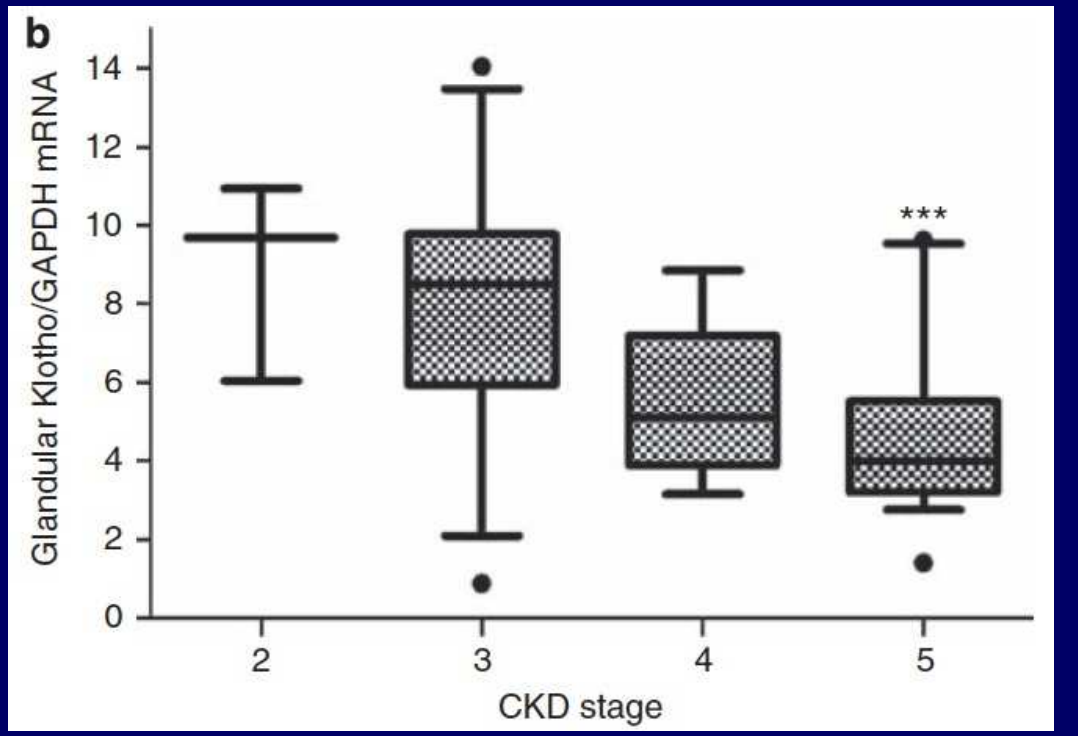


Hu MC et al, JASN 2011;22:124-136

Downregulation of Klotho mRNA expression in parathyroid tissue of patients with CKD

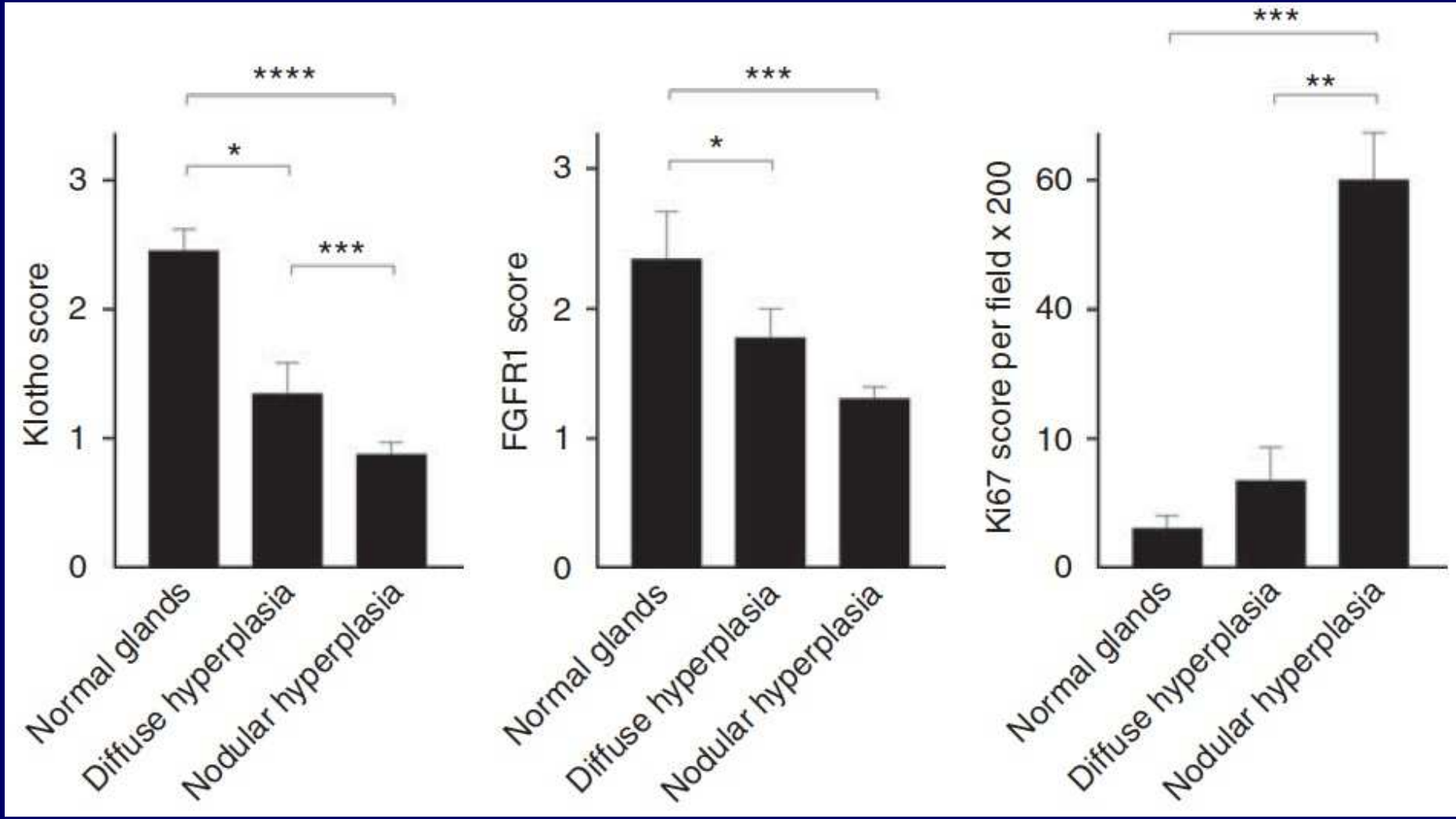
(hyperplastic parathyroid glands ; CKD stages 2-5)





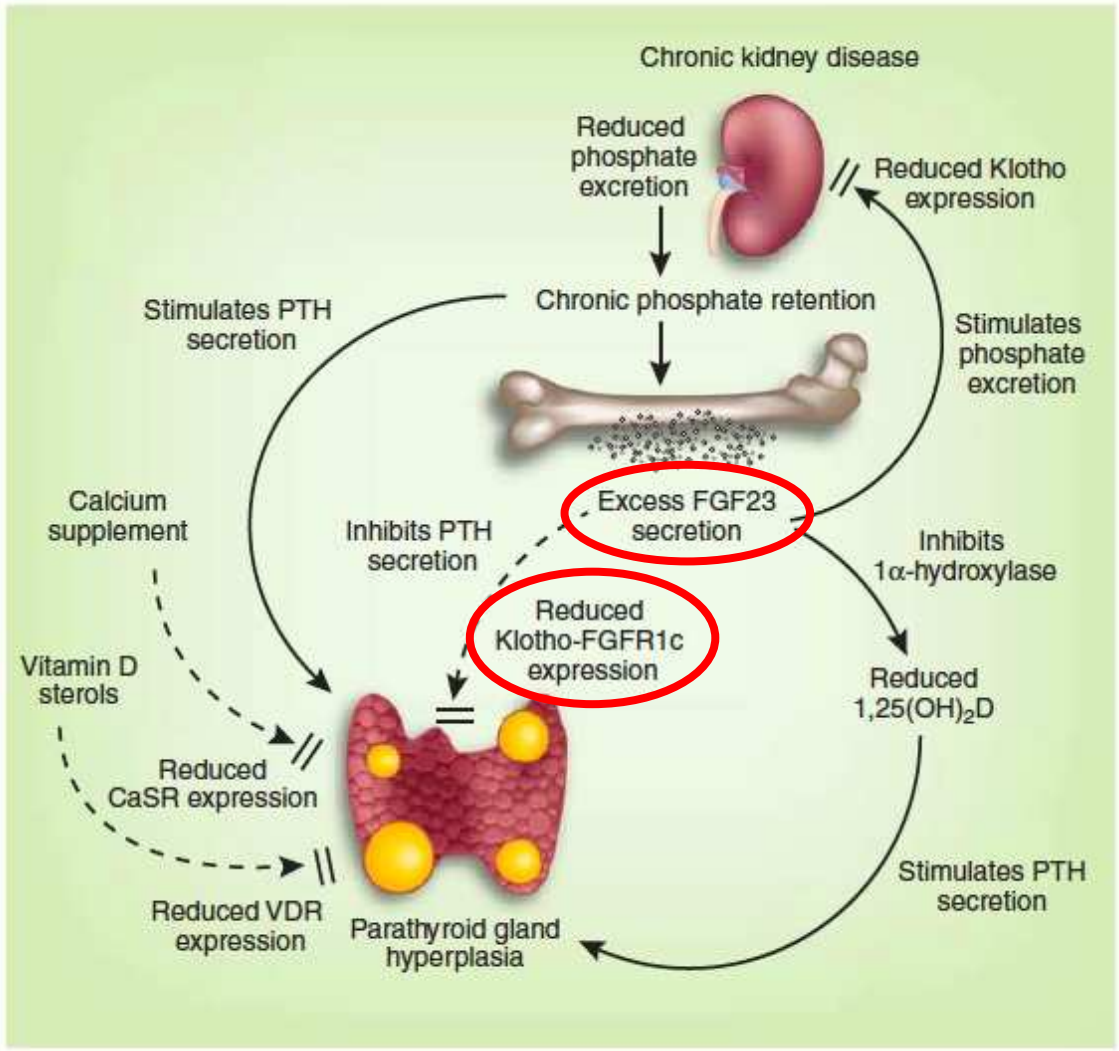
Krajisnik T et al, KI 2010;78:1024-32

Depressed expression of klotho and FGFR1 in parathyroid tissue of hemodialysis patients

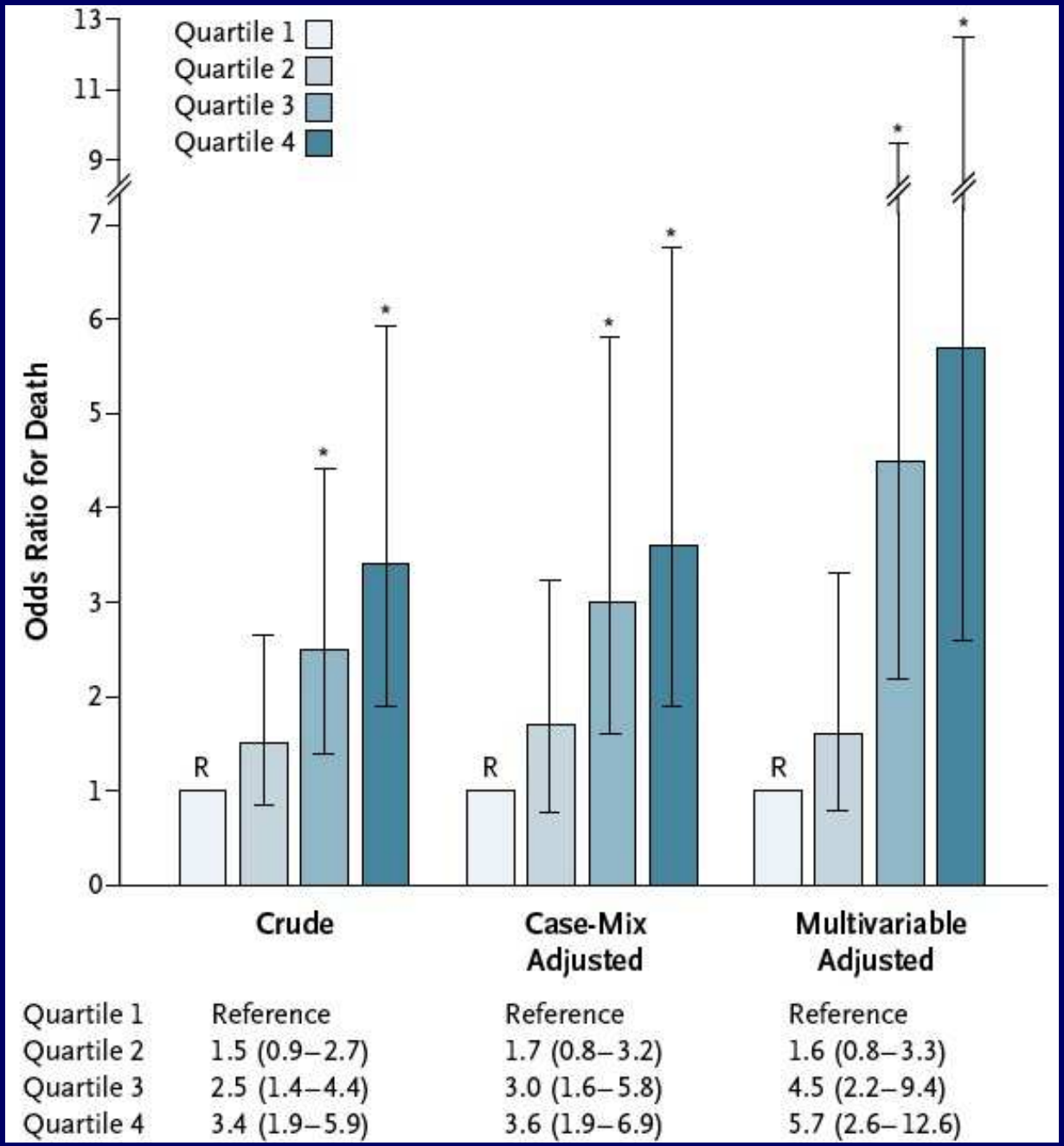


Komaba H et al, Kidney Int 2010; 77: 232-8

FGF23 and Klotho interaction with Ca, Pi, calcitriol and PTH in CKD-MBD



Komaba and Fukagawa. Kidney Int 2010;77:292–298 (slightly modified)

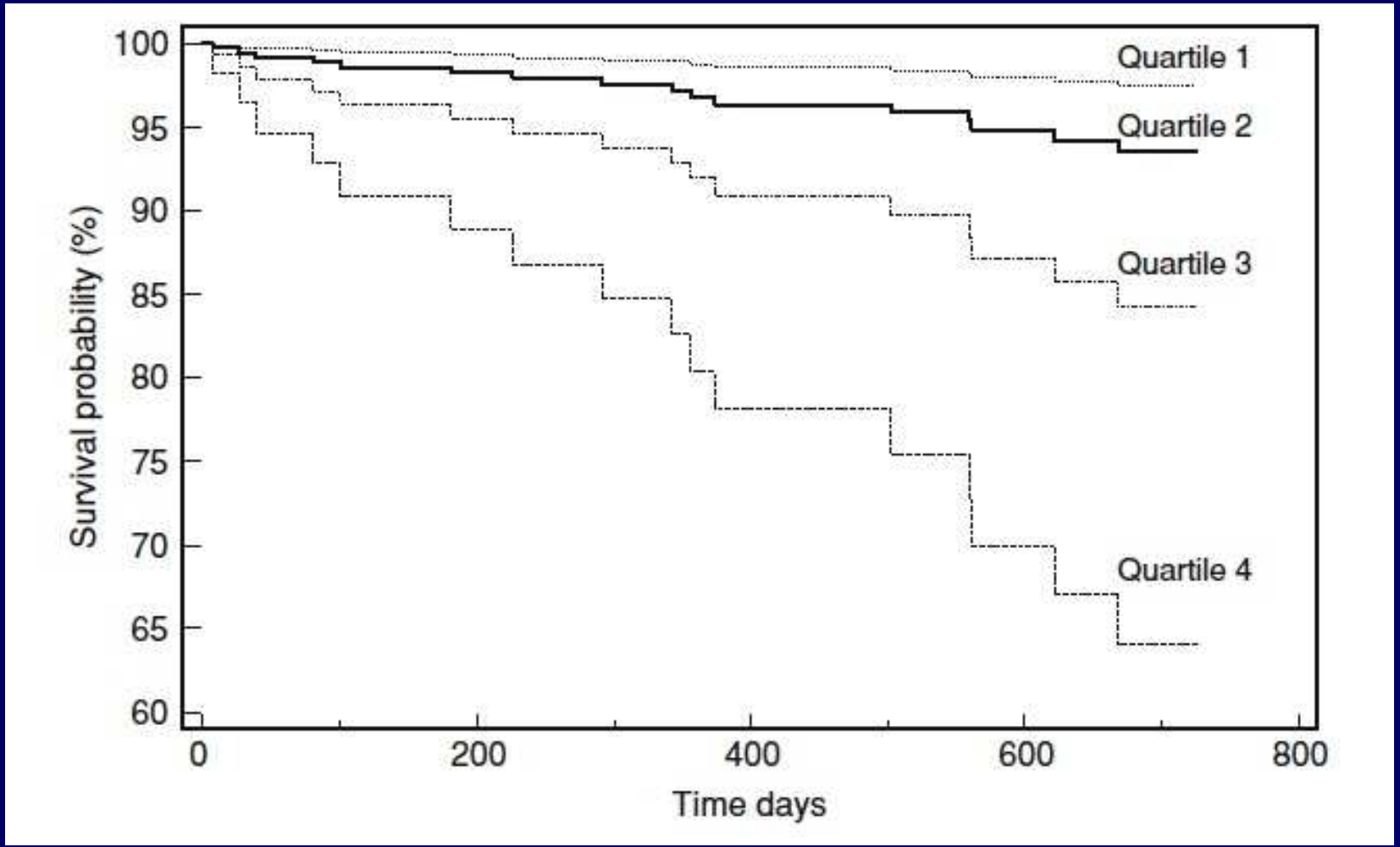


	Crude	Case-Mix Adjusted	Multivariable Adjusted
Quartile 1	Reference	Reference	Reference
Quartile 2	1.5 (0.9–2.7)	1.7 (0.8–3.2)	1.6 (0.8–3.3)
Quartile 3	2.5 (1.4–4.4)	3.0 (1.6–5.8)	4.5 (2.2–9.4)
Quartile 4	3.4 (1.9–5.9)	3.6 (1.9–6.9)	5.7 (2.6–12.6)

Independent association of high FGF-23 with mortality in HD patients *(FGF-23 level quartiles)*

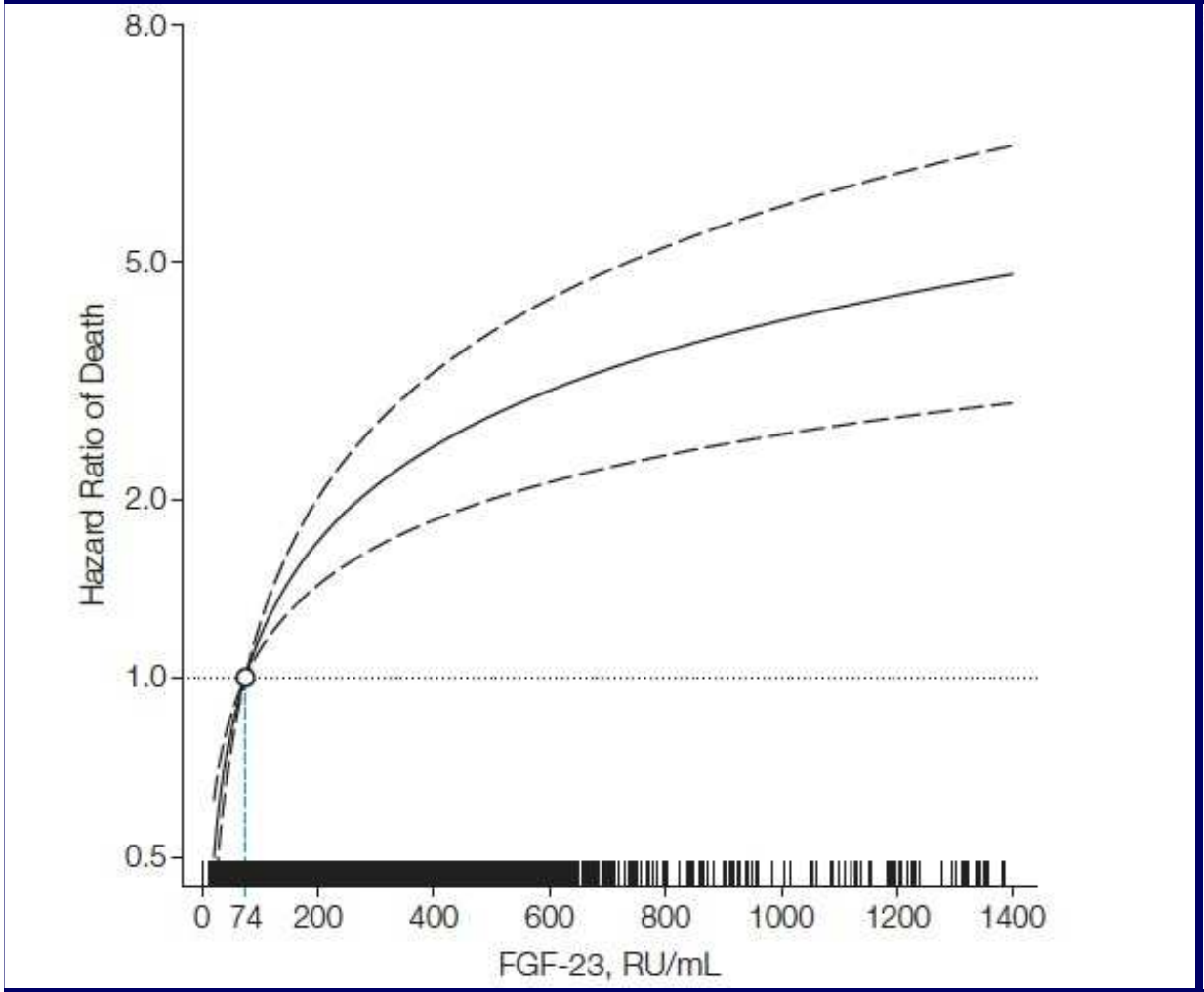
Gutierrez OM et al, NEJM 2008; 359: 584-92

Survival of chronic hemodialysis patients according to serum FGF-23 quartiles

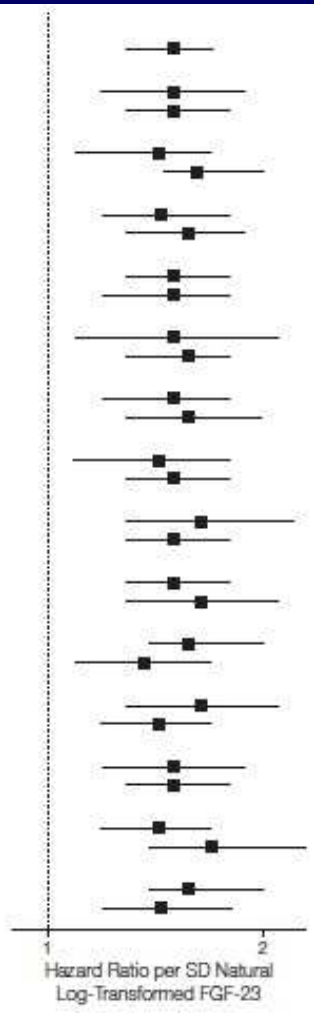


Jean G et al, NDT 2009; 24: 2792-6

Plasma FGF23 is an independent factor of relative mortality risk *(CRIC study; 3879 CKD patients)*

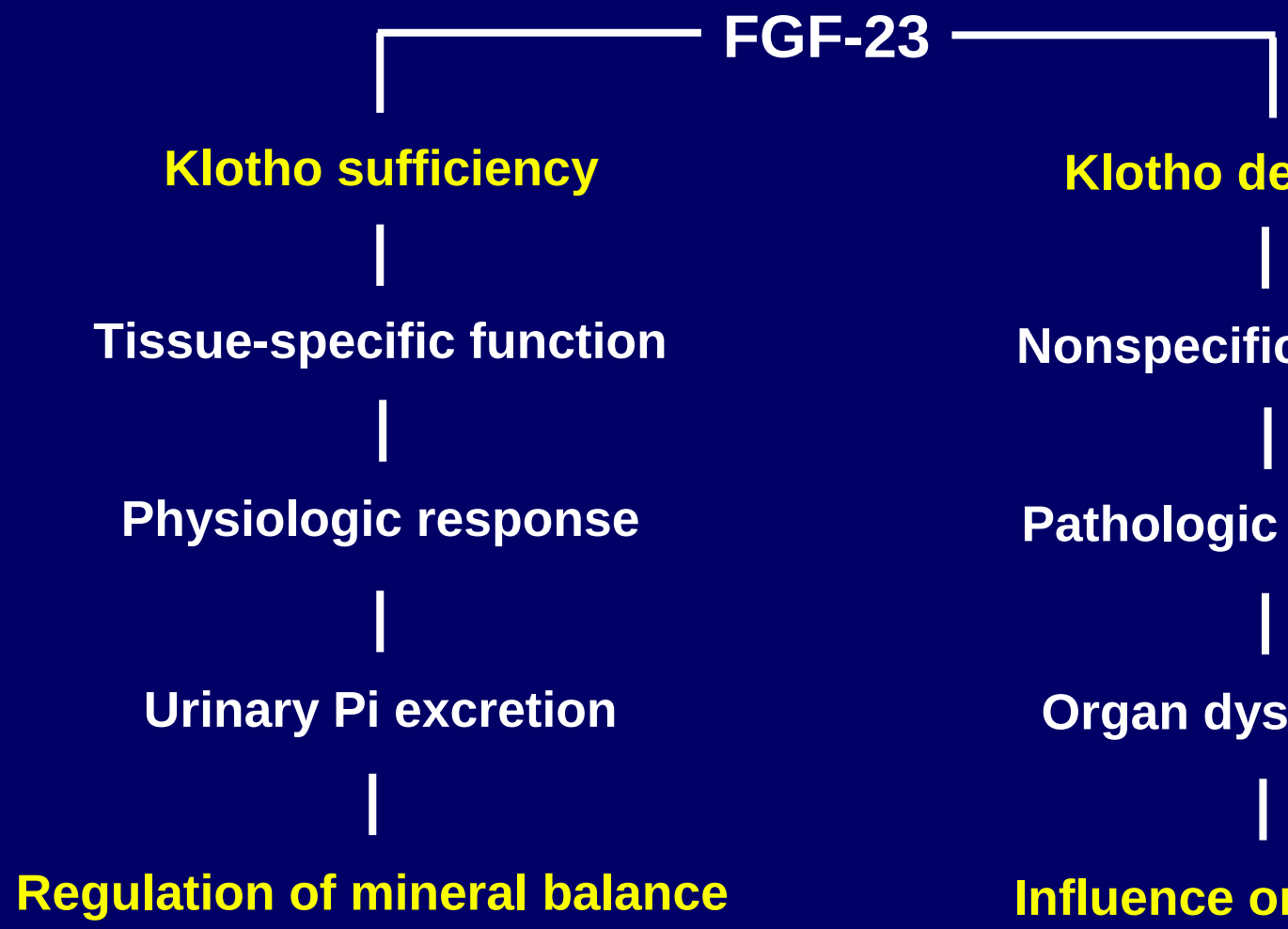


Subgroup	Deaths, No.	Sample Size, No.
Entire cohort	240	3514
Age, y		
<60	82	1658
≥60	158	1856
Sex		
Women	90	1583
Men	150	1931
Race		
Black	118	1454
Nonblack	122	2060
Diabetes		
Yes	149	1690
No	91	1824
Smoking status		
Yes	51	442
No	189	3072
Cardiovascular disease		
Yes	152	1175
No	88	2339
Cystatin C, mg/L		
<1.5	64	1721
≥1.5	176	1793
Albumin-to-creatinine ratio, mg/g		
<52	87	1762
≥52	153	1752
Albumin, g/dL		
<4.0	138	1685
≥4.0	103	1843
Calcium, mg/dL		
<9.2	130	1650
≥9.2	110	1866
Phosphate, mg/dL		
<3.7	87	1693
≥3.7	155	1839
Parathyroid hormone, pg/mL		
<54	95	1823
≥54	151	1769
Hemoglobin, g/dL		
<12.5	135	1691
≥12.5	105	1835
Low-density lipoprotein, mg/dL		
<98	133	1737
≥98	107	1782



Isakova T et al, JAMA 2011; 305:2432-9

Normal and pathologic responses to FGF-23 as a function of klotho – a simplified scheme

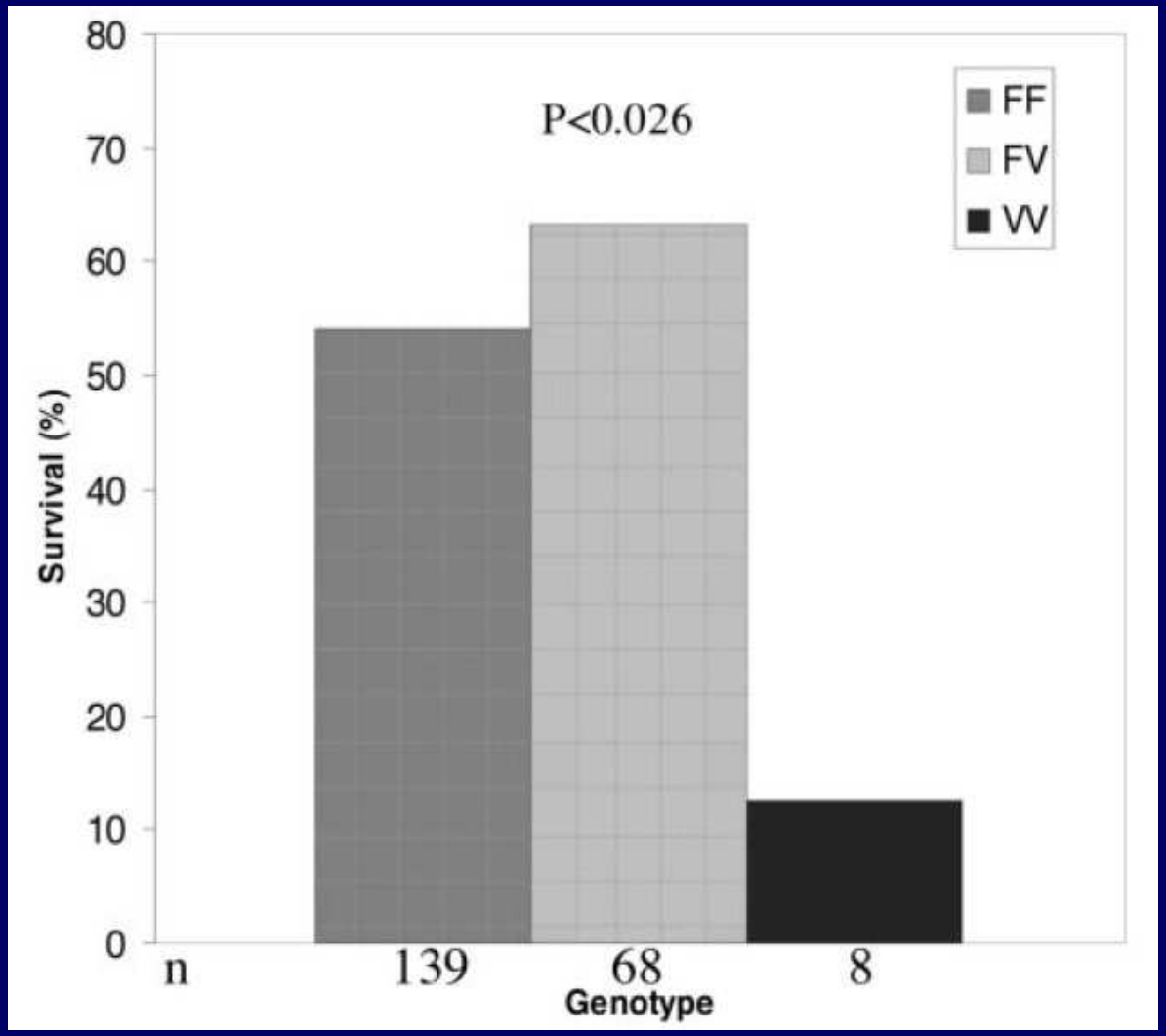


After Razzaque MS, 2008, modified

Klotho, FGF23 and life span

- Role on longevity in humans ?

Association of functional variant KL-VS of Klotho gene with longevity in general population



Arking DE et al, Circ Res 2005;96:412-8

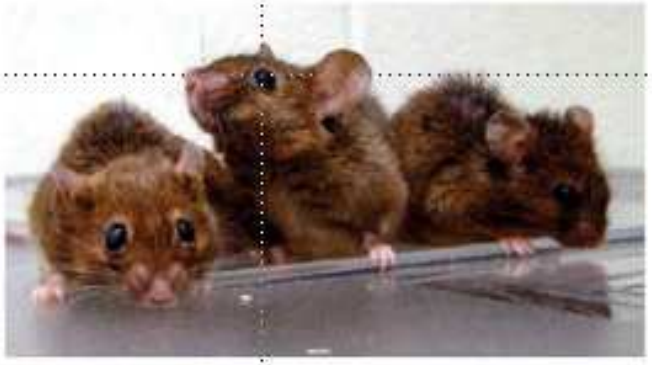
Klotho gene overexpression

NEWS OF THE WEEK

PHYSIOLOGY

Boosting Gene Extends Mouse Life Span

A protein named after the Greek goddess who spins life's thread has joined the short list of ways to extend a mouse's natural life span. Whereas lab mice can live about 2 years, mice engineered to overproduce this protein, called Klotho, have celebrated third birthdays, Makoto Kuro-o of the University of Texas Southwestern Medical Center in Dallas and his colleagues report online in this week's *Science* Express (www.sciencemag.org/cgi/content/abstract/1112766). The mutant rodents represent a rare case of a single gene substantially influencing life span in mammals.

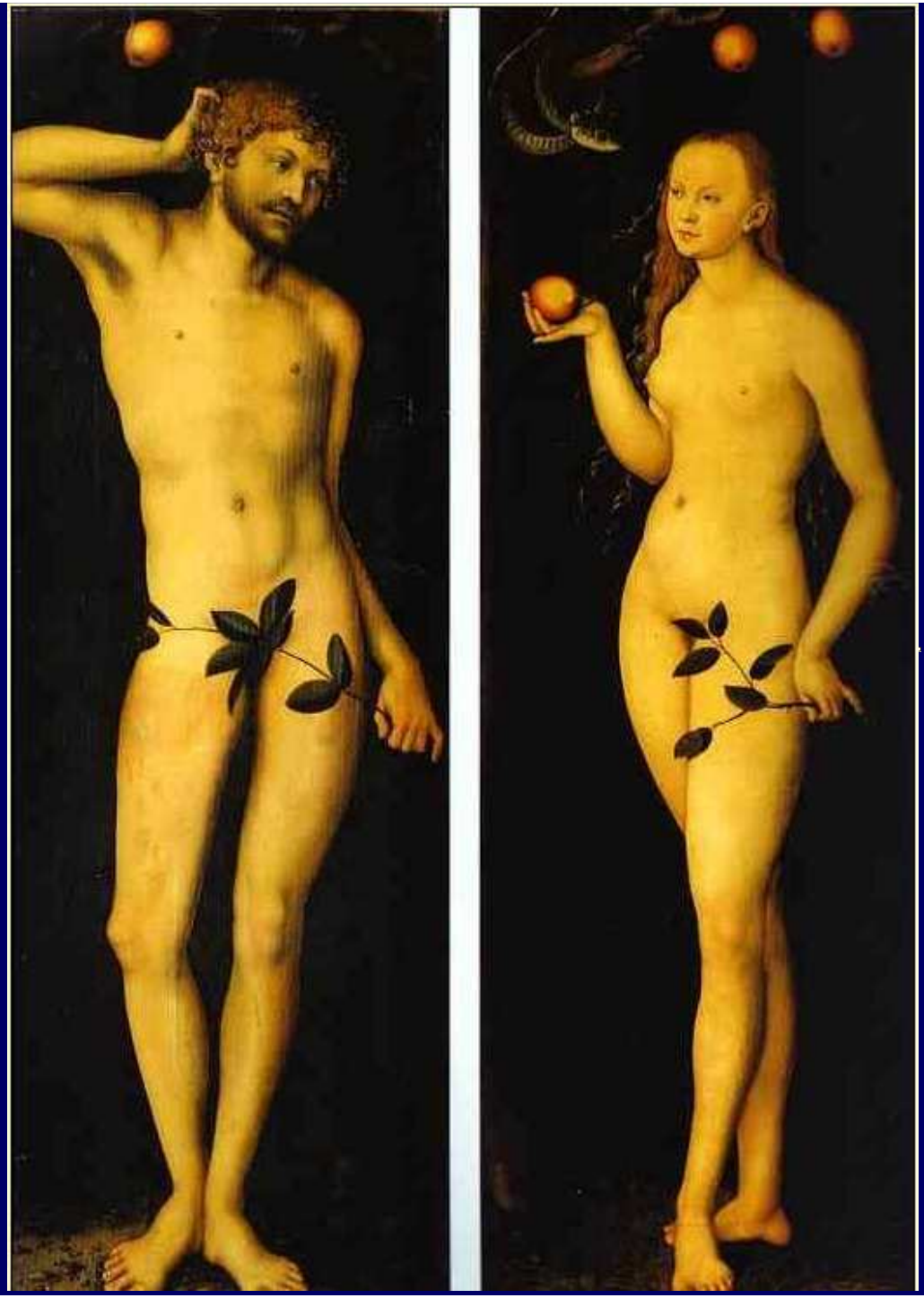


Rare milestone. These mice, which overexpress the gene for Klotho, have celebrated their third birthdays.

into the blood and may act like a hormone. Males making extra Klotho lived up to 30% longer than normal males, and the mutant females survived 20% longer than normal counterparts. As with lab animals coaxed to have lengthy life spans, the altered rodents had fertility problems. They produced about half the expected number of offspring.

Males appeared more affected by Klotho than females did. Their blood, unlike that of females, contained more insulin than normal mice. This suggested that the male mutants were somewhat resistant to insulin—a symptom, in extreme

Science 26 August 2005, Vol 309



Eternal life

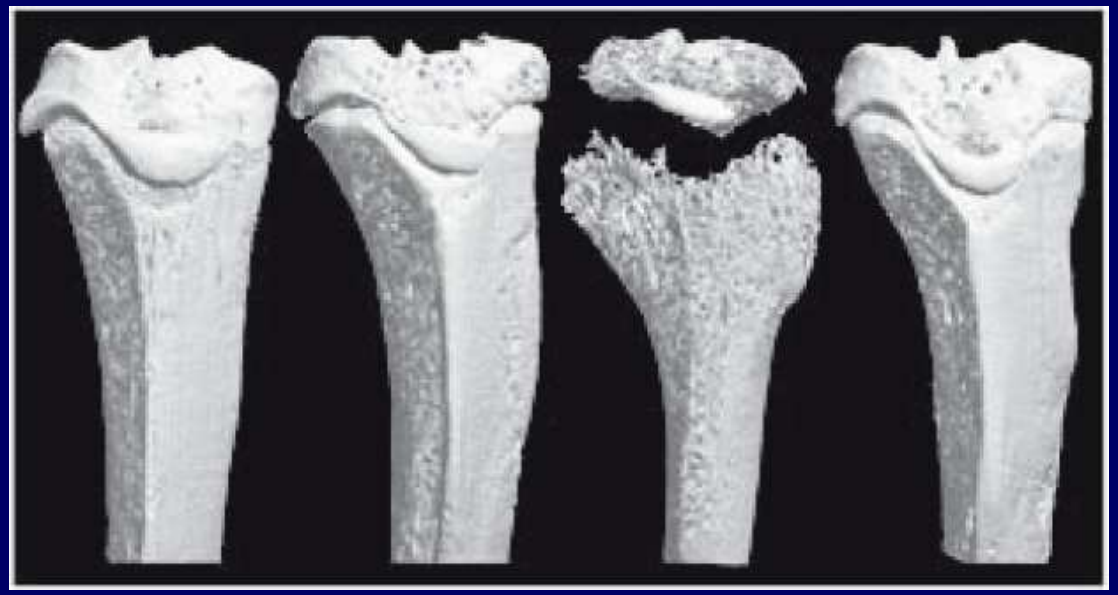
Adam and Eve

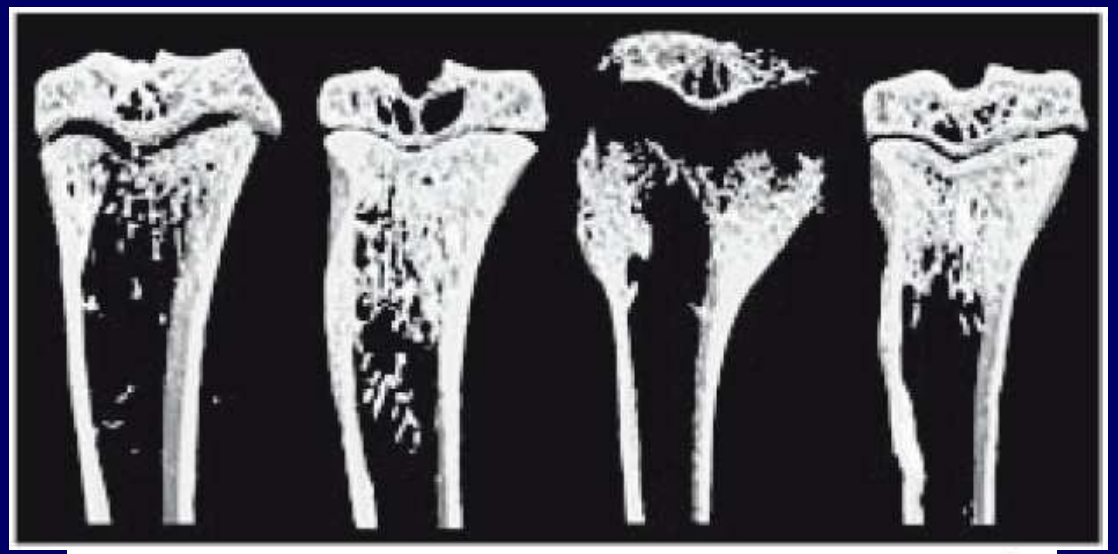
Lucas Cranach the Elder

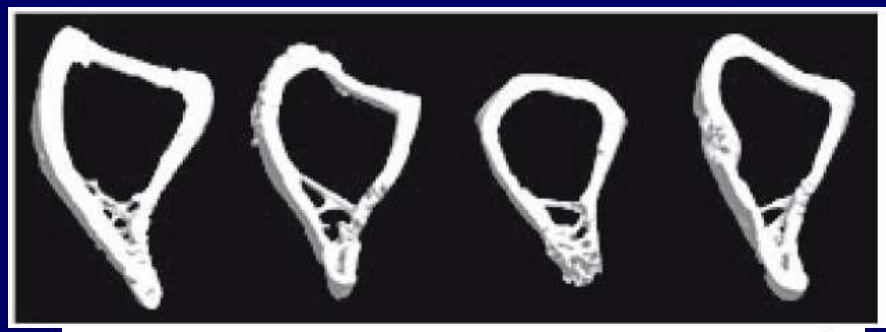
Galleria degli Uffizi, Florence
1528

(http://www.abcgallery.com/C/cranach/cranach66.html)

Klotho ablation converts FGF-23 transgenic phenotype to Klotho-deficient phenotype in mice

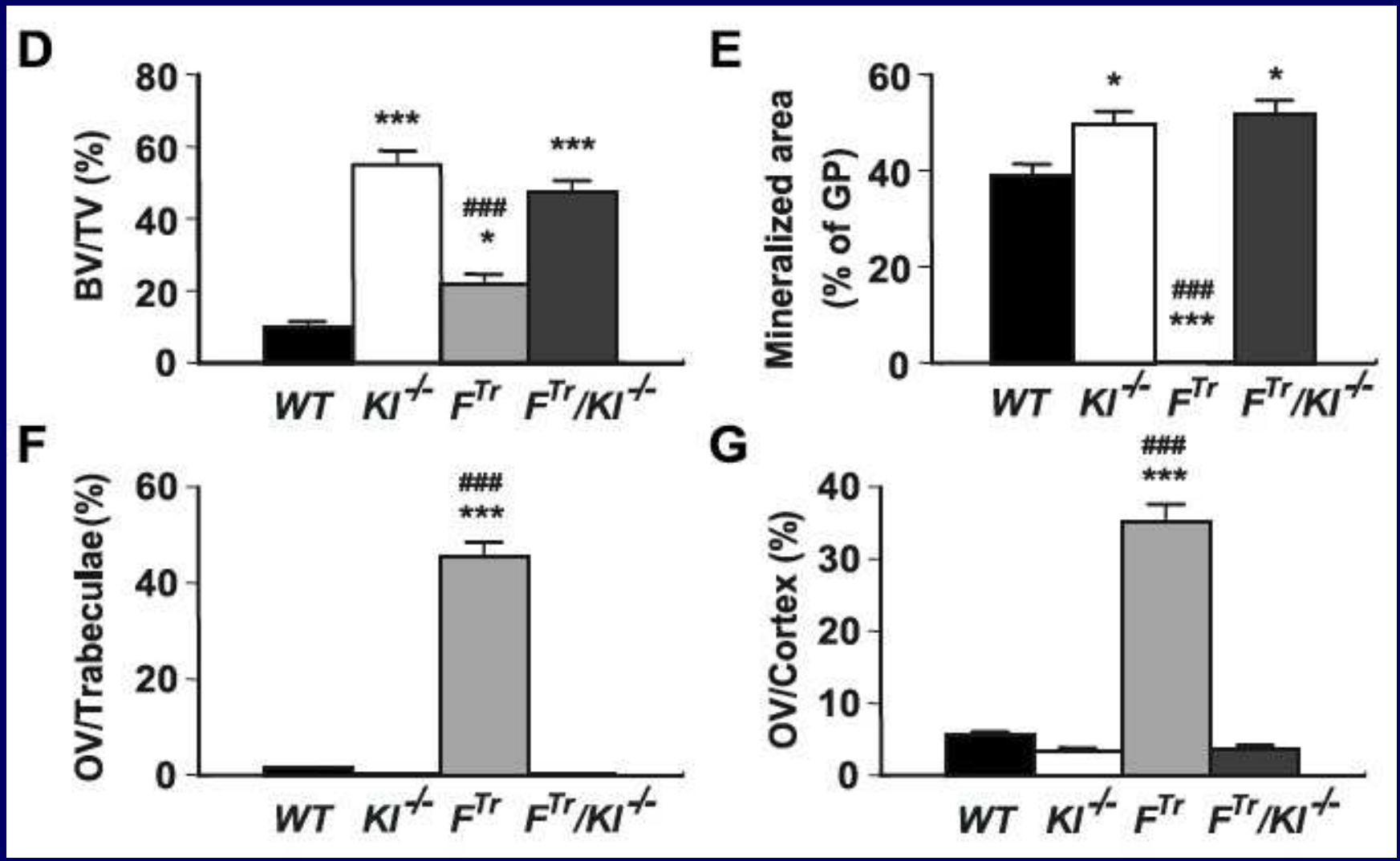






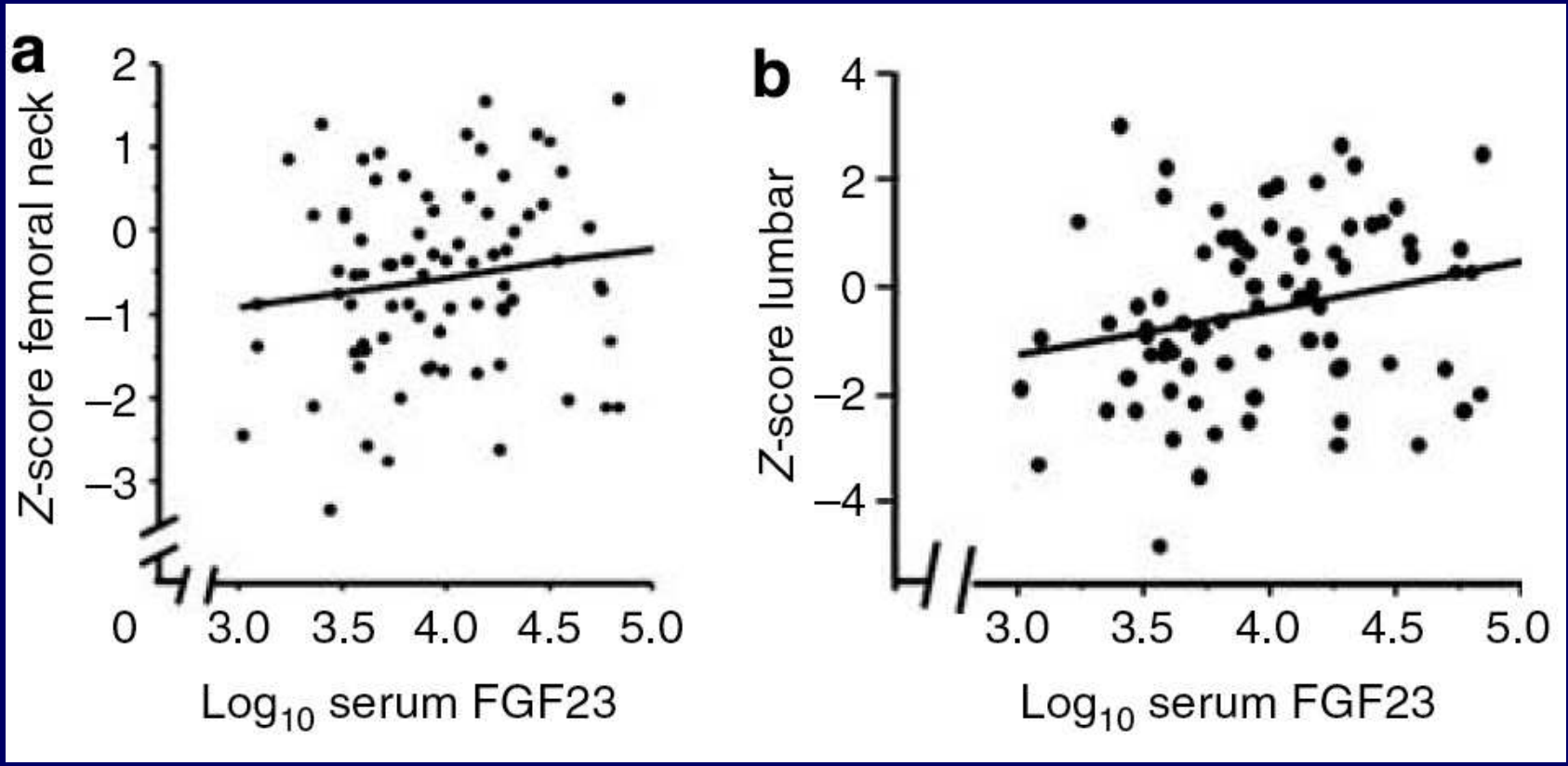
Bai X et al, AJP-Endocr Metab 2009;296:E79-88

Klotho ablation converts FGF-23 transgenic phenotype to Klotho-deficient phenotype in mice



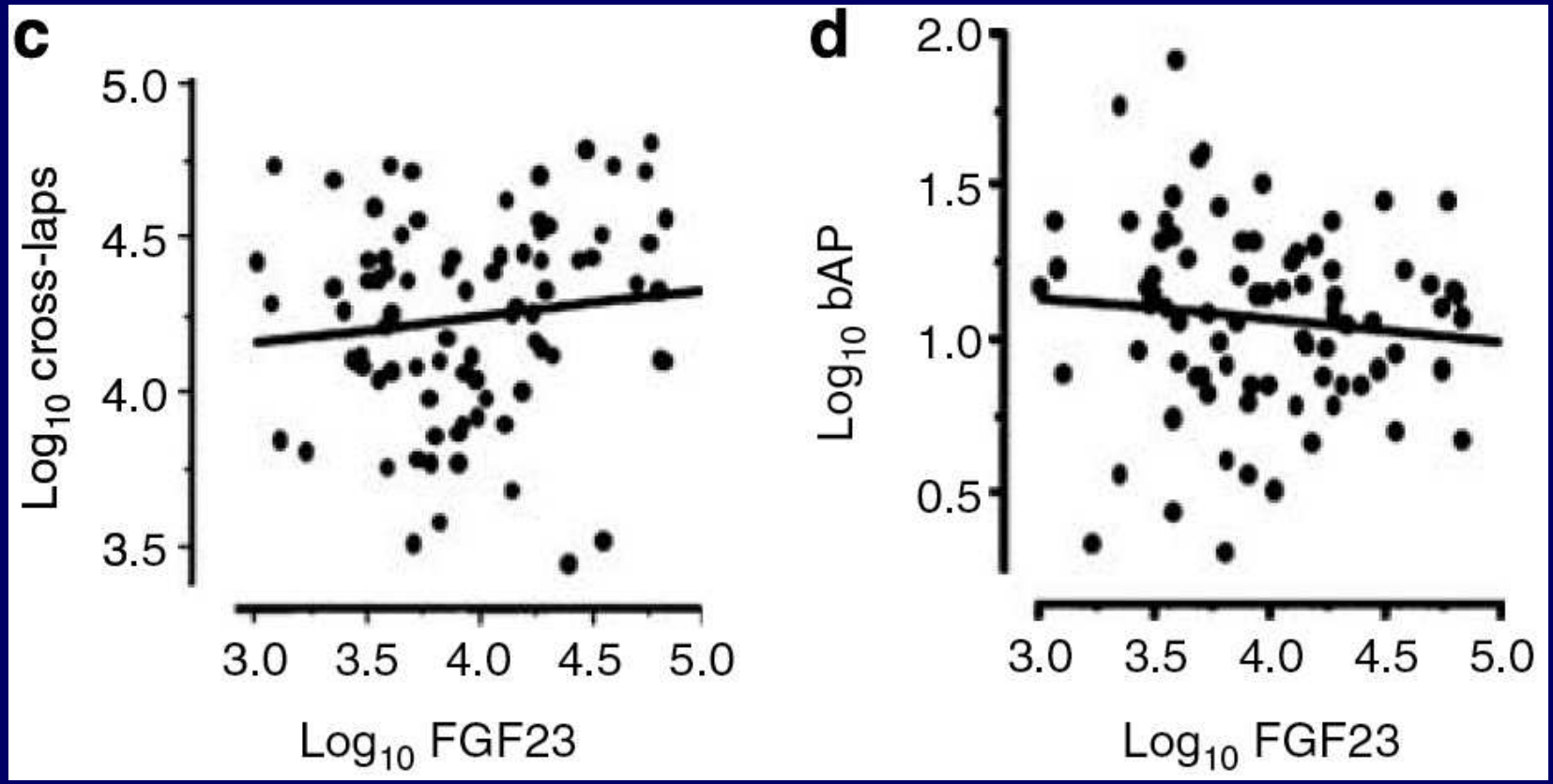
Bai X et al, AJP-Endocr Metab 2009;296:E79-88

Absence of association between serum FGF23 and bone mineral density in chronic HD patients



Ureña P et al, KI 2008;73:102-7

Absence of association between serum FGF23 and cross-laps or bone alk. P'tase in chronic HD patients



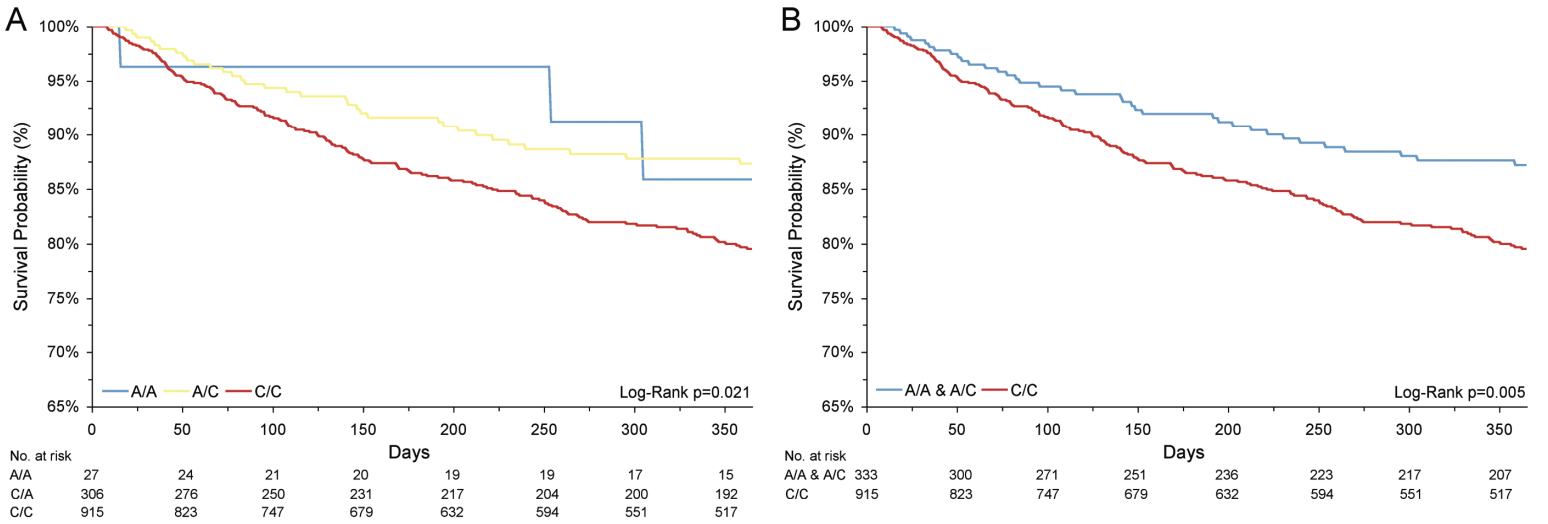
Ureña et al, KI 2008;73:102-7

The future ?

- Prolonged survival
- Long survival with high quality of life

Klotho gene variant rs577912 is associated with increased RR of mortality in chronic hemodialysis patients

FIGURE 1A-1D



A) All subjects, additive model

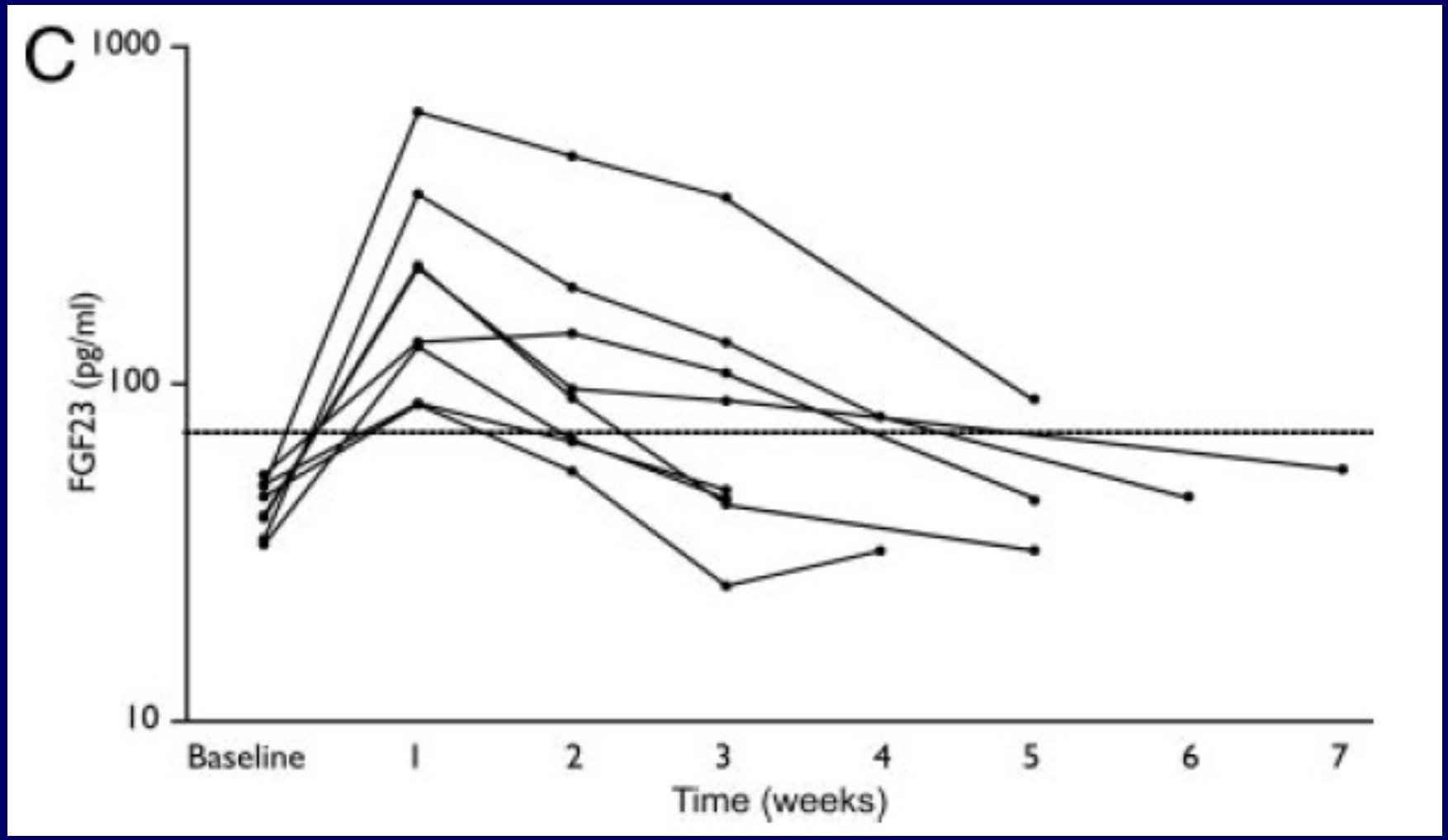
B) All subjects, dominant model

C) Patients on calcitriol therapy

D) Patients not on calcitriol therapy

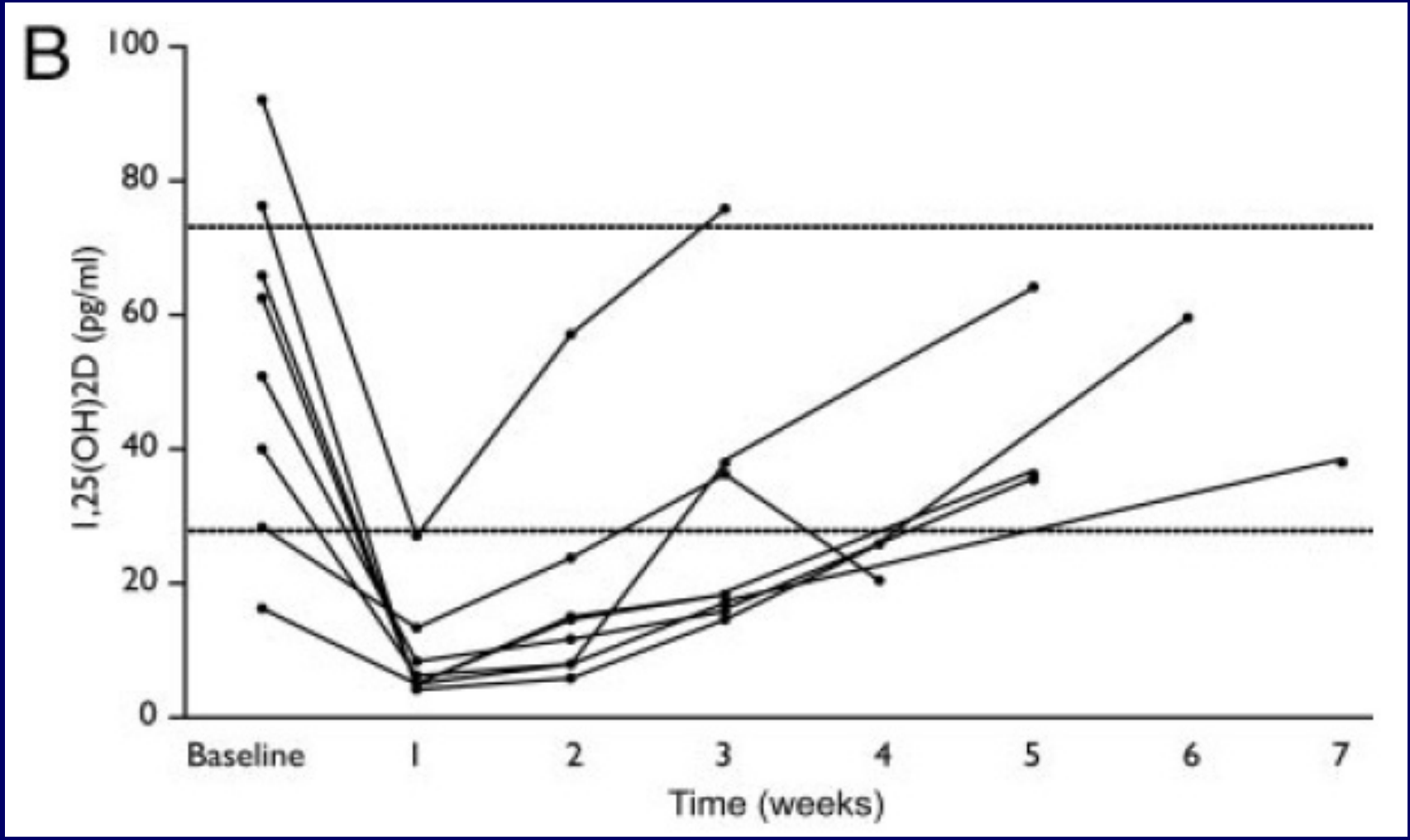
Friedman DJ et al, JBMR 2009;24:1847-55

Increase in serum FGF23 after a single IV iron dose (918 mg) in medical outpatients (GFR > 60 ml/min)



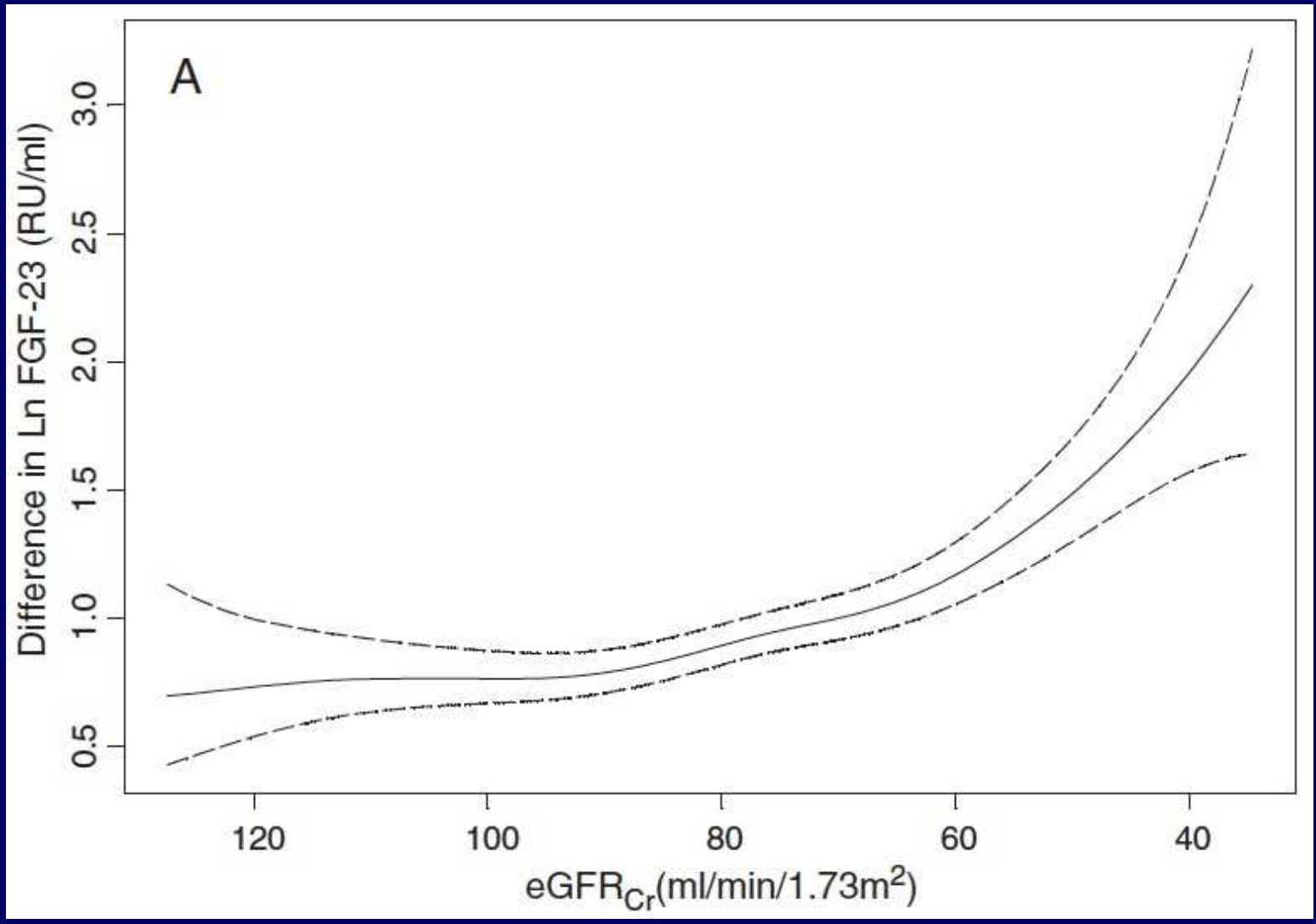
Schouten BJ et al, JCEM 2009:94:2332-7

Decrease in serum calcitriol after a single IV iron dose (918 mg) in medical outpatients (GFR > 60 ml/min)



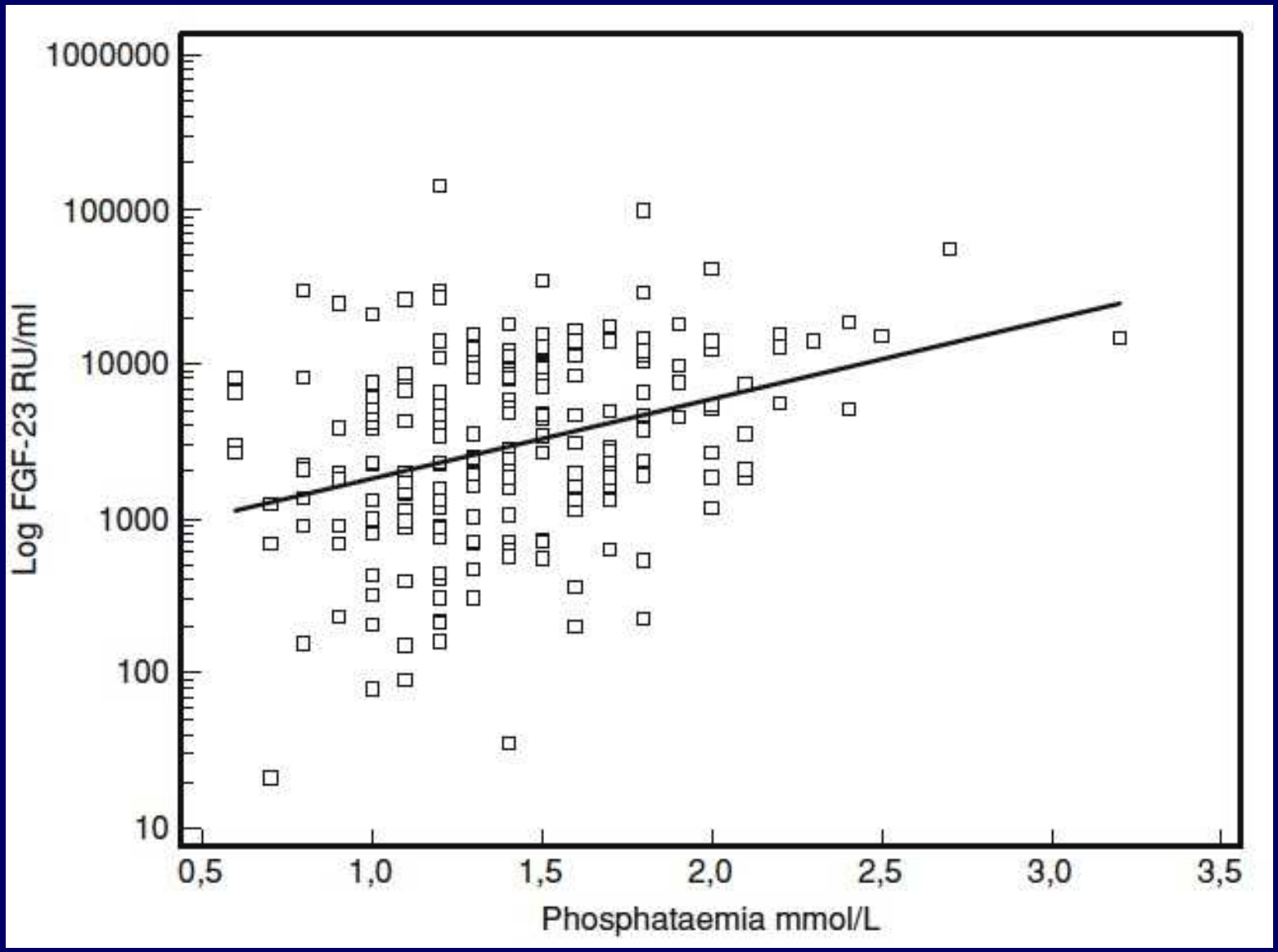
Schouten BJ et al, JCEM 2009:94:2332-7

Serum cFGF-23 as a function of eGFR in patients with early CKD *(MDRD 4-variable formula)*



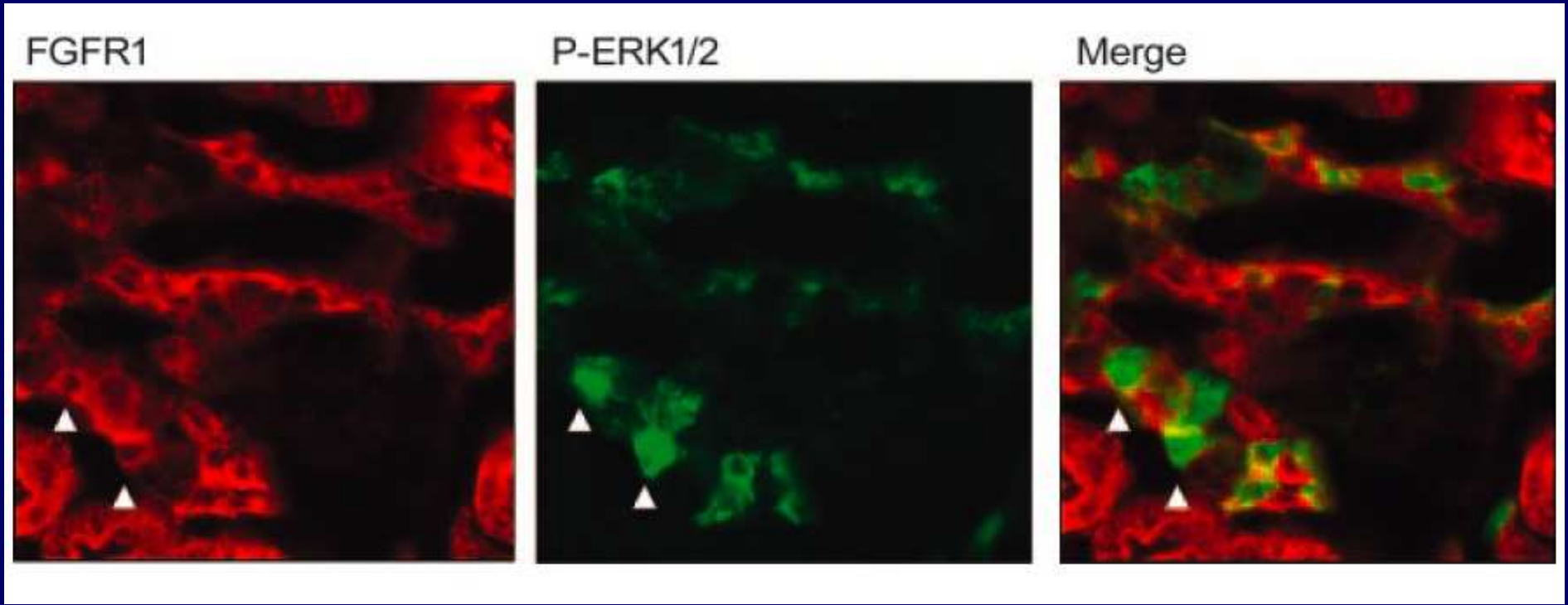
Ix JH et al, NDT 2010;25:993-7

Correlation between serum phosphorus and FGF-23 in chronic hemodialysis patients



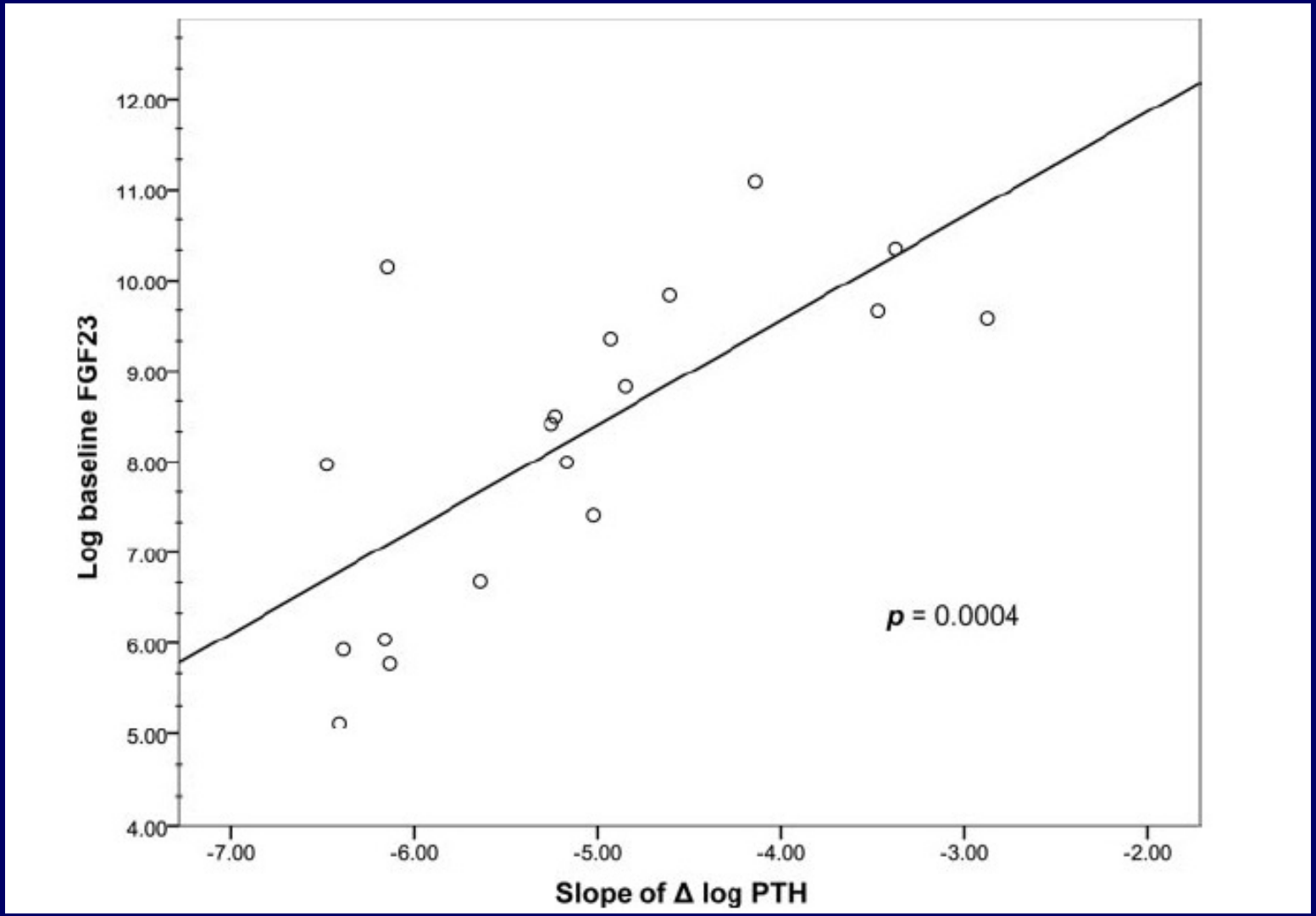
Jean G et al,
NDT 2009;24:2792-6

FGF-23 induces p-ERK1/2 signalling in distal tubular cells at sites of positive Klotho staining



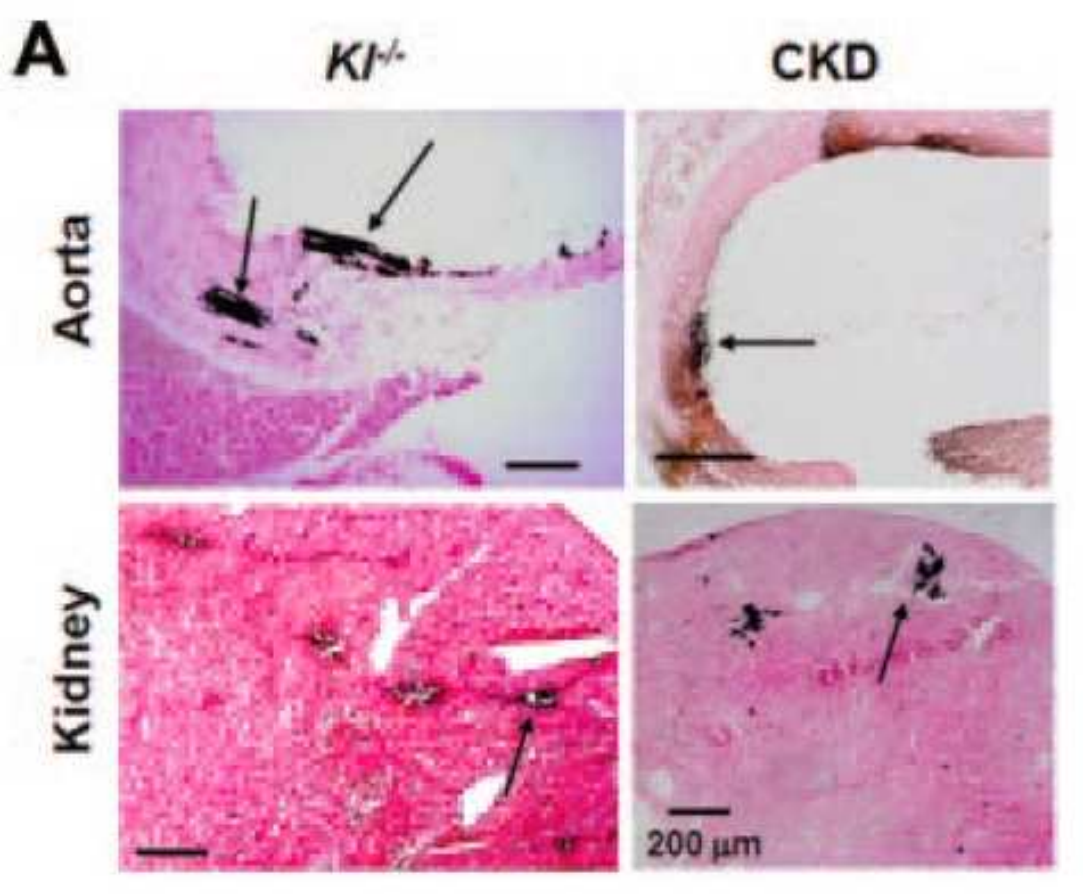
Farrow EG et al, JASN 2009;20:955-60

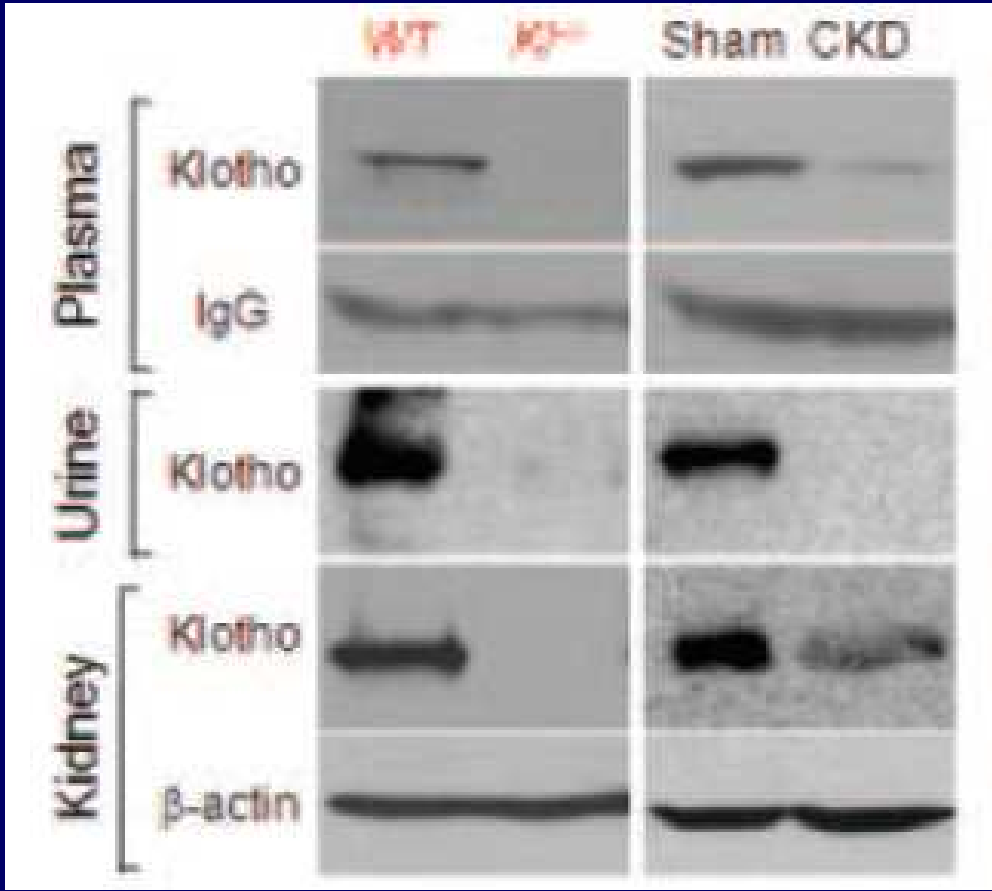
Association between elevated serum FGF23 and calcium-mediated PTH suppression in HD patients



Wetmore JB et al, JCEM 2011;96:E57-64

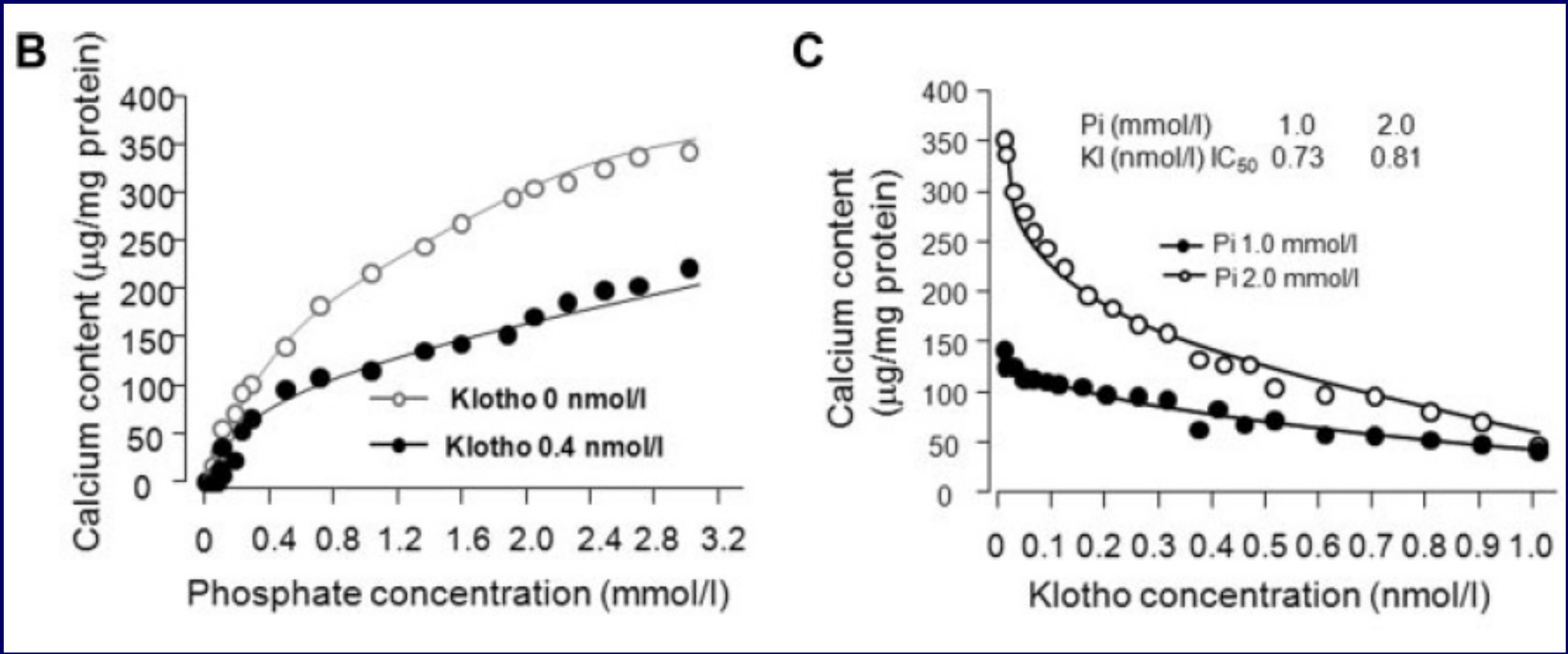
Tissue calcification in Klotho-/- mice and in CKD mice in association with reduced humoral and tissue klotho





Hu MC et al, JASN 2011;22:124-136

Rat VSMC calcification depending on phosphate and klotho concentration in vitro



Hu MC et al, JASN 2011;22:124-136