PTH, Calcium, & Phosphate Goals in CKD?

Balancing Arteriosclerosis, Renal Function, & Bone Integrity

Disclosures: none

Objectives: Introduction to arterial osteogenesis

1. To discuss the mechanisms for osteogenesis, bone maintenance, & calcium-phosphate homeostasis
2. To identify the hormonal, cellular, & mineral modulators of arterial calcification
3. To propose possible therapies to reduce CV events & renal dysfunction w/o adversely affecting bone

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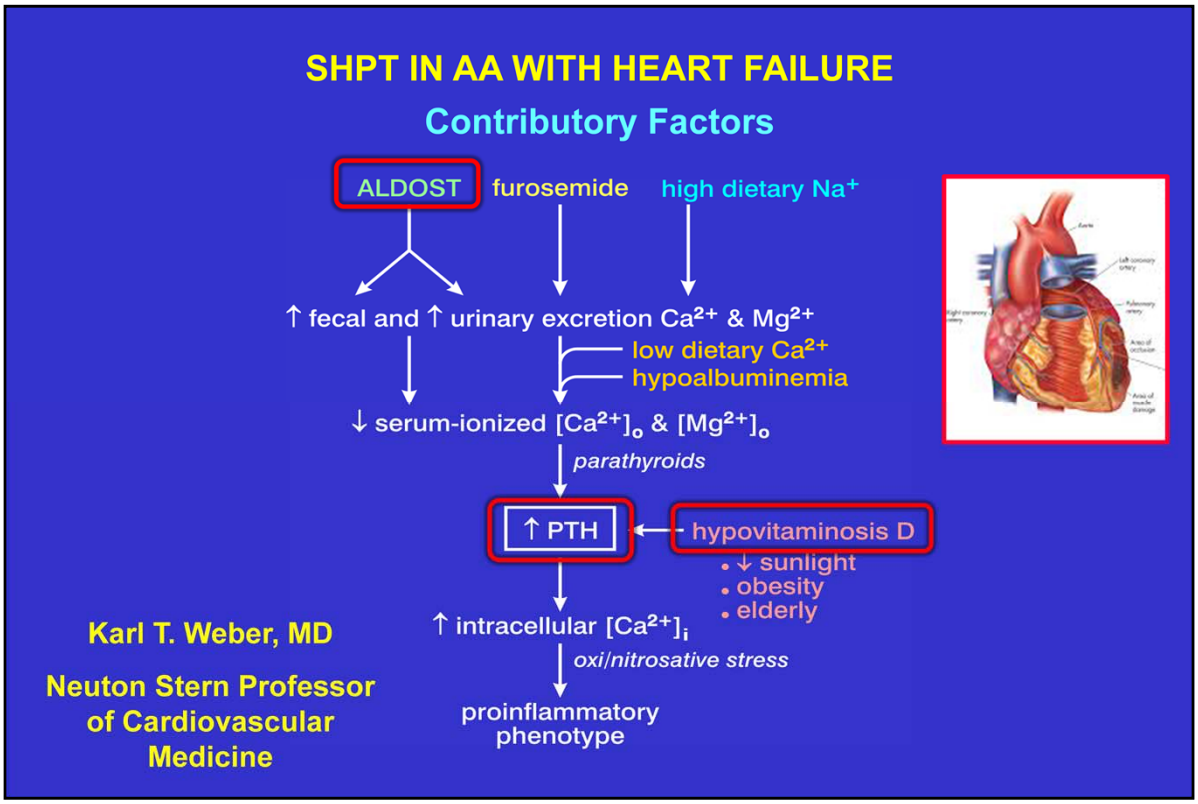


Increasing aldosterone → increased urinary calcium & magnesium excretion → reducing serum levels which induces increased PTH → increase intracellular calcium → toxicity

Loop diuretics and/or high sodium intake will promote this process.

Low calcium intake and/or vitamin D deficiency will also promote this process.

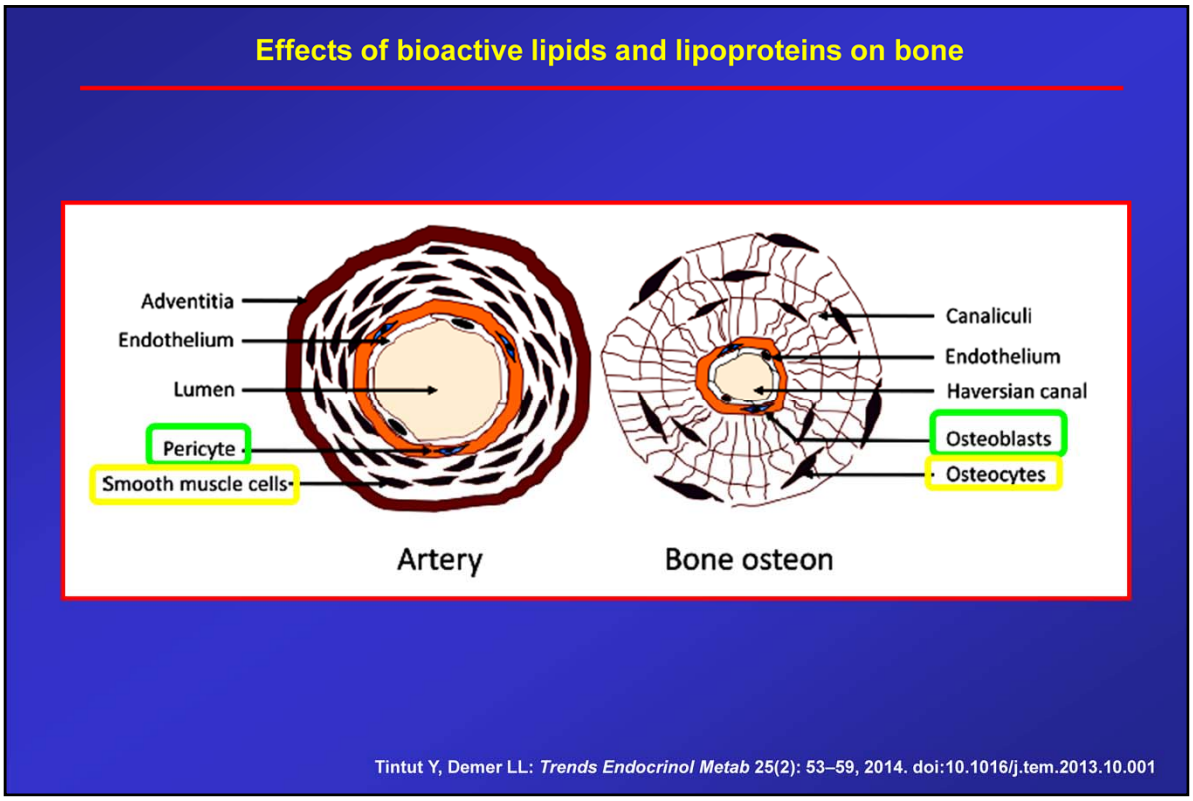


Figure 1. Schematic comparing the anatomic structure of an artery and bone osteon

Both the artery and osteon are centered on a blood lumen, which is surrounded by a single layer of endothelium. This, in turn, is surrounded by a basement membrane housing immature mesenchymal cells. In arteries, the immature cells are pericytes and/or smooth muscle cells, whereas, in bone, they are pericytes and/or preosteoblasts.

(Modified from Parhami et al., Arteriosclerosis, Thrombosis, and Vascular Biology, 1997; 17:680–687).

Arteriosclerosis & Bone Physiology

Arteriosclerosis: arterial mechanical stiffening from any cause

1. Atherosclerosis: Eccentric intimal–medial atherosclerotic plaques with calcification, fibrosis, and cholesterol-laden lipoprotein deposition
2. Concentric medial & adventitial fibrosis with medial calcification, elastinolysis, & mural thickening (DM & CKD)

Bone Physiology: development and maintenance of bone

1. Osteogenesis → *de-novo* develop of bone, both normal & pathological (arteries)

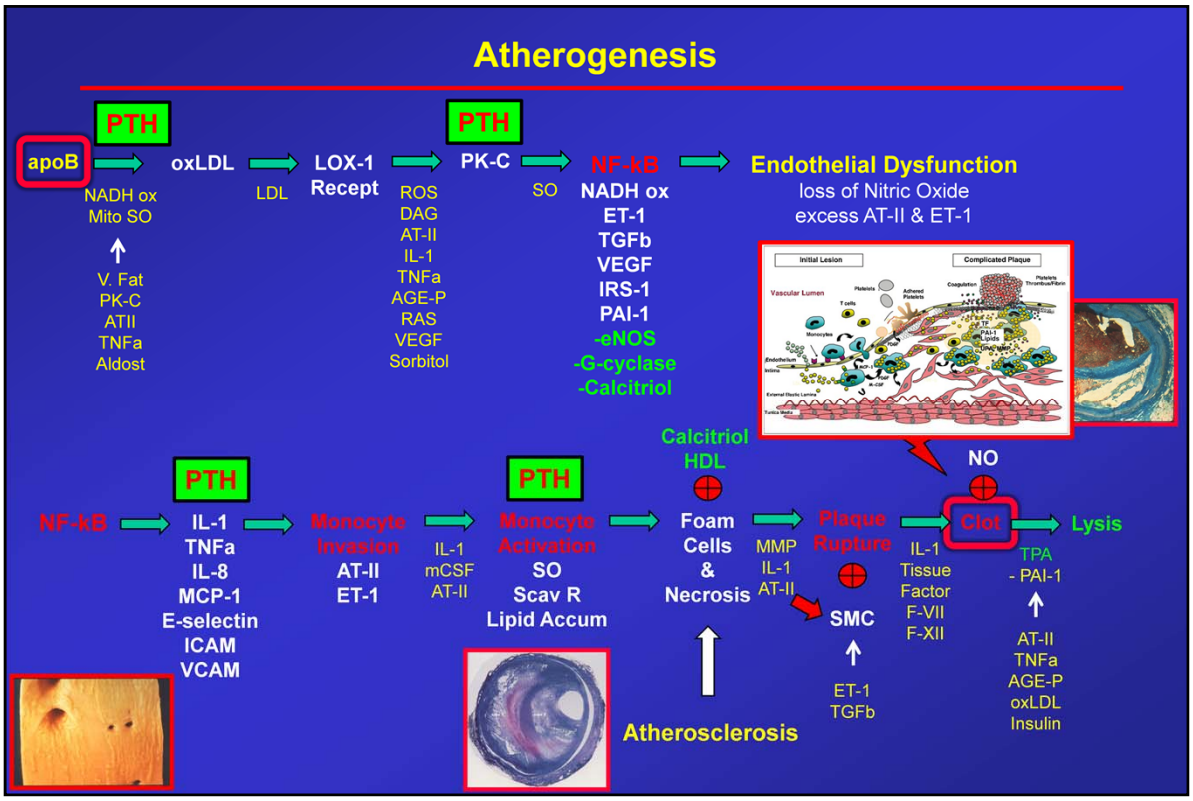
   Osteoblasts & BMP-2 (bone morphogenetic protein)

2. Bone Maintenance & Integrity → repair damage & respond to changing forces

   Osteocytes & PTHrp

3. Calcium & Phosphate Homeostasis → osteocytes, osteoclasts, osteoblasts, & circulating PTH

   Sacrifices bone for serum calcium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ET-1
TGFb
VEGF
IRS-1
PAI-1
-eNOS
-G-cyclase
-Calcitriol
Endothelial Dysfunction
loss of Nitric Oxide
excess AT-II & ET-1
Initial Lesion
Complicated Plaque
Vascular Lumen
Platelets
T cells
Monocytes
Endothelium
Lesion
Adhered Platelets
Coagulation
Platelets Thrombus/Fibrin
PAI-1
Lipids
Smooth Muscle
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Calcitriol
HDL
PTH
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IL-8
MCP-1
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ICAM
VCAM
Monocyte Invasion
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ET-1
IL-1
mCSF
AT-II
PTH
Monocyte Activation
SO
Scav R
Lipid Accum
Foam Cells & Necrosis
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IL-1
AT-II
Plaque Rupture
SMC
ET-1
TGFb
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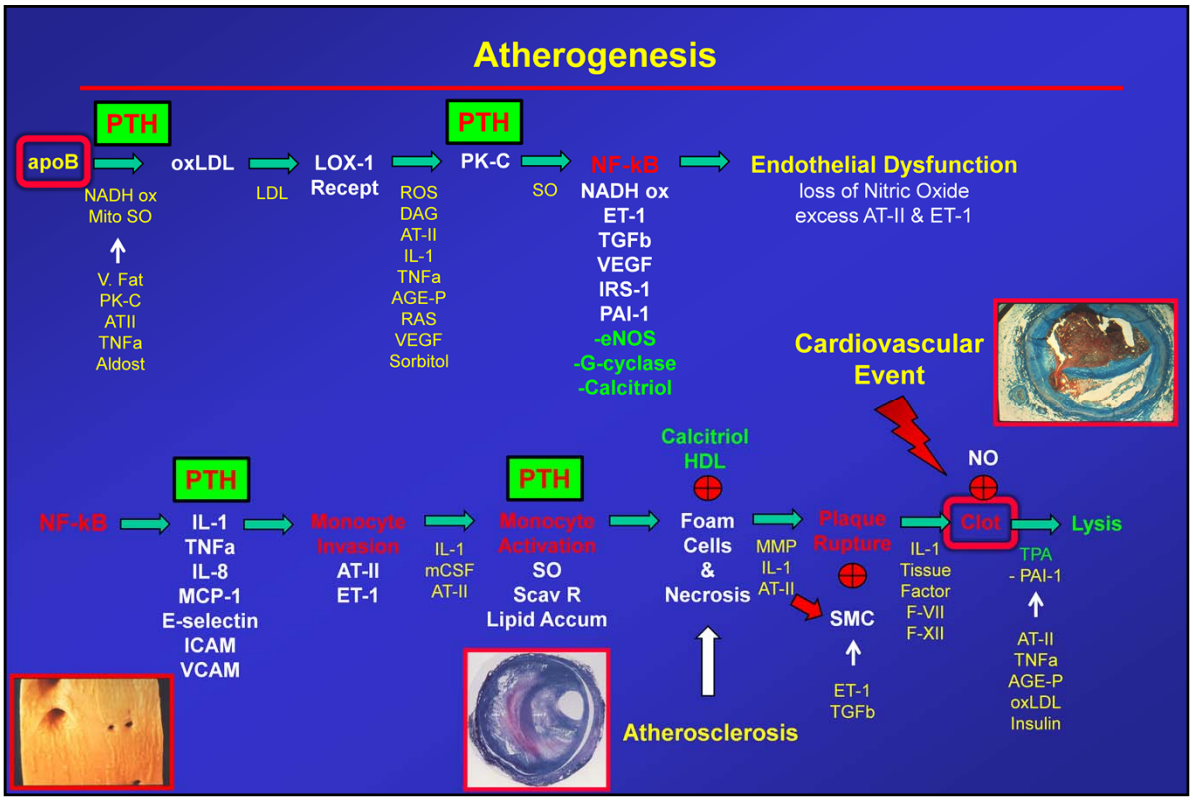
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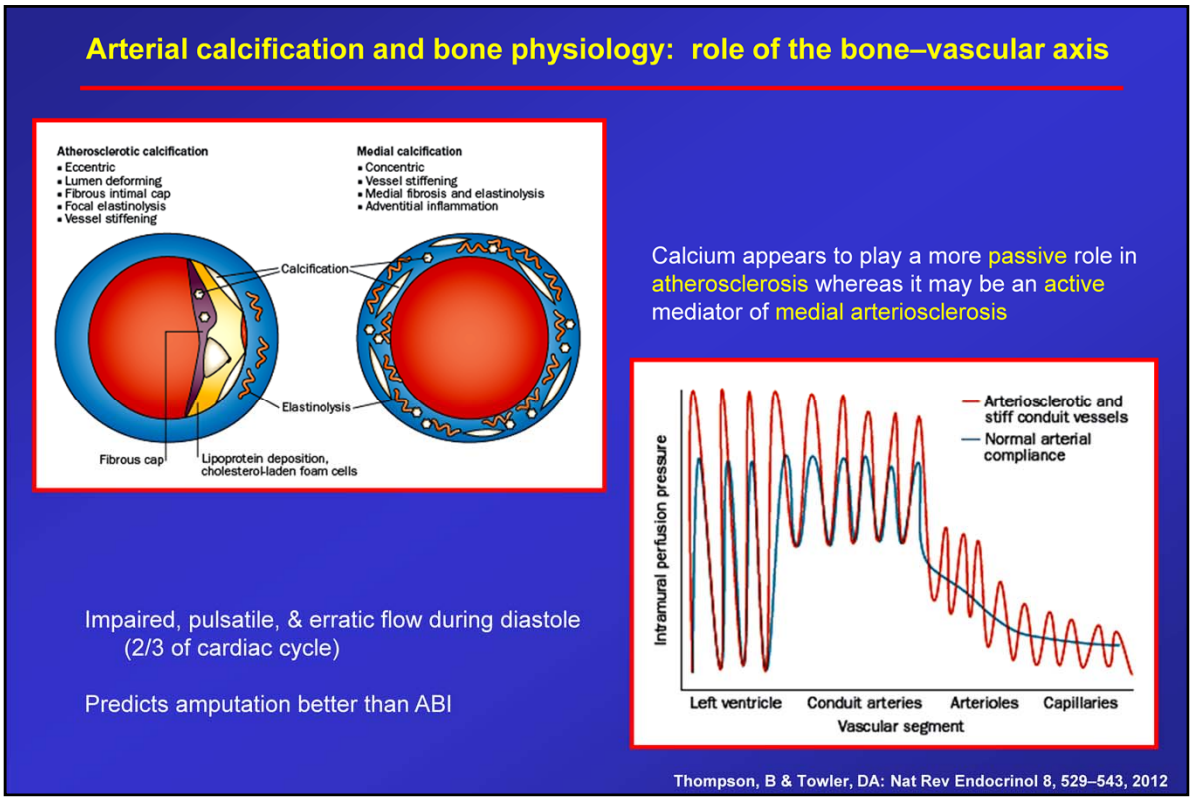


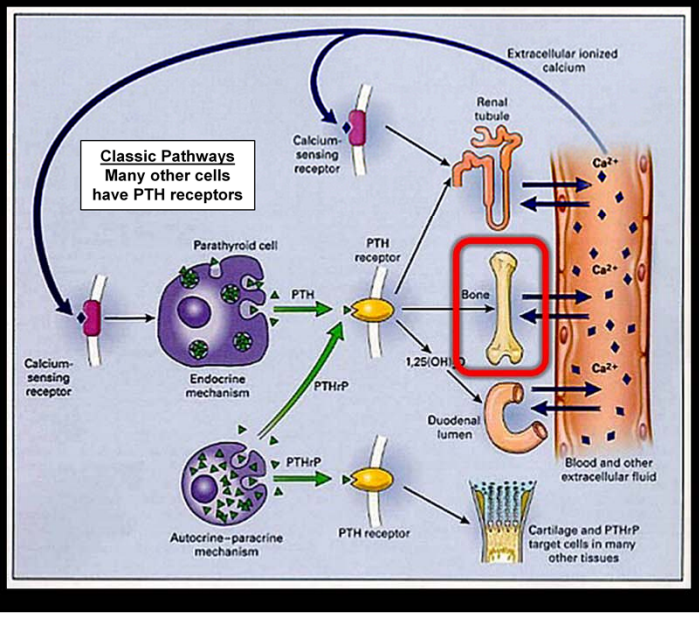
Figure 1 | Consequences of arterial stiffening and impaired Windkessel physiology. During systole, some kinetic energy is stored as potential energy in the elastic conduit arteries. This stored energy permits not only coronary perfusion but also smooth distal capillary perfusion during diastole (blue tracing). With arteriosclerotic stiffening (red tracing), less potential energy is stored during systole, giving rise to impaired, pulsatile and erratic flow during diastole (two-thirds of the cardiac cycle). Systolic blood pressure is also increased.

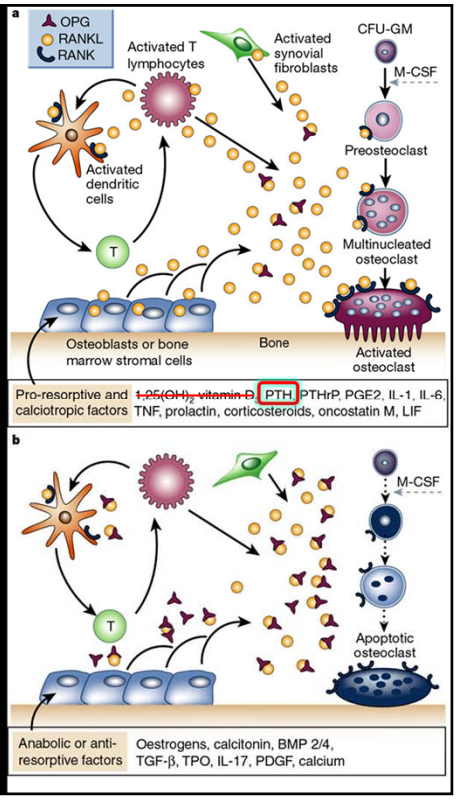
Calcium – Phosphate Homeostasis: Goal is to maintain serum [Ca^{++}]

PTH increases secretion of RANKL while inhibiting OPG (osteoprotegerin) → increasing TOTAL body bone resorption

cAMP / PK-A pathway → MCP-1

RANKL - Receptor activator of nuclear factor kappa-B ligand (**TNF family**)





Osteoclasts are detrimental to bone integrity in this setting but osteoblasts control this osteoclastic activity so they are actually the enablers of this injurious action.

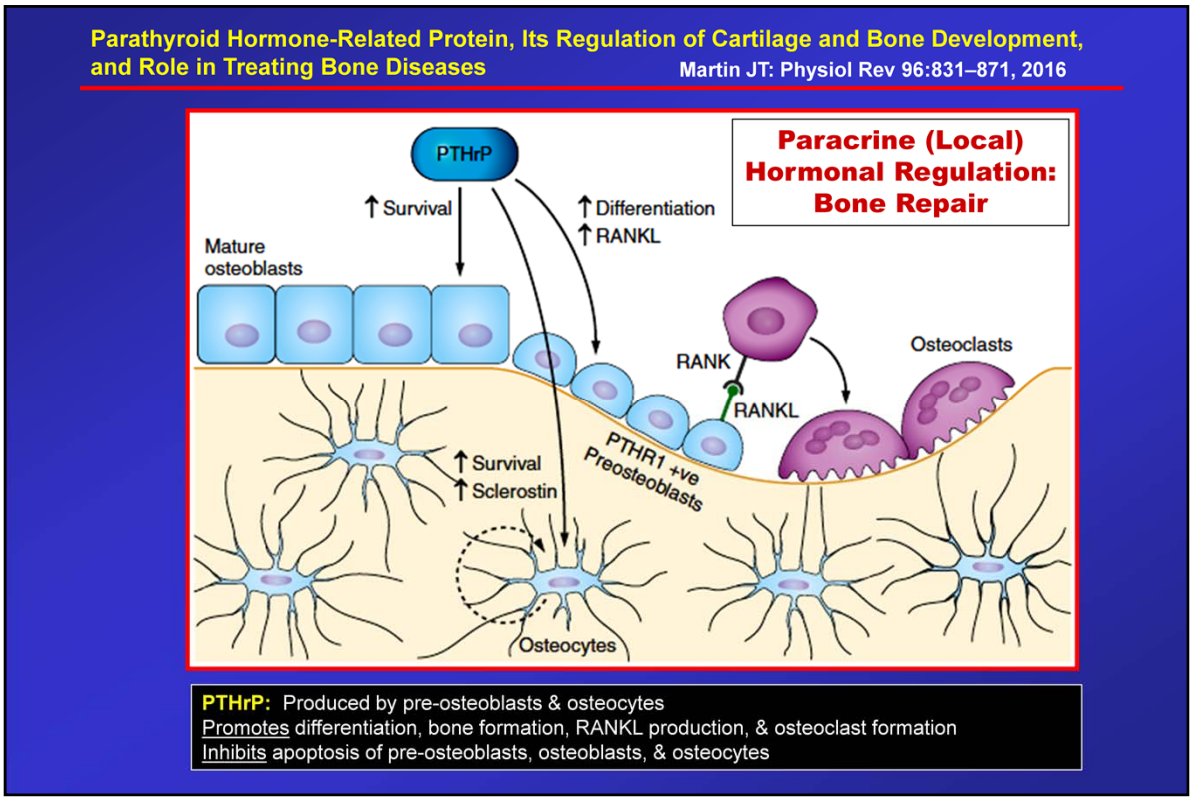


FIGURE 12. Paracrine actions of PTHrP in bone remodeling. PTHrP produced by cells early in the osteoblast lineage acts on cells of the lineage that have differentiated to the stage of possessing the PTH1R, promoting their differentiation and therefore bone formation, as well as increasing production of RANKL and osteoclast formation. PTHrP also inhibits apoptosis of mature osteoblasts, of earlier cells, and of osteocytes.

Osteoclasts are integral components of the bone repair process so their actions in this setting are beneficial to bone integrity. Again, osteoblasts and osteocytes control the osteoclastic activity.

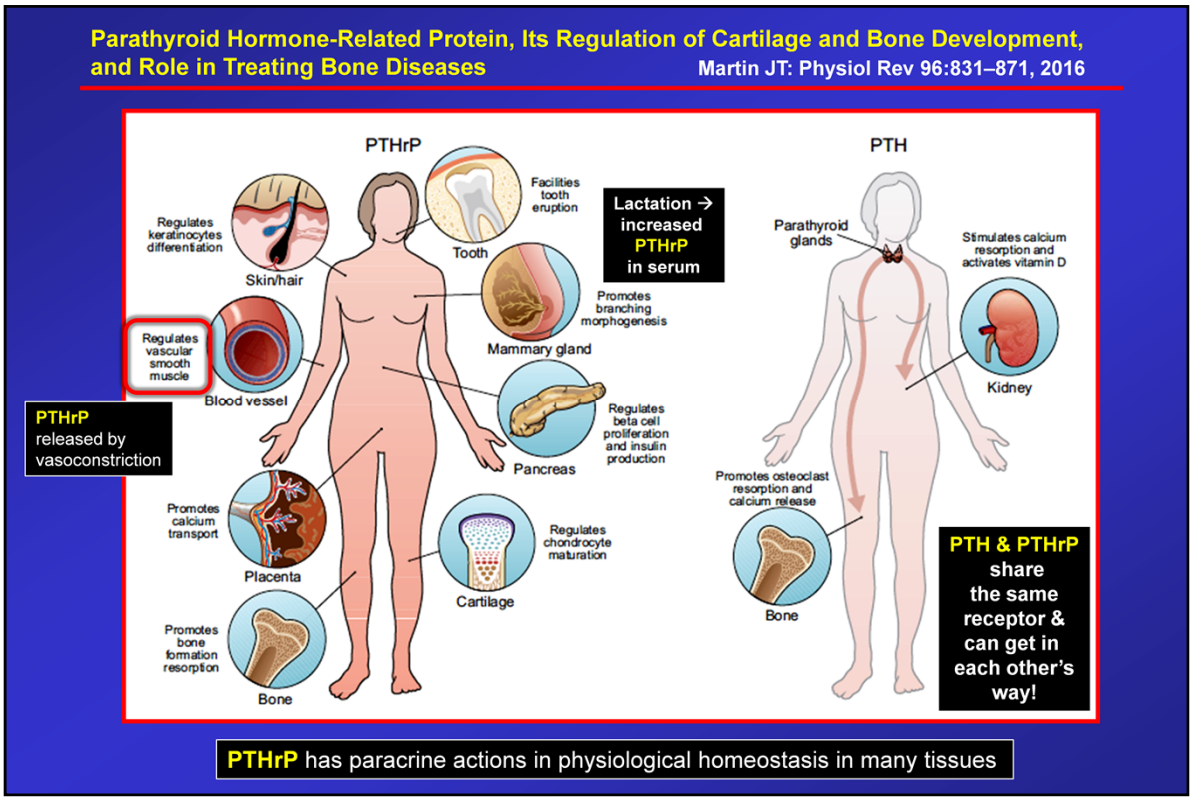
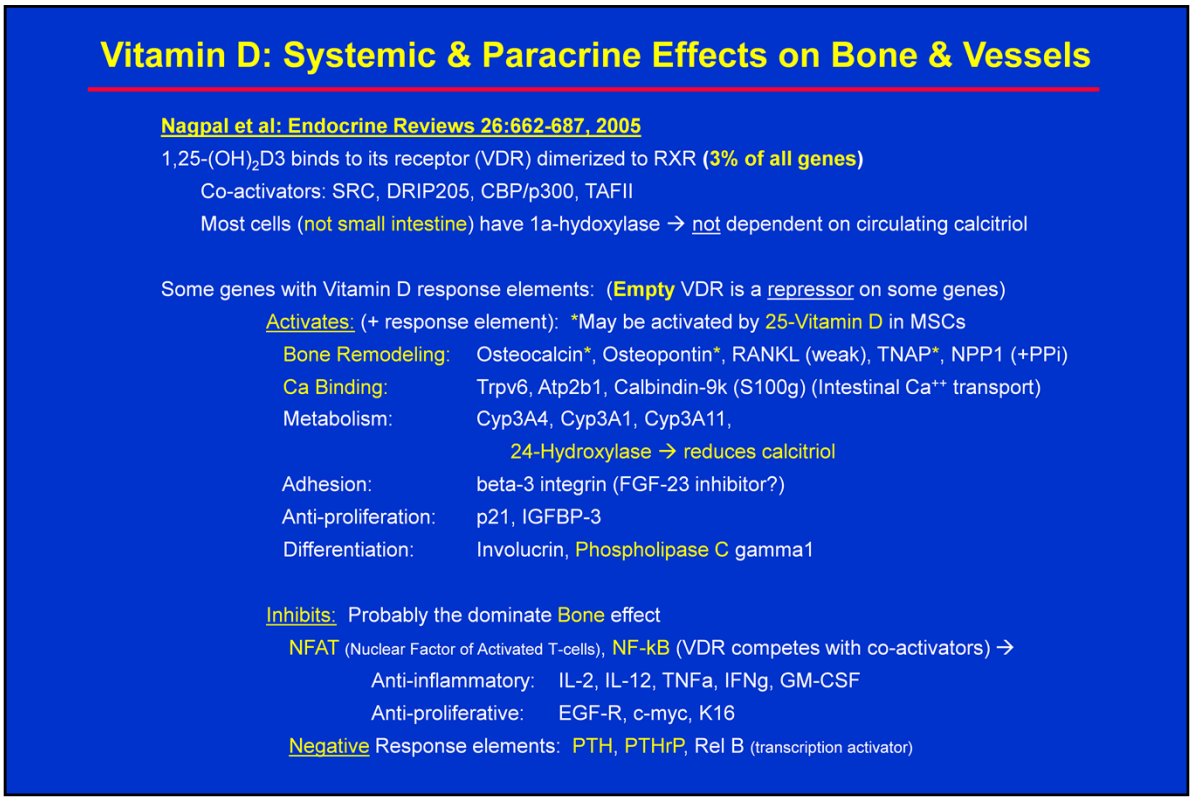


FIGURE 8. Paracrine actions of PTHrP and endocrine actions of PTH. PTHrP has paracrine actions in physiological homeostasis in many tissues, including keratinocytes/hair follicles, cartilage, vascular smooth muscle, bone, mammary gland development, tooth eruption, and pancreas, whereas PTH has relatively fewer physiological actions through its role as a circulating hormone. The summary diagram omits important details such as the role of PTHrP in lactation



S.M. Lee, J.W. Pike / Journal of Steroid Biochemistry & Molecular Biology 164 (2016) 265–270

The vitamin D receptor functions as a transcription regulator in the absence of 1,25-dihydroxyvitamin D3

25-Hydroxyvitamin D3 induces osteogenic differentiation of human mesenchymal stem cells; Yan-Ru Lou, Tai Chong Toh, Yee Han Tee, & Hanry Yu

Scientific Reports | 7:42816 | DOI: 10.1038/srep42816 2017

Wikipedia: Nuclear factor of activated T-cells (**NFAT**) is a general name applied to a family of transcription factors shown to be important in immune response. One or more members of the NFAT family is expressed in most cells of the immune system. NFAT is also involved in the development of cardiac, skeletal muscle, and nervous systems. The NFAT transcription factor family consists of five members NFATc1, NFATc2, NFATc3, NFATc4, and NFAT5. NFATc1 through NFATc4 are regulated by calcium signaling. Calcium signaling is critical to NFAT activation because calmodulin (CaM), a well-known calcium sensor protein, activates the serine/threonine phosphatase Calcineurin (CN). Activated CN rapidly dephosphorylates the serine-rich region (SRR) and SP-repeats in the amino termini of NFAT proteins, resulting in a conformational change that exposes a nuclear localization signal, resulting in NFAT nuclear import. Nuclear import of NFAT

proteins is opposed by maintenance kinases in the cytoplasm and export kinases in the nucleus. Export kinases, such as PKA and GSK-3β, must be inactivated for NFAT nuclear retention.

NFAT proteins have weak DNA-binding capacity. Therefore, to effectively bind DNA, NFAT proteins must cooperate with other nuclear resident transcription factors generically referred to as NFATn. This important feature of NFAT transcription factors enables integration and coincidence detection of calcium signals with other signaling pathways such as ras-MAPK or PKC. In addition, this signaling integration is involved in tissue-specific gene expression during development. A screen of ncRNA sequences identified in EST sequencing projects discovered a 'ncRNA repressor of the nuclear factor of activated T cells' called NRON.

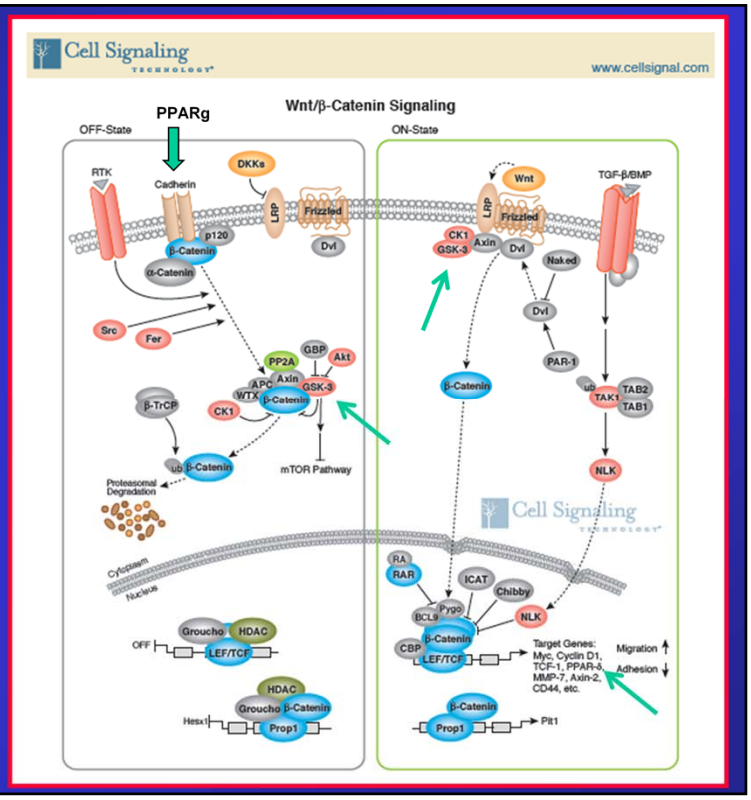
Cellular Control of Arterial Calcification

Wnt Pathway

Present in slime molds
Controls cell-cell communication
Embryonic Development
Maintains Adult Cell Differentiation
Cell Polarity

Wnt controls beta-Catenin
Wnt5a → PL-C → IP3, DAG
(increased in some cancers)

APC defic or b-Catenin mutation → excess stem cell renewal & proliferation



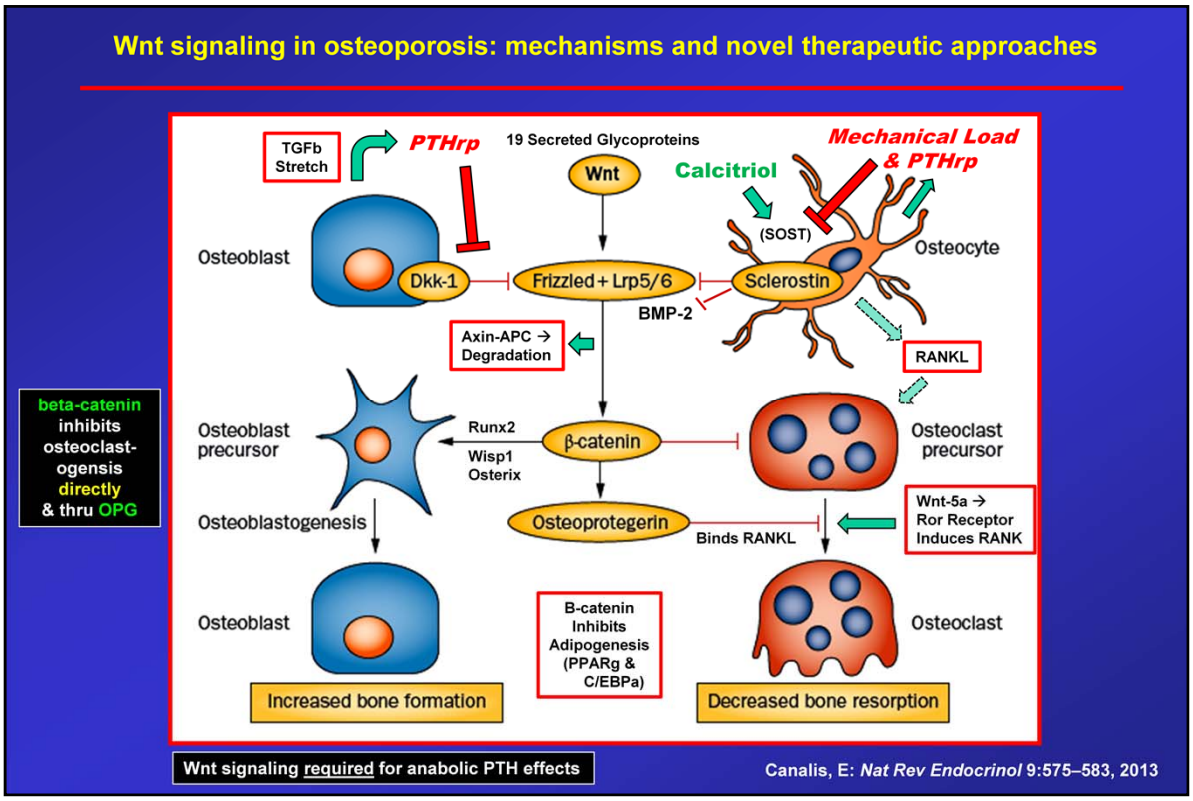


Figure 1 | Canonical Wnt signalling and bone remodelling. Wnt induces osteoblastogenesis and thereby enhances bone formation. Canonical Wnt signalling suppresses osteoclastogenesis by inducing osteoprotegerin. In addition, Wnt signalling suppresses bone resorption by an osteoprotegerin-independent mechanism acting directly on osteoclast precursors. The dual effect of Wnt on cells of the osteoblast and osteoclast lineage results in an increase in bone mass. Sclerostin and Dkk-1 bind to Wnt co-receptors and thereby prevent Wnt-receptor interaction and signalling.

Abbreviations: Dkk-1, Dickkopf-related protein 1; LRP-5, LDL receptor related protein co-receptor 5; LRP-6, LDL receptor related protein co-receptor 6. Ror: receptor tyrosine kinase-like orphan receptor

Osteoporosis is a skeletal disorder characterized by bone loss, which results in architectural deterioration of the skeleton, compromised bone strength and an increased risk of fragility fractures. Most current therapies for osteoporosis stabilize the skeleton by inhibiting bone resorption (antiresorptive agents), but the development of anabolic therapies that can increase bone formation and bone mass is of great interest. Wnt signalling induces differentiation of bone-forming cells (osteoblasts) and suppresses the development of bone-resorbing cells (osteoclasts). The Wnt pathway is controlled by antagonists that interact either directly with Wnt

proteins or with Wnt co-receptors. The importance of Wnt signalling in bone formation is indicated by skeletal disorders such as sclerosteosis and van Buchem syndrome, which are caused by mutations in the gene encoding the Wnt antagonist sclerostin (SOST). Experiments in mice have shown that downregulation or neutralization of Wnt antagonists enhances bone formation. Phase II clinical trials show that 1-year treatment with antisclerostin antibodies increases bone formation, decreases bone resorption and leads to a substantial increase in BMD. Consequently, Wnt signalling can be targeted by the neutralization of its extracellular antagonists to obtain a skeletal anabolic response.

PTHrp is made by all SMCs in response to vasoconstriction (stretch) & causes relaxation → anti-AT-II

PTHrp is made by osteoblasts in response to TGFb, EGF, calcitriol, cortisol, & stretch

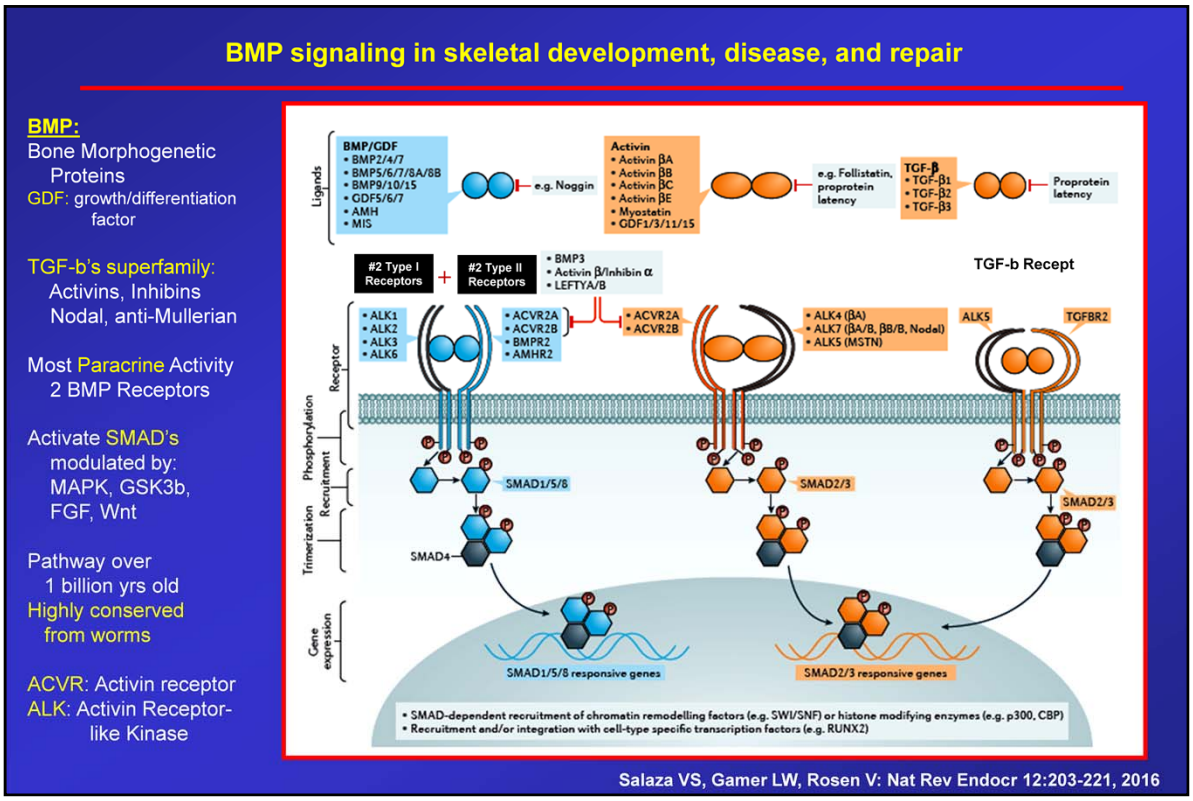


Figure 2 | **Fundamental mechanisms of canonical BMP superfamily signalling.** Over 30 bone morphogenetic protein (BMP) superfamily ligands have been discovered in humans. Most are secreted as mature disulfide-linked dimers, with the exception of TGF-β1, TGF-β2 and TGF-β3, which can be secreted in a latent form and require proteolytic activation. BMPs signal through a multimeric cell surface complex consisting of two type I receptors and two type II receptors. Type I and type II BMP receptors are single pass transmembrane proteins with an intracellular serine/ threonine kinase domain. After ligand binding, type II receptors phosphorylate (P) the type I receptors. Activated type I receptors recruit and phosphorylate pathway-specific R-SMADs (SMAD1, SMAD5 and SMAD8 (blue pathway), and SMAD2 and SMAD3 (orange pathway)), which can form trimers with SMAD4 and translocate to the nucleus. SMADs have intrinsic DNA-binding activity and are able to regulate gene expression by recruitment of chromatin-remodelling machinery and integration with tissue-specific transcription factors. SMAD8 is also known as SMAD9. The pathway can be antagonized by many mechanisms including neutralization of ligands by secreted traps such as noggin or follistatin, secretion of latent ligands bound to their propeptides, or via titration of receptors by non-signalling ligands such as BMP3, activin β/inhibin α dimers or LEFTY monomers.

ACVR, activin receptor; ALK, activin receptor-like kinase; AMH, anti-Müllerian hormone; AMHR2, AMH receptor 2; BMPR, BMP receptor; GDF, growth/differentiation factor; TGF, transforming growth factor; TGFBR, TGF-β

receptor.

Since the identification in 1988 of bone morphogenetic protein 2 (BMP2) as a potent inducer of bone and cartilage formation, BMP superfamily signalling has become one of the most heavily investigated topics in vertebrate skeletal biology. Whereas a large part of this research has focused on the roles of BMP2, BMP4 and BMP7 in the formation and repair of endochondral bone, a large number of BMP superfamily molecules have now been implicated in almost all aspects of bone, cartilage and joint biology. As modulating BMP signalling is currently a major therapeutic target, our rapidly expanding knowledge of how BMP superfamily signalling affects most tissue types of the skeletal system creates enormous potential to translate basic research findings into successful clinical therapies that improve bone mass or quality, ameliorate diseases of skeletal overgrowth, and repair damage to bone and joints. This Review examines the genetic evidence implicating BMP superfamily signalling in vertebrate bone and joint development, discusses a selection of human skeletal disorders associated with altered BMP signalling and summarizes the status of modulating the BMP pathway as a therapeutic target for skeletal trauma and disease.

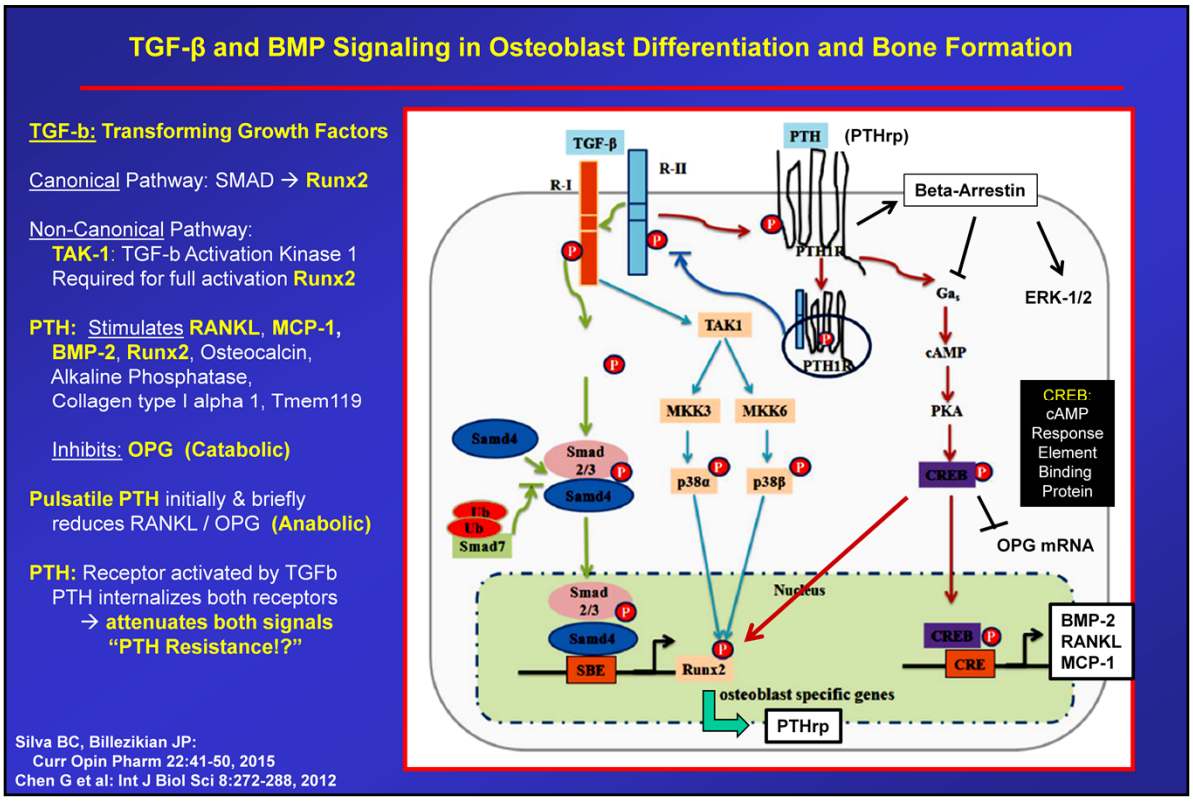


Figure 1. **TGF-β signaling and negative regulation in bone formation.**

Canonical Smad-dependent TGF-β signaling first binds to receptor type II (R-II) and receptor type I (R-I), and then signaling transduces to their Smads. Activated Smads form a complex with Smad4 and then translocate into the nucleus where they interact with other transcription factors to trigger target gene expression. Smad7 disrupts the activated Smad2/3 to form a complex with Smad4. The non-Smad-dependent TAK1 signaling pathway also regulates bone formation. PTH binding activates PTH1R to stimulate several downstream effectors. PTH binding also drives internalization of PTH1R-TGFβRII complex, which attenuates both TGF-β and PTH signaling on bone development. Transcriptional factor cAMP response element binding protein (CREB) mediates PTH signaling in osteoblasts.

Parathyroid hormone: anabolic and catabolic actions on the skeleton.

Parathyroid hormone (PTH) is essential for the maintenance of calcium homeostasis through, in part, its actions to regulate bone remodeling. While PTH stimulates both bone formation and bone resorption, the duration and periodicity of exposure to PTH governs the net effect on bone mass, that is whether it is catabolic or anabolic. PTH receptor signaling in osteoblasts and osteocytes can increase the RANKL/OPG ratio, increasing both osteoclast recruitment and osteoclast activity, and thereby stimulating bone resorption. In contrast, PTH-induced bone formation is explained, at least in part, by its ability to downregulate SOST/sclerostin

expression in osteocytes, permitting the anabolic Wnt signaling pathway to proceed. The two modes of administration of PTH, that is, continuous vs. intermittent, can regulate, in bone cells, different sets of genes; alternatively, the same sets of genes exposed to PTH in sustained vs. transient way, will favor bone resorption or bone formation, respectively. This article reviews the effects of PTH on bone cells that lead to these dual catabolic and anabolic actions on the skeleton.

TGF-β and BMP signaling in osteoblast differentiation and bone formation.

Transforming growth factor-beta (TGF-β)/bone morphogenic protein (BMP) signaling is involved in a vast majority of cellular processes and is fundamentally important throughout life. TGF-β/BMPs have widely recognized roles in bone formation during mammalian development and exhibit versatile regulatory functions in the body. Signaling transduction by TGF-β/BMPs is specifically through both canonical Smad-dependent pathways (TGF-β/BMP ligands, receptors and Smads) and non-canonical Smad-independent signaling pathway (e.g. p38 mitogen-activated protein kinase pathway, MAPK). Following TGF-β/BMP induction, both the Smad and p38 MAPK pathways converge at the Runx2 gene to control **mesenchymal precursor cell differentiation**. The coordinated activity of Runx2 and TGF-β/BMP-activated Smads is critical for formation of the skeleton. Recent advances in molecular and genetic studies using gene targeting in mice enable a better understanding of TGF-β/BMP signaling in bone and in the signaling networks underlying osteoblast differentiation and bone formation. This review summarizes the recent advances in our understanding of TGF-β/BMP signaling in bone from studies of genetic mouse models and human diseases caused by the disruption of TGF-β/BMP signaling. This review also highlights the different modes of cross-talk between TGF-β/BMP signaling and the signaling pathways of MAPK, Wnt, Hedgehog, Notch, and FGF in osteoblast differentiation and bone formation.

P: phosphorylation; Ub: ubiquitination.

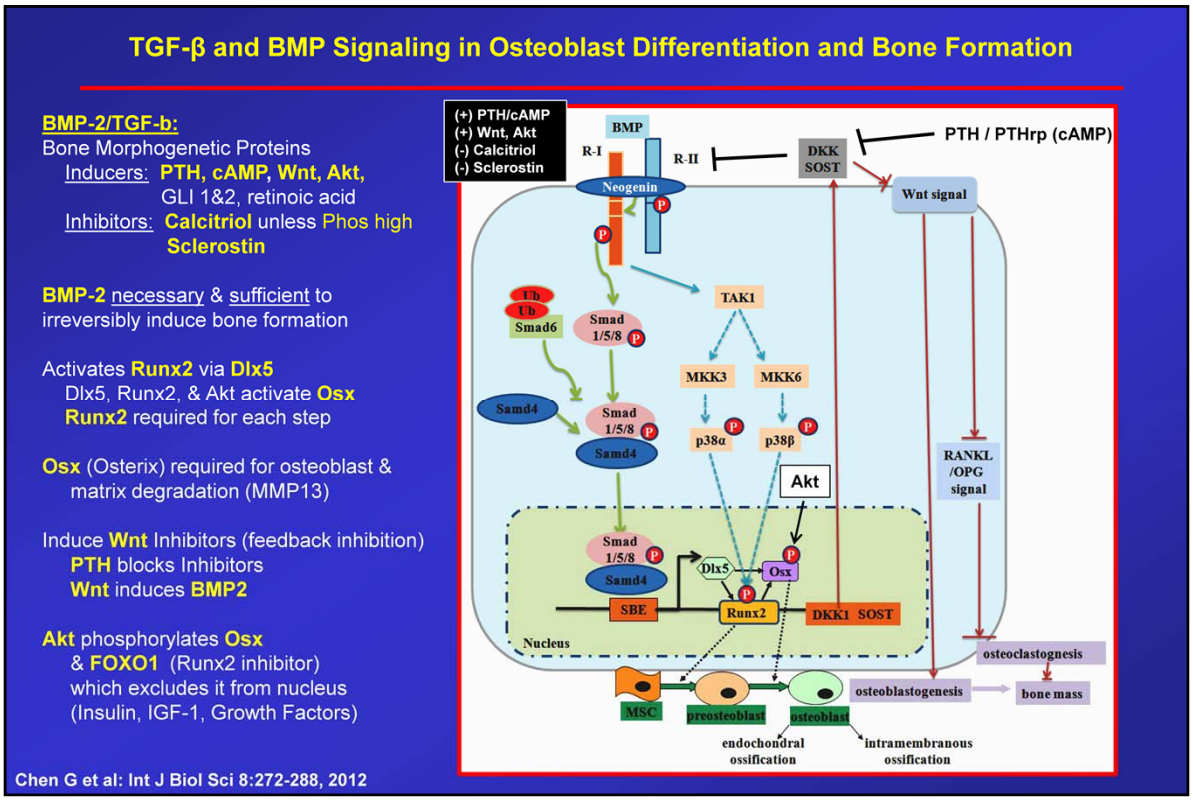


Figure 2. **BMP signaling and negative regulation in bone formation.**

Smad-dependent-BMP signaling binds to receptor type II (R-II) and receptor type I (R-I) and then the signaling transduces to their Smads. Activated Smads form a complex with Smad4 and then translocate into the nucleus where they interact with other transcription factors to trigger target gene expression. Neogenin regulates BMP receptor association and Smad1/5/8 signaling. Activated Smads regulate expression of transcriptional factors and transcriptional coactivators important in osteoblasts (Dlx5, Runx2 and Osx). Smad6 binds type I BMP receptor and prevents Smad1/5/8 to be activated. Non-Smad-dependent TAK1 signaling pathway also regulates bone formation. The interplay between BMPs and Wnt signaling affects bone formation (99). BMPR1a signaling upregulates Sost expression primarily through Smad-dependent signaling, while it upregulates DKK1 through Smad-dependent and non-Smad-dependent signaling. Both Sost and DKK1 inhibit canonical Wnt signaling, leading to a decrease in bone mass. P: phosphorylation; Ub: ubiquitination.

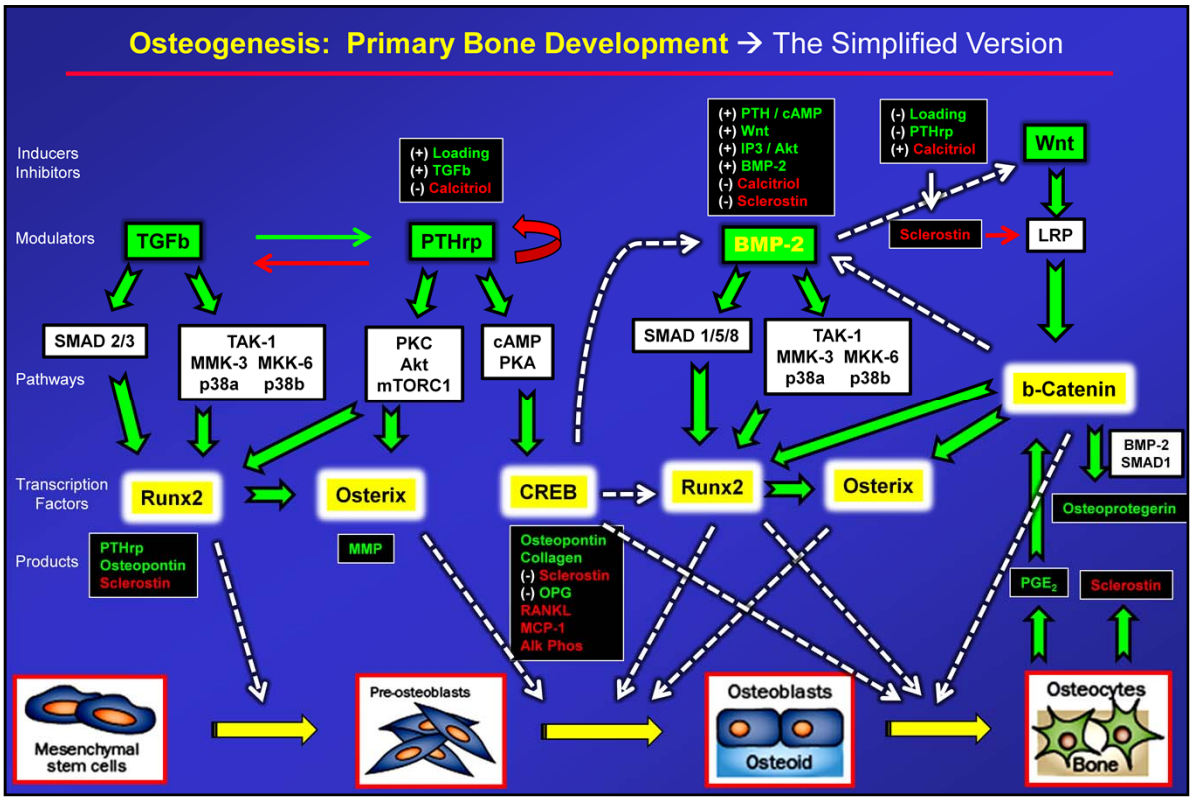
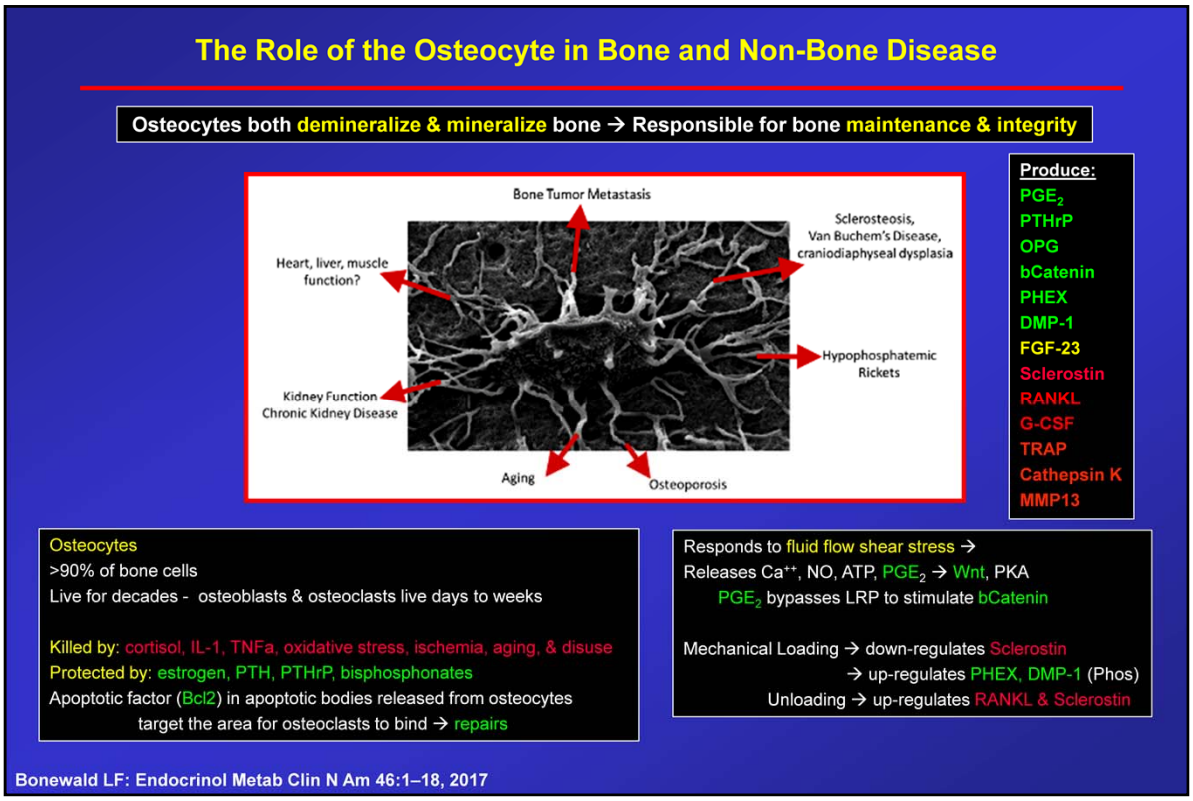


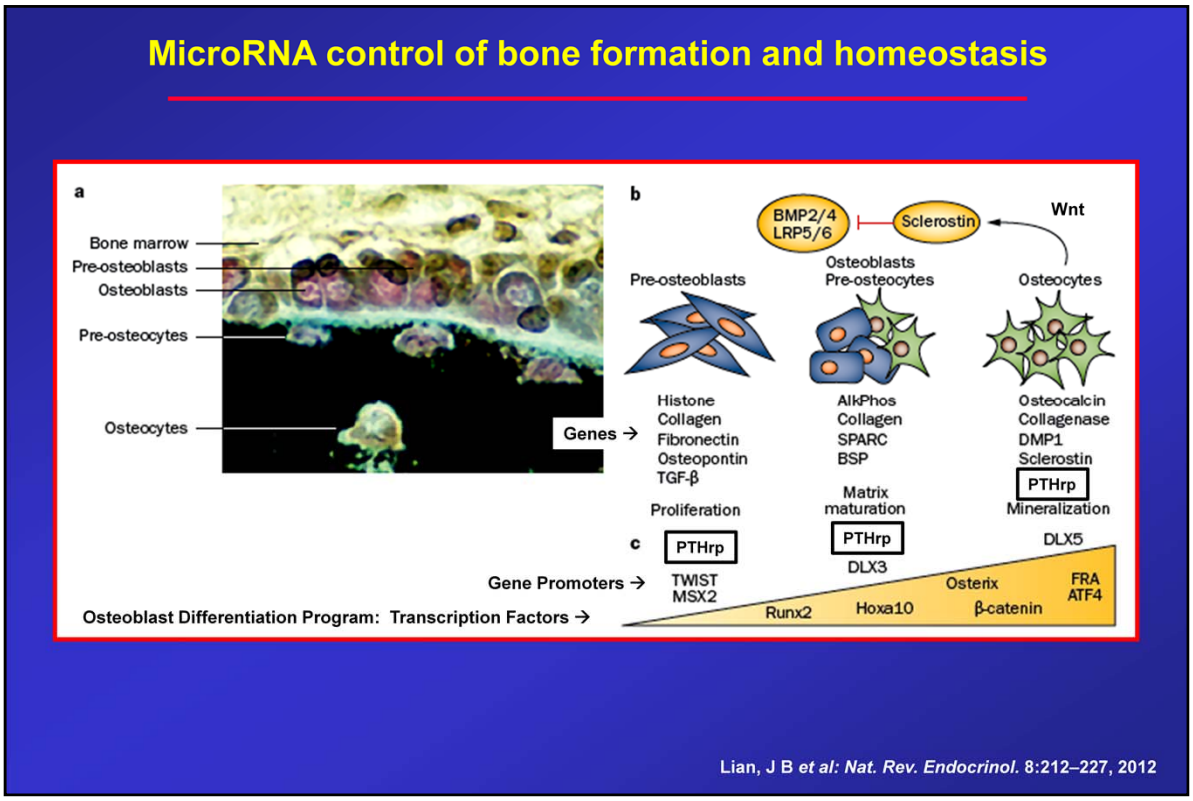
Figure 2 | The osteoblast differentiation program. **a** | *In vivo*: bone surface shows organization of indicated osteoblast lineage cells (black, mineralized tissue). Mesenchymal stem cells and osteoprogenitor cells cannot be seen. **b** | *In vitro*: stages of differentiation of committed preosteoblast cells isolated from newborn rodent calvarium or bone marrow stromal cells. Peak expression of genes that are markers for the three major stages are shown. At mineralization, a feedback signal from sclerostin secreted by osteocytes inhibits BMP and Wnt osteogenic-mediated bone formation by regulating the number of cells entering the osteoblast lineage. **c** | Examples of transcription factors regulating osteoblast differentiation and *in vivo* bone formation are shown. Within the triangle are those that increase during differentiation, whereas those above the triangle are functional on gene promoters at the indicated stages of maturation. Permission obtained from American Society for Bone and Mineral Research © Favus, M. J. (Ed.) *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism*, 6th edn (2006).



Fluid flow shear stress activates the Wnt/b-catenin signaling pathway through the rapid release of prostaglandin, which acts through EP receptors to bypass LDL receptor-related protein activation. Components of the b-catenin pathway are essential for osteocyte viability, mechano-sensation, transduction, and release of important factors essential for bone homeostasis. The central molecule through which all molecules must go is b-catenin. b-Catenin regulates expression of both the positive activators of this pathway, the wnts, and the negative regulators of this pathway, sclerostin and Dkk1 (for a review see 16). Global deletion of b-catenin is embryonically lethal, but deletion in osteocytes using the Dmp1-Cre results in dramatic bone loss characterized by perforated cortises. Interestingly, deletion of only 1 allele in osteocytes results in mice with a normal skeleton but a completely abrogated response to anabolic loading. b-Catenin plays an important role in bone integrity, osteocyte communication, and osteocyte viability, but also in bone response to loading. This role extends to other components of this signaling pathway.

Before osteocytes were recognized as active essential bone cells necessary for bone health, it was assumed that all the action took place on the bone surface and not within the bone. Osteoblasts and osteoclasts were the major players, osteoblasts making bone and osteoclasts resorbing bone to maintain bone homeostasis. It was

assumed that osteoblasts and osteoclasts were regulated by external factors such as parathyroid hormone (PTH) or 1,25 dihydroxyvitamin D3, and other external regulatory factors. It has also been proposed that osteoblasts make factors that regulate osteoclast activity and, conversely, that osteoclasts make factors that could regulate osteoblast activity. Therapeutics were generated that would target either osteoclasts or osteoblasts. Osteocytes were left out of the picture.



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c | Examples of transcription factors regulating osteoblast differentiation and *in vivo* bone formation are shown. Within the triangle are those that increase during differentiation, whereas those above the triangle are functional on gene promoters at the indicated stages of maturation.

Abstract MicroRNAs (miRNAs) repress cellular protein levels to provide a sophisticated parameter of gene regulation that coordinates a broad spectrum of biological processes. Bone organogenesis is a complex process involving the

differentiation and crosstalk of multiple cell types for formation and remodeling of the skeleton. Inhibition of mRNA translation by miRNAs has emerged as an important regulator of developmental osteogenic signaling pathways, osteoblast growth and differentiation, osteoclast-mediated bone resorption activity and bone homeostasis in the adult skeleton. miRNAs control multiple layers of gene regulation for bone development and postnatal functions, from the initial response of stem/progenitor cells to the structural and metabolic activity of the mature tissue. This Review brings into focus an emerging concept of bone-regulating miRNAs, the evidence for which has been gathered largely from in vivo mouse models and in vitro studies in human and mouse skeletal cell populations. Characterization of miRNAs that operate through tissue-specific transcription factors in osteoblast and osteoclast lineage cells, as well as intricate feedforward and reverse loops, has provided novel insights into the supervision of signaling pathways and regulatory networks controlling normal bone formation and turnover. The current knowledge of miRNAs characteristic of human pathologic disorders of the skeleton is presented with a future goal towards translational studies.

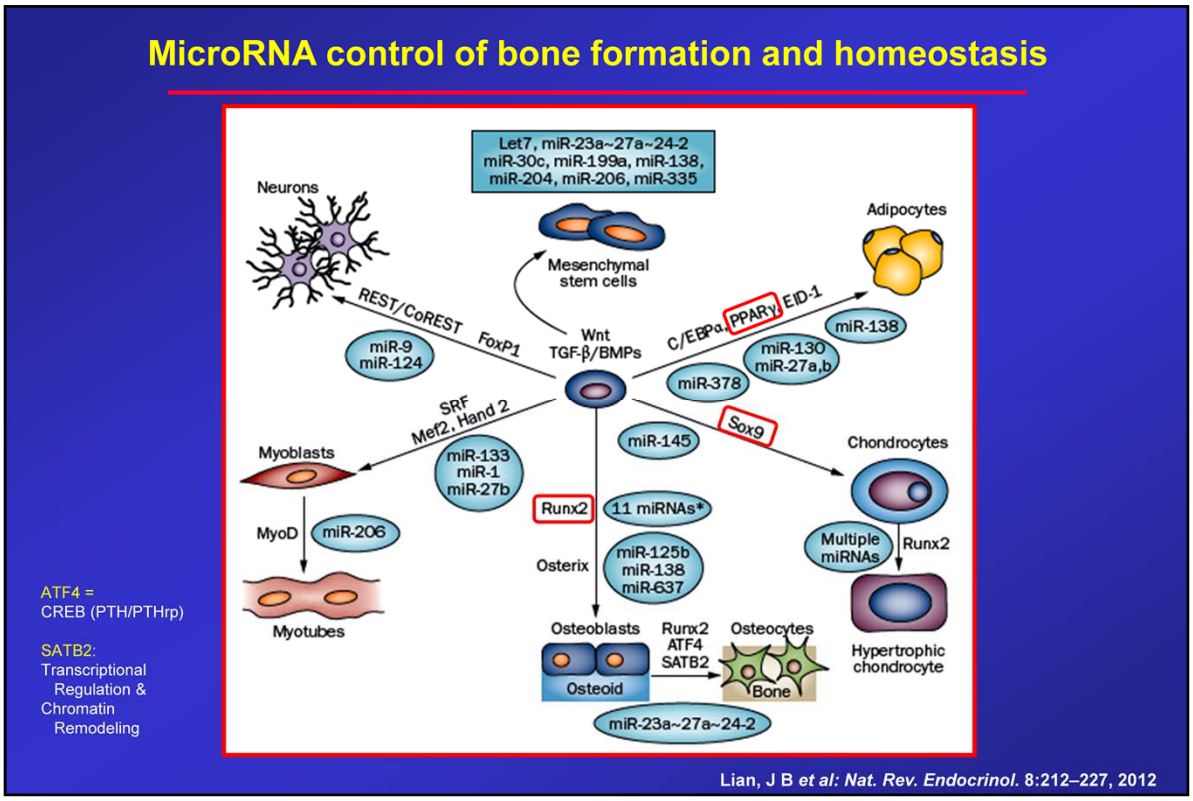


Figure 6 | Allocation of mesenchymal stem cells (MSCs) to lineage-specific phenotypes by transcription factors and microRNAs. Schematic illustration of MSC lineages directed by cell-type specific transcription factors (arrows). Selected miRNAs highly expressed in MSCs are shown because they are downregulated during differentiation into phenotype-committed cells. Cell-type-related miRNAs targeting the transcription factors or their coregulatory proteins are indicated. Although the transcription factors are attenuated by miRNAs at different stages of maturation, they are critical for regulating a normal program of differentiation and for cell specification within a tissue. Relevant references to support this concept for the indicated tissues are as follows: muscle, nerve, fat, bone, and cartilage (Table 1). *Many miRNAs repress Runx2 directly (shown in Figure 7), and to date three miRNAs have been shown to repress osterix: miR-125b140 in vascular smooth muscle cells prevents calcification and miR-138 in MSCs retains stemness, and miR-637 is expressed in adipocytes.

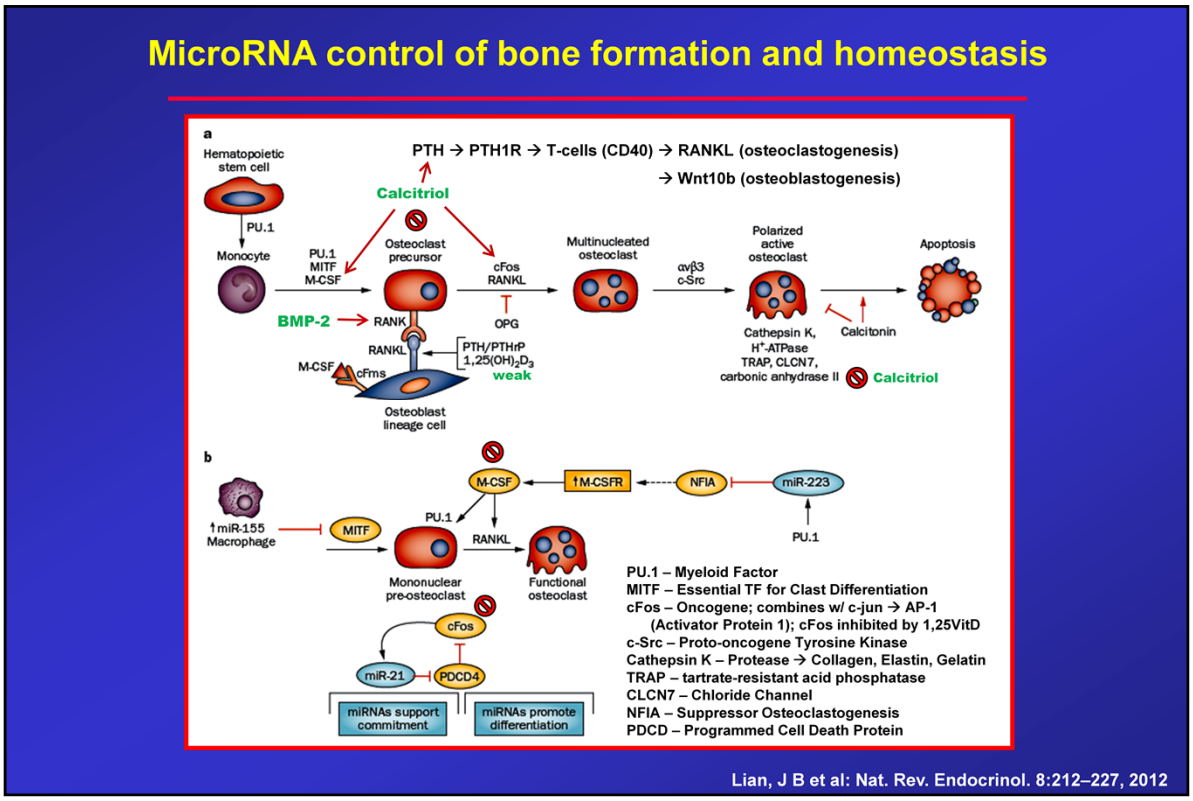
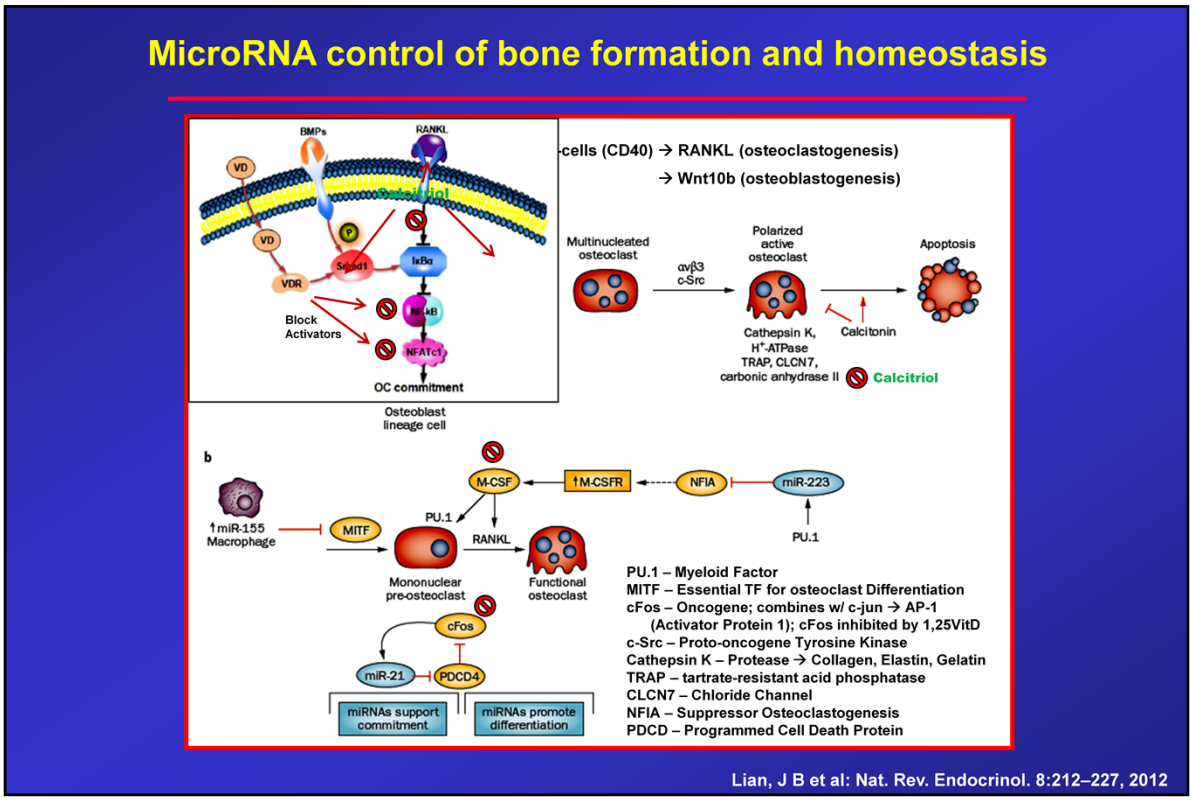


Figure 3 | Osteoclast differentiation sequence and effect of microRNAs.

a | Stages in the differentiation of the multinucleated osteoclast from its hematopoietic precursor are illustrated with key transcription factors and regulatory proteins established as critical for progression to the activated osteoclast. These include the RANK–RANKL interaction regulated by the indicated hormones and the inhibitor of RANK signaling osteoprotegerin. Integrin (αvβ3) mediates attachment of osteoclasts to bone surface and c-Src signaling induces polarization of the osteoclast and formation of the characteristic ruffled border for active bone resorption.

b | MicroRNAs regulating commitment to osteoclastogenesis have been identified. Indicated are three different mechanisms. miR-155 functions as an inhibitor of osteoclastogenesis, being highly expressed in macrophages to support robust expression of this phenotype by inhibiting MITF, essential for preosteoclast differentiation. PU.1 initiates a feedforward mechanism increasing miR-223 which downregulates an inhibitor of osteoclast differentiation, NFIA, resulting in an increase in M-CSFR and thereby M-CSF functional activity. Also shown is a regulatory loop between miR-21 and cFos (AP-1), which activates many osteoclast genes essential for multinucleated cell formation and promotes resorptive activity.

Abbreviations: αvβ3, integrin αvβ3; CLNC7, chloride channel; OPG, osteoprotegerin; TRAP, tartrate-resistant acid phosphatase.



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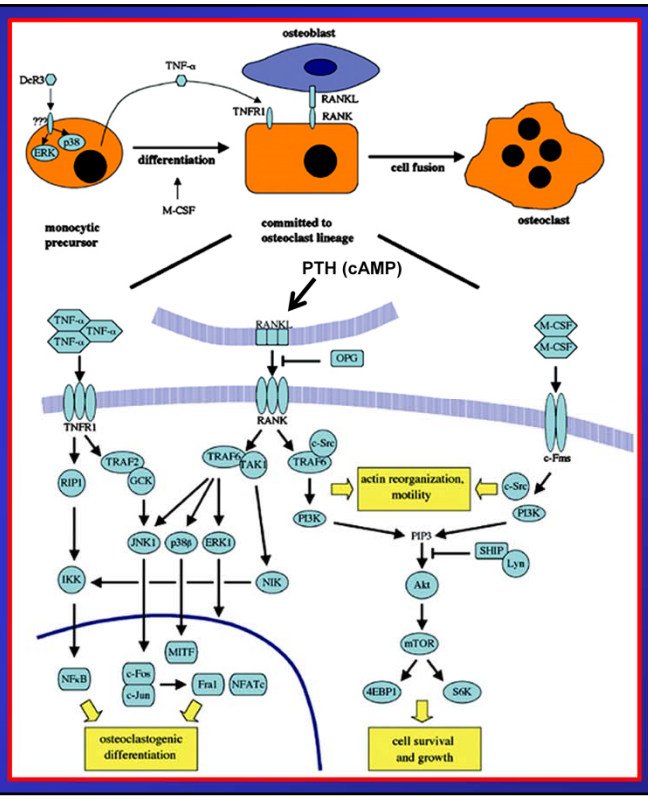
Bone Metabolism

Osteoclast Activation

TNFa + RANKL + mCSF

RIPk1
TRAF
TAK1
Akt

Similar to Foam Cell Development!



Mineral Control of Arterial Calcification

Promotors:

- Hyperphosphatemia
- FGF-23
- Hypercalcemia or Hypocalcemia??

- PTH / PTHrp
- BMP
- Wnt
- High or Low Bone Turnover

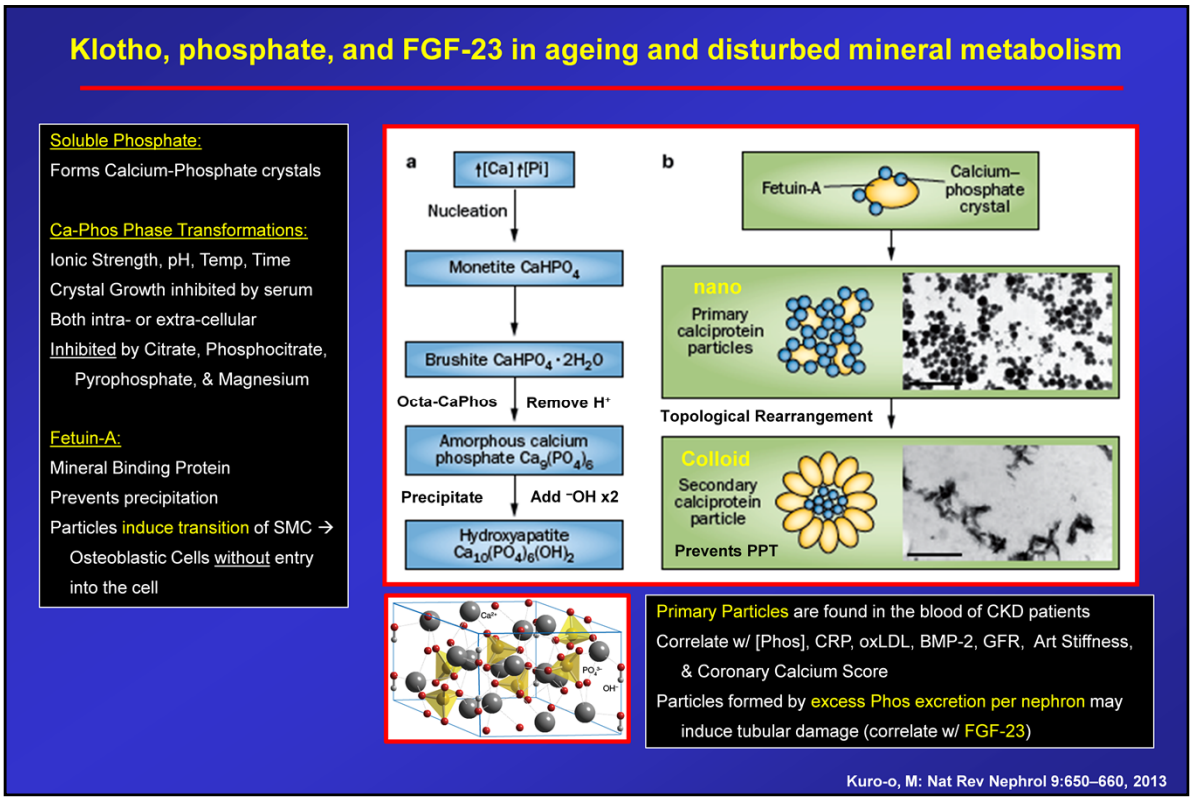
- Hyperlipidemia & Hypertension
- Super Oxide & H_2O_2
- Oxysterols (oxLDL)
- IL-1 & TNFa
- Angiotensin II (RAAS)
- Glucose (RAGE)
- Hypoxia

- Indoxyl Sulfate (renal toxins)

Inhibitors:

- Magnesium (high normal)
- Citrate
- Pyrophosphate (PPi)
- Phospho-citrate
- Acidosis

- 25 Vitamin D & 1-25 Vitamin D (20Vitamin D?)
- Fetuin-A ??
- mGP (matrix gamma-carboxyglutamic acid protein)
- Osteopontin (OPN) ??
- Klotho – increased by:
  - Pioglitazone
  - ARB's
  - Statins



Klotho, phosphate and FGF-23 in ageing and disturbed mineral metabolism. Kuro-o, M. *Nat. Rev. Nephrol.* 9, 650–660 (2013)

Figure 2 | The formation of calciprotein particles.

a | When the concentration of free calcium and phosphate exceed the concentration of the formation product, calcium–phosphate crystals are generated by nucleation. Calcium–phosphate crystals are transformed from monetite to hydroxyapatite through different phases and eventually precipitate.

b | In the presence of serum, calcium–phosphate crystals bind to fetuin-A and form colloidal nanoparticles. The calcium–phosphate-crystal-laden fetuin-A molecules aggregate to form nanoparticles (50–100 nm diameter; scale bar 500 nm), which are called primary calciprotein particles. Primary calciprotein particles undergo topological rearrangement to form a stable structure, in which a densely packed fetuin-A monolayer covers a mineral core, thereby preventing further crystal growth.[83,84] These particles are referred to as secondary calciprotein particles and are 100–200 nm diameter.

The image in panel b is republished with permission of the American Society of Nephrology, from © Nanoparticle-based test measures overall propensity for calcification in serum. Pasch, A. *J. Am. Soc. Nephrol.* 10, 1744–1752 (2012); permission conveyed through Copyright Clearance Center.

Abstract High concentrations of extracellular phosphate are toxic to cells. Impaired urinary phosphate excretion increases serum phosphate level and induces a premature-ageing phenotype. Urinary phosphate levels are increased by dietary phosphate overload and might induce tubular injury and interstitial fibrosis. Extracellular phosphate exerts its cytotoxic effects by forming insoluble nanoparticles with calcium and fetuin-A; these nanoparticles are referred to in this Review as calciprotein particles. Calciprotein particles are highly bioactive ligands that can induce various cellular responses, including the osteogenic transformation of vascular smooth muscle cells and cell death of vascular endothelial cells and renal tubular epithelial cells. Calciprotein particles are detected in the serum of animal models of kidney disease and in patients with chronic kidney disease (CKD) and might be associated with a (mal)adaptation of the endocrine axes mediated by fibroblast growth factors and Klothos that regulate phosphate homeostasis and ageing. These observations raise the possibility that calciprotein particles contribute to the pathogenesis of CKD. This theory, if verified, is expected to provide novel diagnostic markers and therapeutic targets in CKD.

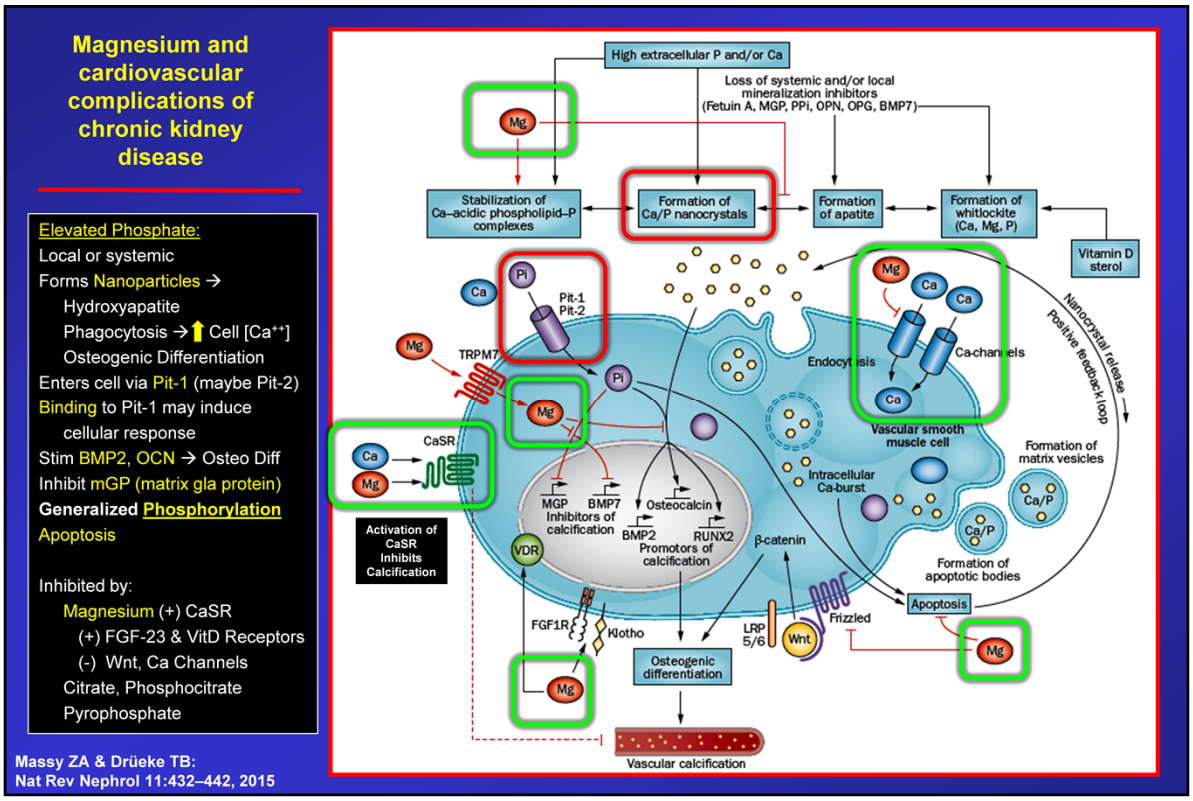


Figure 1 | The putative inhibitory effects of magnesium on the process of vascular calcification. Abnormalities in mineral metabolism, particularly hyperphosphataemia, and loss of inhibitors of mineralization leads to the formation and deposition of Ca/P nanocrystals, which are taken up by VSMCs. Lysosomal degradation of the endocytosed crystals results in intracellular release of Ca and Pi. In addition, Pi accumulates in the cell via uptake through Pit-1 and probably also Pit-2. To compensate for excess Ca/P, VSMCs form matrix vesicles loaded with Ca/P products and the mineralization inhibitors. The intracellular Ca-burst induced by endocytosed nanocrystals and Pi uptake triggers apoptosis, resulting in the formation of Ca/P-containing apoptotic bodies. Matrix vesicles and apoptotic bodies cause a positive feedback loop through nanocrystal release into the surrounding milieu, thus amplifying the calcification process. Furthermore, Ca/P nanocrystals and Pi induce the expression of genes that promote the calcification–mineralization process and repress the expression of factors that inhibit calcification, resulting in transdifferentiation of VSMCs to osteoblast-like cells and, ultimately, vessel calcification. Mg interferes with the process of vascular calcification by inhibiting transformation of amorphous Ca/P to apatite and by forming Mg-substituted whitlockite crystals, which result in smaller, more soluble deposits. Secondly, Mg functions as a Ca-channel antagonist and thus inhibits the entry of Ca into the cells. Thirdly, Mg enters the cell via TRPM7 and restores the balance between expression of calcification promoters and inhibitors by neutralizing phosphate-induced inhibition of MGP and BMP7 and enhanced expression of RUNX2 and BMP2. These effects prevent osteoblastic conversion and calcification

of VSMCs. In addition, Mg acts on the CaSR; activation of this receptor by calcimimetics has been shown to inhibit VSMC calcification but the molecular mechanisms have not yet been identified.

Abbreviations: BMP, bone morphogenetic protein; Ca, calcium; CaSR, calcium-sensing receptor; FGF1R, fibroblast growth factor receptor-1; LRP 5/6, LDL receptor-related protein 5/6; Mg, magnesium; MGP, matrix gla protein; OPG, osteoprotegerin; OPN, osteopontin; Pi, inorganic phosphate; Pit, sodium-dependent phosphate transporter; PPi, pyrophosphate; RUNX2, runt-related transcription factor 2; TRPM7, transient receptor potential cation channel subfamily M member 7; VDR, vitamin D receptor; VSMC, vascular smooth muscle cell. Permission obtained from Oxford University Press © Massy, Z. A. & Drüeke, T. B. *Clin. Kidney J*. 5 (Suppl. 1), i52–i61 (2013).

Abstract Cardiovascular complications are the leading cause of death in patients with chronic kidney disease (CKD). Abundant experimental evidence suggests a physiological role of magnesium in cardiovascular function, and clinical evidence suggests a role of the cation in cardiovascular disease in the general population. The role of magnesium in CKD-mineral and bone disorder, and in particular its impact on cardiovascular morbidity and mortality in patients with CKD, is however not well understood. Experimental studies have shown that magnesium inhibits vascular calcification, both by direct effects on the vessel wall and by indirect, systemic effects. Moreover, an increasing number of epidemiologic studies in patients with CKD have shown associations of serum magnesium levels with intermediate and hard outcomes, including vascular calcification, cardiovascular events and mortality. Intervention trials in these patients conducted to date have had small sample sizes and have been limited to the study of surrogate parameters, such as arterial stiffness, vascular calcification and atherosclerosis. Randomized controlled trials are clearly needed to determine the effects of magnesium supplementation on hard outcomes in patients with CKD.

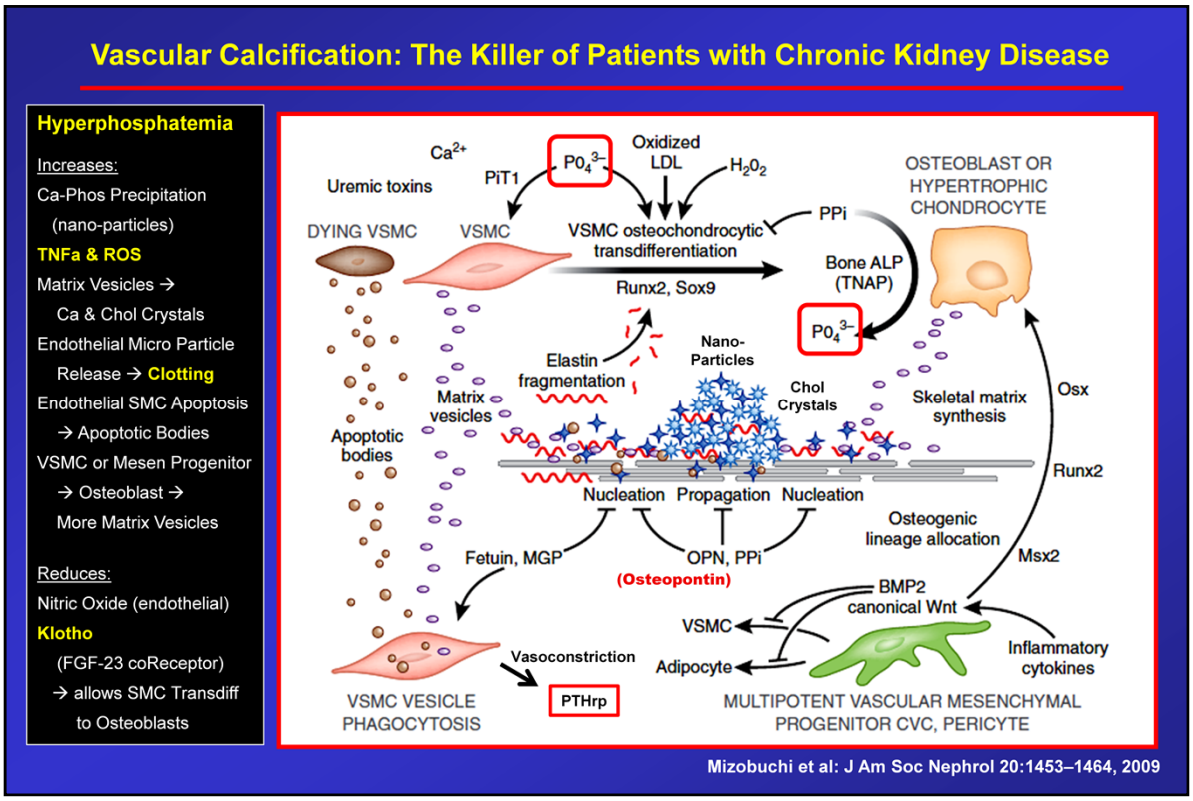
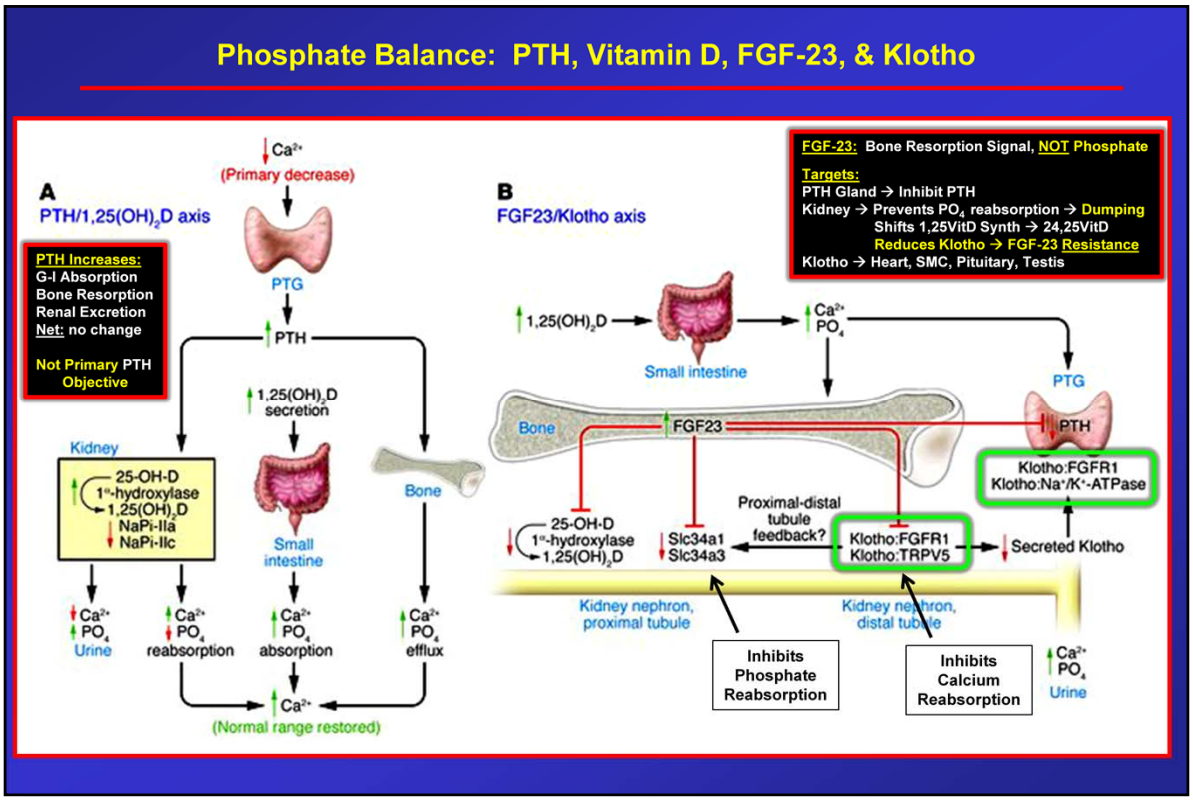
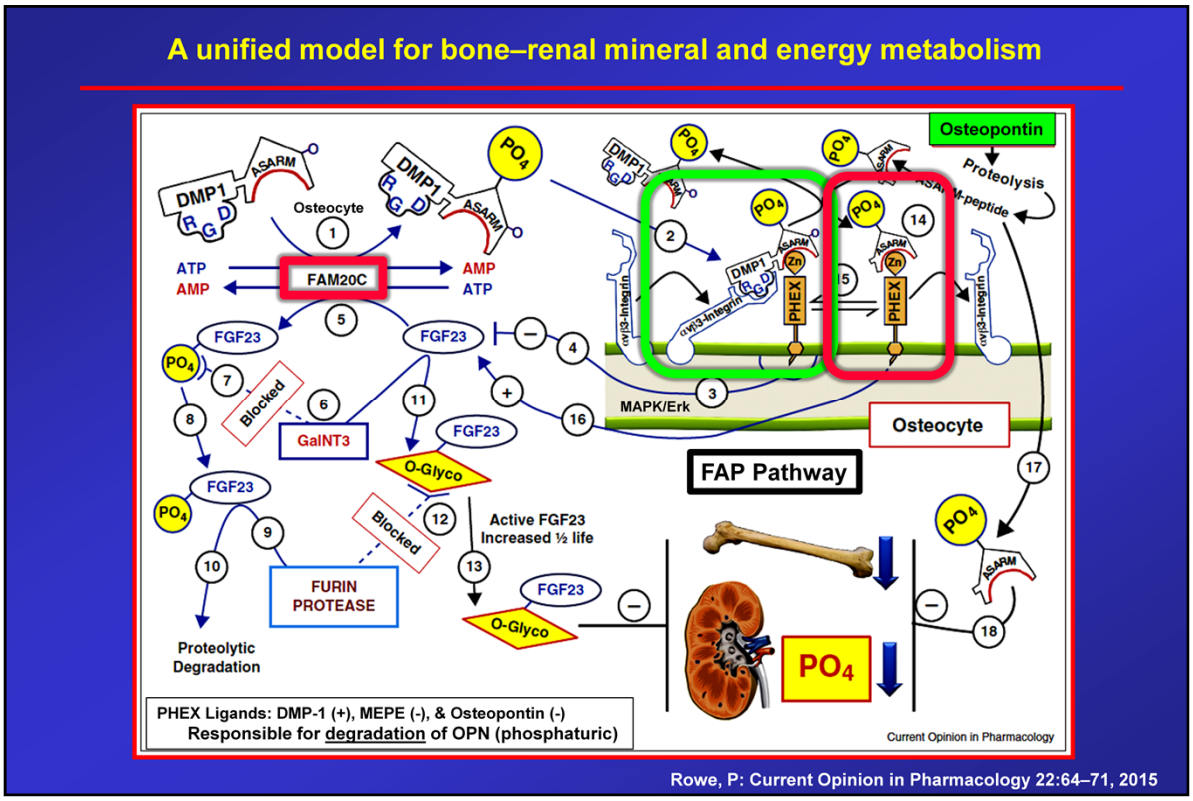


Figure 1. Cell fate, function, and phenotype in vascular calcification. In response to uremic toxins or elevated calcium and phosphate levels, VSMCs elaborate lipidaceous vesicles from apoptotic cells or produce matrix vesicle. The latter, approximately one third the diameter of apoptotic bodies, are much more efficient in nucleating mineral deposition. These vesicles nucleate calcium deposition in the form of a poorly crystalline hydroxyapatite, associated with the elastin-rich extracellular matrix of arteries. The process of elastinolysis not only creates sites for vesicle, mediated nucleation, but also released EDPs that promote osteochondrogenic "transdifferentiation" of VSMCs. This latter process is stimulated by oxidized LDL and ROS, *viz*., hydrogen peroxide. With osteochondrogenic differentiation, gene expression profiles change dramatically, with induction of bone ALP, production of a highly collagenous extracellular matrix, and elaboration of matrix vesicle. Bone ALP locally degrades inorganic pyrophosphate, an important inhibitor of mineralization and transdifferentiation. In addition, multipotent vascular mesenchymal progenitors called calcifying vascular cells (CVC) or pericytes can yield cells of the osteoblast and chondrocyte lineage. This occurs through paracrine BMP and canonical Wnt signals that "shunt" these proliferating progenitors away from other fates, such as the mature VSMC, and toward osteogenic lineages. Inflammatory cytokines such and TNF play critical roles. Of note, as shown by Shanahan's group, the phagocytic clearance of matrix vesicles by VSMCs is critical in limiting the number of sites that nucleate mineral deposition. In severely advanced atherosclerotic lesions, cholesterol crystals have also been shown to nucleate calcium phosphate deposition as well.

Abstract Cardiovascular complications are the leading cause of death in patients with chronic kidney disease (CKD). Vascular calcification is a common complication in CKD, and investigators have demonstrated that the extent and histo-anatomic type of vascular calcification are predictors of subsequent vascular mortality. Although research efforts in the past decade have greatly improved our knowledge of the multiple factors and mechanisms involved in vascular calcification in patients with kidney disease, many questions remain unanswered. No longer can we accept the concept that vascular calcification in CKD is a passive process resulting from an elevated calcium-phosphate product. Rather, as a result of the metabolic insults of diabetes, dyslipidemia, oxidative stress, uremia, and hyperphosphatemia, "osteoblast-like" cells form in the vessel wall. These mineralizing cells as well as the recruitment of undifferentiated progenitors to the osteochondrocyte lineage play a critical role in the calcification process. Important transcription factors such as Msx 2, osterix, and RUNX2 are crucial in the programming of osteogenesis. Thus, the simultaneous increase in arterial osteochondrocytic programs and reduction in active cellular defense mechanisms creates the "perfect storm" of vascular calcification seen in ESRD. Innovative clinical studies addressing the combined use of inhibitors that work on vascular calcification through distinct molecular mechanisms, such as fetuin-A, osteopontin, and bone morphogenic protein 7, among others, will be necessary to reduce significantly the accrual of vascular calcifications and cardiovascular mortality in kidney disease. In addition, the roles of oxidative stress and inflammation on the fate of smooth muscle vascular cells and their function deserve further translational investigation.



Both PTH and FGF-23 impact serum phosphate concentrations but neither responds to the phosphate concentration.



Scheme illustrating the ASARM-model and the FAM20C-kinase link to the FAP pathway: the numbers highlighted in the circles refer to the explanations in the following text. The interactions depicted on the osteocyte cell-surface between DMP1, PHEX, integrin and ASARM-peptides are dynamic and competitive occurring on the extracellular cell-surface. Arrows linking other factors represented in the cartoon illustrate positive and negative effector relationships (paracrine, autocrine, allosteric, signal transduction or gene expression):

(1) FAM20C-kinase phosphorylates the DMP1 C-terminal ASARM-motif

(2) phosphorylation of the DMP1–ASARM motif is required for binding to PHEX and the RGD motif of DMP1 binds to a5b3 integrin to form a [PHEX–DMP1–a5b3-integrin] trimeric complex. This interaction occurs on the cell surface of the osteocyte where PHEX and a5b3-integrin have an intramembranous domain, a short intracellular domain, and a large extracellular domain

(3 and 4) formation of the [PHEX–DMP1–a5b3 integrin] trimeric complex initiates a signaling pathway (MAPK/Erk) that suppresses FGF23 expression

(5) FAM20C-kinase also phosphorylates FGF-23 (Ser180)

(6 and 7) FAM20C phosphorylation of Ser180 inhibits O-glycosylation of FGF-23 by polypeptide N-acetylgalactosaminyltransferase 3 (GalNT3)

(8–10) the under-glycosylated and phosphorylated FGF-23 is targeted for Furin degradation and proteolysis

(6 and 11) in contrast, non-phosphorylated FGF-23 is O-glycosylated by GalNT3

(12 and 13) O-glycosylation of FGF-23 increases resistance to Furin degradation and increases ½ life of full length active FGF-23. Of relevance, mutations in GalNT3 are responsible for reduced circulating full-length FGF-23 resulting in hyperphosphatemia and tumoral calcinosis [48,78,79]. This is the opposite phenotype to high FGF-23 or hypophosphatemia.

In summary, FAM20C is responsible for suppressing FGF-23 expression via the [PHEX–DMP1–a5b3-integrin] trimeric complex and decreasing full-length active FGF-23 by targeting the hormone for furin degradation. This is consistent with the high circulating active FGF23, increased FGF23 mRNA expression, rickets and hypophosphatemia (ARHR 2) reported in mice that are null for bone expressed FAM20C [45 ,46];

(14 and 15) the binding of PHEX to the DMP1–ASARM-motif is also competitively regulated by free ASARM-peptide. Specifically, free ASARM-peptide can directly bind to PHEX preventing the binding to DMP1 and thereby disrupting the [PHEX–DMP1–a5b3-integrin] trimeric complex;

(16) this in turn results in increased expression of FGF23;

(17 and 18) also, free ASARM-peptide inhibits mineralization and renal phosphate uptake. In X-linked and autosomal recessive rickets there are high levels of circulating ASARM-peptides. Both in vitro and in vivo (bolus and infusion) administration of ASARM-peptides and transgenic mice over expressing ASARM-peptides causes mineralization defects and hypophosphatemia [4,8–10,16–18,22,80]. The bimodal effects of SPR4-peptide administration are not shown in the scheme but are discussed in detail in recent publications [10,13,14]. The dual pharmacological effects depend on phosphate diet and mode of administration (bolus or continuous fusion). Briefly, SPR4-peptide binds to either the DMP1–ASARM motif or free ASARM-peptide. Binding to the DMP1–ASARM motif results in increased expression of FGF-23 whereas binding to ASARM-peptides decreased FGF-23 expression.

Abstract The beginning of the millennium saw the discovery of a new bone-matrix protein, **Matrix Extracellular PhosphoglycoprotEin (MEPE)** and an associated C-terminal motif called ASARM. This motif and other distinguishing features occur in a group of proteins called SIBLINGs. These proteins include dentin matrix protein 1 (DMP1), osteopontin, dentin-sialophosphoprotein (DSPP), statherin, bone sialoprotein (BSP) and MEPE. MEPE, DMP1 and ASARM-motifs regulate expression of a phosphate regulating cytokine FGF23. Further, a trimeric interaction between phosphate regulating endopeptidase homolog X-linked (PHEX), DMP1, and α5β3-integrin that occurs on the plasma-membrane of the osteocyte mediates FGF23 regulation (FAP pathway). ASARM-peptides competitively inhibit the trimeric complex and increase FGF23. A second pathway involves specialized structures, matrix vesicles pathway (MVP). This review will discuss the FAP and MVP pathways and present a unified model for mineral and energy metabolism.

Journal of Bone and Mineral Research, Vol. 28, No. 3, March 2013, pp 688–699 Barros NMT et al

We report that OPN is a full-length protein substrate for PHEX. Degradation of OPN was essentially complete, including hydrolysis of the ASARM motif, resulting in only very small residual fragments. Western blotting of Hyp (the murine homolog of human XLH) mouse bone extracts having no PHEX activity clearly showed accumulation of an 35 kDa OPN fragment that was not present in wild-type mouse bone. Immunohistochemistry and immunogold labeling (electron microscopy) for OPN in Hyp bone likewise showed an accumulation of OPN and/or its fragments compared with normal wild-type bone. Incubation of Hyp mouse bone extracts with PHEX resulted in the complete degradation of these fragments. In conclusion, these results identify full-length OPN and its fragments as novel, physiologically relevant substrates for PHEX, suggesting that accumulation of mineralization-inhibiting OPN fragments may contribute to the mineralization defect seen in the osteomalacic bone characteristic of XLH/HYP.

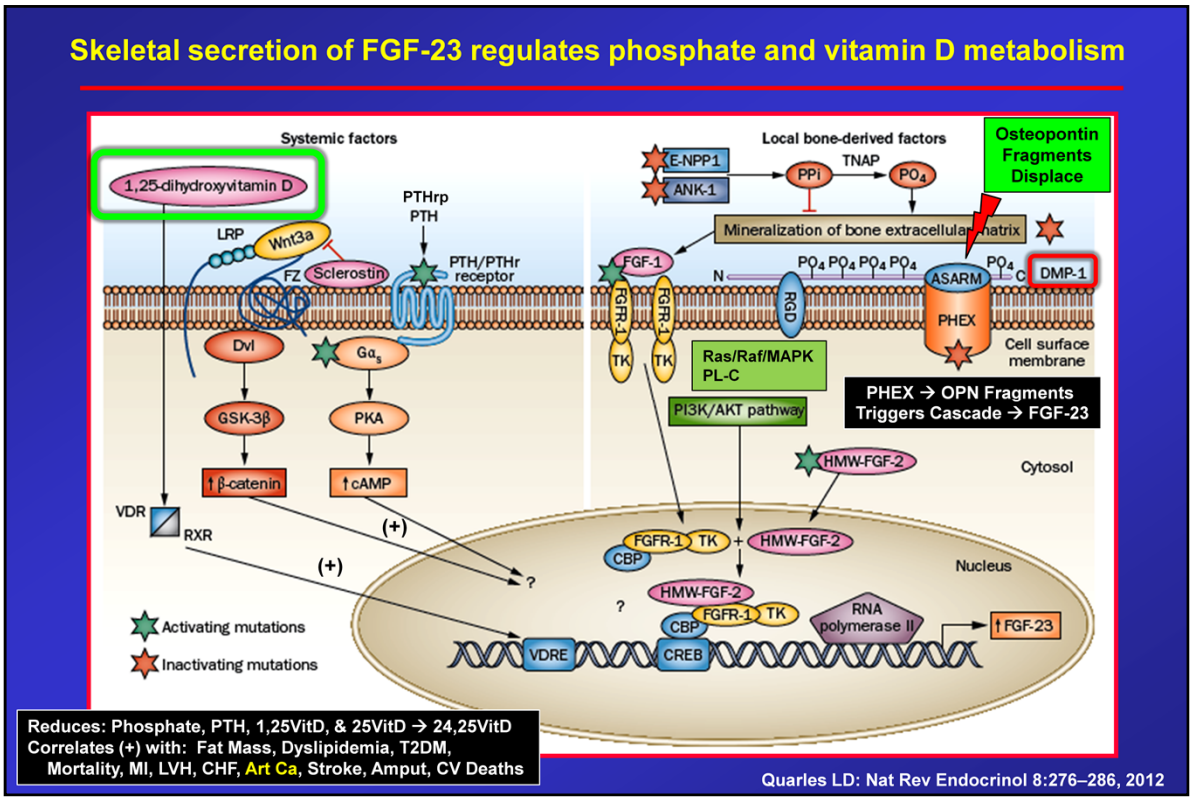
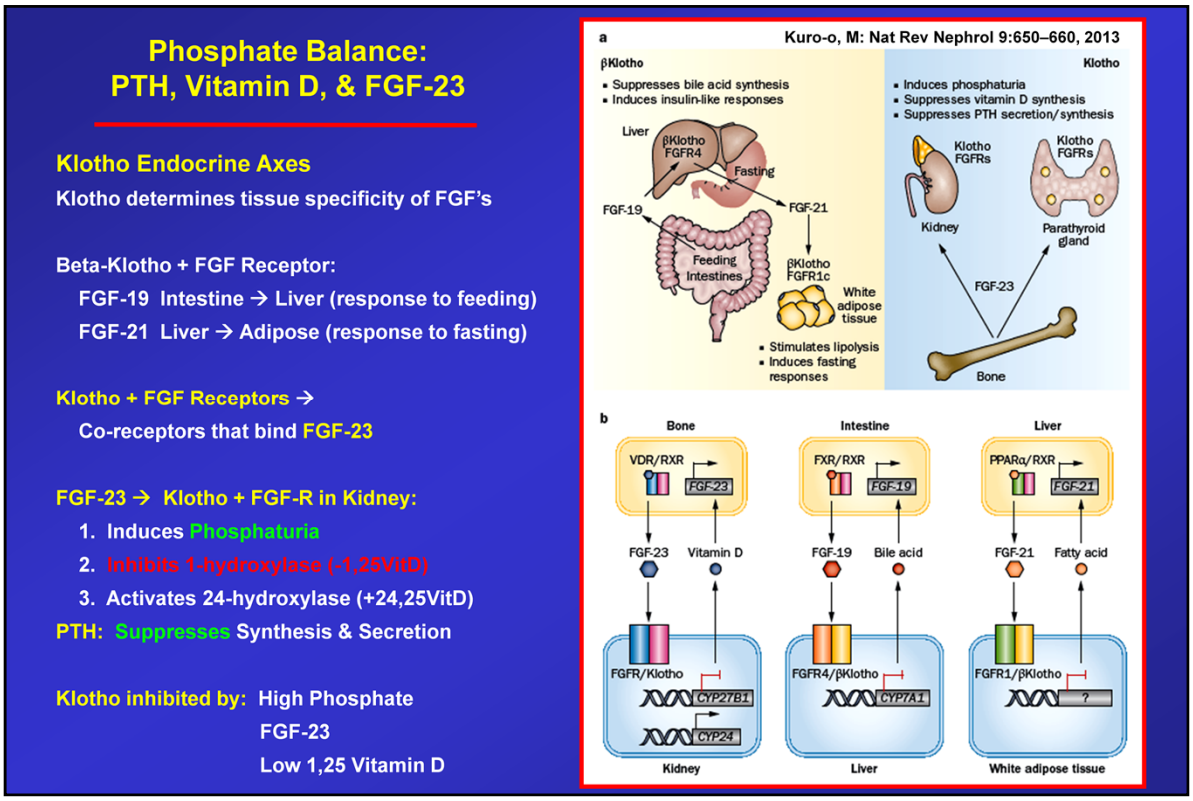


Figure 1 | A speculative model of *FGF23* gene transcriptional regulation. Four activating mutations or pathways (involving FGFR-1, Gαs encoded by *GNAS*, PTH/PTHr receptor and HMW-FGF-2) and four inactivating mutations (involving PHEX, DMP-1, E-NPP1 and ANK-1) are associated with increased FGF-23 expression in bone. Local bone-derived factors that are linked to mineralization are shown on the right-hand side. ANK-1 and E-NPP1 regulate the transport and biosynthesis of pyrophosphate, and TNAP regulates the conversion of pyrophosphate to phosphate in the extracellular matrix mineralization process, whereas both **PHEX** and **DMP-1** regulate bone mineralization through mechanisms that remain to be fully elucidated. Evidence exists in osteoblasts derived from the Hyp mouse model that defective mineralization is linked to the activation of FGFR-1 as well as **HMW-FGF-2** integrative nuclear signaling pathways. The left-hand side of the figure shows systemic factors involved in FGF-23 regulation. 1,25-dihydroxyvitamin D is an important regulator of FGF-23 expression, acting through the VDR and VDRE. PTH can also stimulate FGF-23 through a sclerostin-dependent mechanism involving the Wnt–β-catenin pathway, or through stimulation of GNAS and cAMP-dependent signaling pathways, as well as indirectly through stimulation of 1,25-dihydroxyvitamin D. Intrinsic and systemic factors are integrated at the levels of *cis*-acting elements in the proximal *FGF23* promoter that remain to be elucidated. A question mark (?) indicates areas of uncertainty. Abbreviations: Hyp, mouse model of X-linked hypophosphatemic rickets; PO4, phosphate; PPi, pyrophosphate; TK, tyrosine kinase.

Abstract The discovery of fibroblast growth factor 23 (FGF-23) has expanded our understanding of phosphate and vitamin D homeostasis and provided new insights into the pathogenesis of hereditary hypophosphatemic and hyperphosphatemic disorders, as well as acquired disorders of phosphate metabolism, such as chronic kidney disease. FGF-23 is secreted by **osteoblasts and osteocytes** in bone and principally **targets the kidney** to regulate the reabsorption of phosphate, the production and catabolism of 1,25-dihydroxyvitamin D and the expression of **α-Klotho**, an anti-ageing hormone. Secreted FGF-23 plays a central role in complex endocrine networks involving local bone-derived factors that regulate mineralization of extracellular matrix and systemic hormones involved in mineral metabolism. Inactivating mutations of PHEX, DMP1 and ENPP1, which cause hereditary hypophosphatemic disorders and primary defects in bone mineralization, stimulate FGF23 gene transcription in osteoblasts and osteocytes, at least in part, through canonical and intracrine FGF receptor pathways. These FGF-23 regulatory pathways may enable systemic phosphate and vitamin D homeostasis to be coordinated with bone mineralization. FGF-23 also functions as a counter-regulatory hormone for 1,25-dihydroxyvitamin D in a bone-kidney endocrine loop. FGF-23, through regulation of additional genes in the kidney and extra-renal tissues, probably has broader physiological functions beyond regulation of mineral metabolism that account for the association between FGF-23 and increased mortality and morbidity in chronic kidney disease.



Klotho, phosphate and FGF-23 in ageing and disturbed mineral metabolism.
Makoto Kuro-o Kuro-o, M. *Nat. Rev. Nephrol*. 9, 650–660 (2013)

Figure 1 | FGF–Klotho endocrine axes.

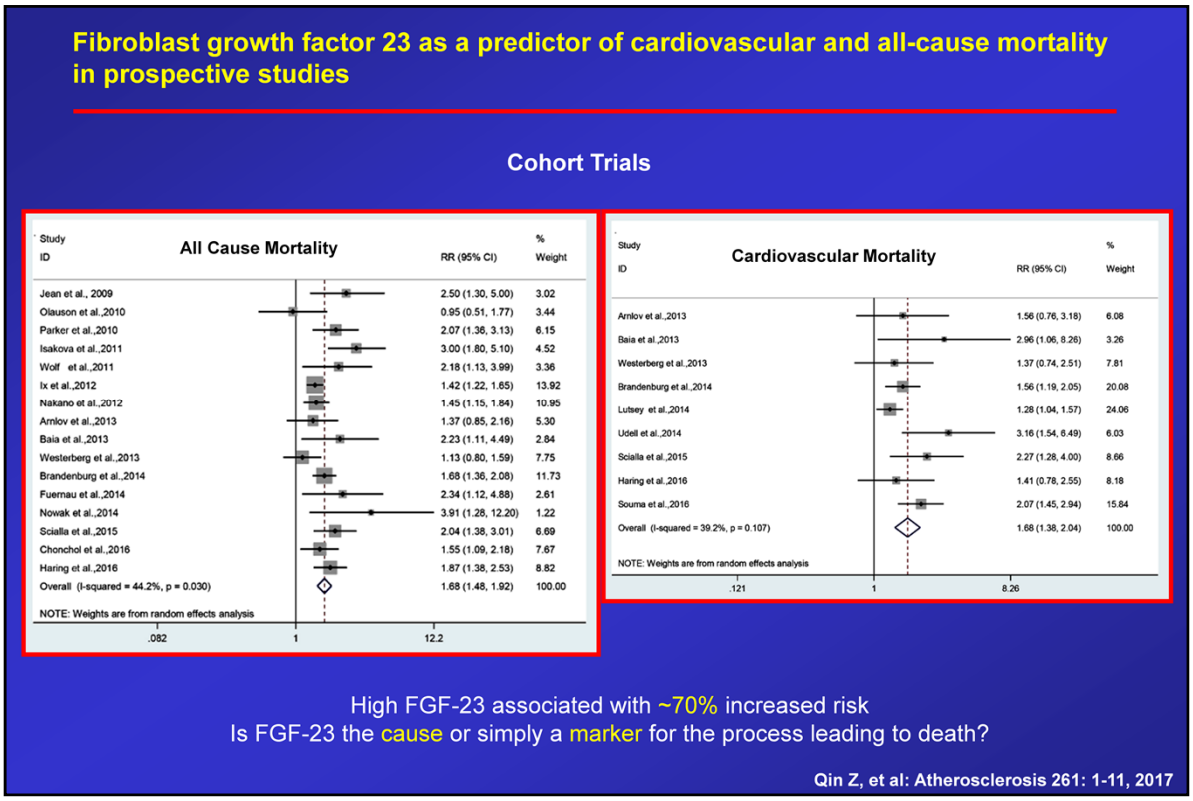
a | Endocrine axes mediated by βKlotho (left) and Klotho (right). FGF-19 is an intestine-derived satiety hormone targeting the liver to induce metabolic responses to feeding. FGF-21 is a liver-derived hunger hormone targeting white adipose tissue to induce metabolic responses to fasting. These hormones require βKlotho to bind to their cognate FGFRs with high affinity. Similarly, FGF-23 requires Klotho for binding to FGFRs. FGF-23 secreted from bone acts on the kidney and the parathyroid gland to regulate mineral homeostasis.

b | Analogous feedback loops in the FGF–Klotho endocrine axes involving FGF-23, FGF-19 and FGF-21. Whether or not there is a *CYP* gene(s) that is downregulated by FGF-21 in white adipose tissue remains unknown. Permission to reproduce panel b obtained from Elsevier Ltd ©

Kuro-o, M. *Trends Endocrinol. Metab.* 19, 239–245 (2008).

Abbreviations: CYP, cytochrome P450 superfamily; FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; FXR, farenoid X receptor; PTH, parathyroid hormone; PPAR, peroxisome proliferator-activated receptor; RXR, retinoid X receptor; VDR, vitamin D receptor.

Abstract High concentrations of extracellular phosphate are toxic to cells. Impaired urinary phosphate excretion increases serum phosphate level and induces a premature-ageing phenotype. Urinary phosphate levels are increased by dietary phosphate overload and might induce tubular injury and interstitial fibrosis. Extracellular phosphate exerts its cytotoxic effects by forming insoluble nanoparticles with calcium and fetuin-A; these nanoparticles are referred to in this Review as calciprotein particles. Calciprotein particles are highly bioactive ligands that can induce various cellular responses, including the osteogenic transformation of vascular smooth muscle cells and cell death of vascular endothelial cells and renal tubular epithelial cells. Calciprotein particles are detected in the serum of animal models of kidney disease and in patients with chronic kidney disease (CKD) and might be associated with a (mal)adaptation of the endocrine axes mediated by fibroblast growth factors and Klothos that regulate phosphate homeostasis and ageing. These observations raise the possibility that calciprotein particles contribute to the pathogenesis of CKD. This theory, if verified, is expected to provide novel diagnostic markers and therapeutic targets in CKD.



Abstract BACKGROUND AND AIMS: The prognostic value of fibroblast growth factor 23 (FGF23) for mortality remains controversial. We performed a meta-analysis of cohort studies to examine the controversial relationship between FGF23 and mortality.

METHODS: PubMed, EMBASE, the Cochrane Library databases and reference bibliographies were searched through September 2016 to identify prospective cohort studies with relative risks (RRs) and 95% confidence intervals (CIs) for FGF23 and mortality. A random effects model was used to pool the risk estimates. A dose-response analysis of the risk for all-cause mortality associated with FGF23 was conducted using the generalized least squares trend estimation method.

RESULTS: Nineteen prospective cohort studies were eligible for inclusion in this meta-analysis, of which 16 reported all-cause mortality and 9 reported cardiovascular mortality. During the follow-up periods ranging from 1 to 18.6 years, 5606 deaths occurred among 22,805 participants and 2458 cardiovascular deaths occurred among 28,845 participants. Elevated FGF23 was associated with an increased risk of all-cause mortality (RR 1.68; 95% CI 1.48-1.92) and cardiovascular mortality (RR 1.68; 95% CI 1.38-2.04) with moderate heterogeneity. These associations were not markedly modified by the geographic location, follow-up length, patient predisposition, FGF23 measurement or study quality. A sensitivity

analysis yielded a similar effect on the pooled risk estimate. Evidence of a nonlinear relationship between FGF23 and all-cause mortality was observed in the dose-response analysis, with the risk gradually increasing as FGF23 increased.

CONCLUSIONS: This meta-analysis showed that individuals with increased plasma FGF23 levels might suffer a higher risk of all-cause mortality and cardiovascular mortality.

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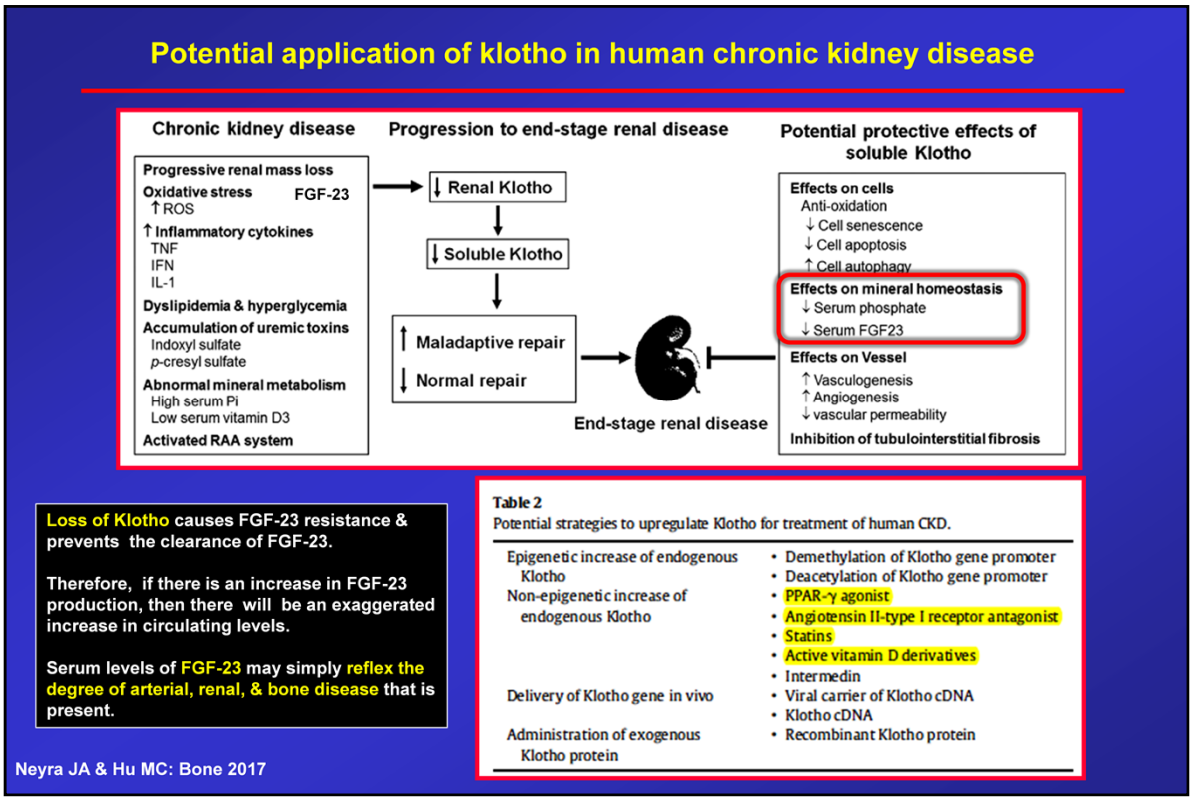
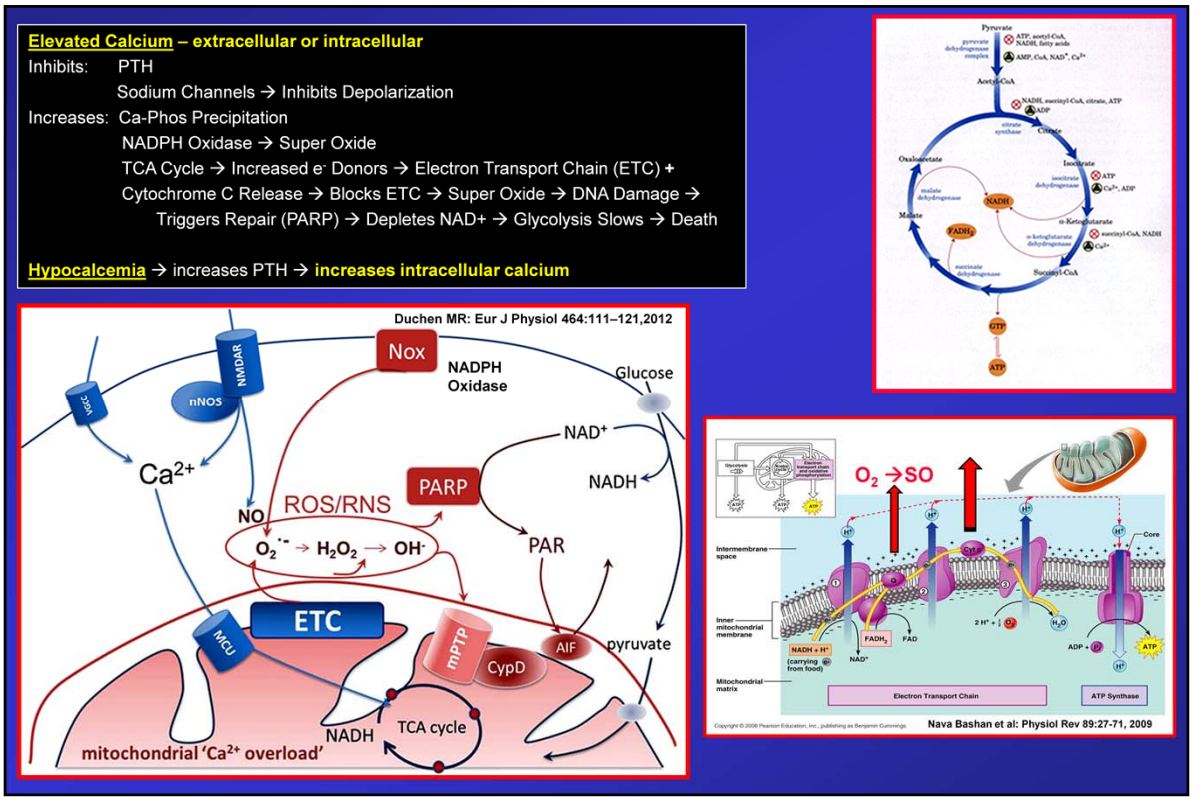


Fig. 2. Potential mechanisms of Klotho downregulation in CKD, and beneficial effects of soluble Klotho on CKD. Left panel: Loss of renal mass, over production of reactive oxygen species (ROS) as well as pro-inflammatory cytokines including tumor necrosis factor (TNF), interferon (IFN) and interleukin 1 (IL-1), dyslipidemia and hyperglycemia, and elevation of uremic toxins including indoxyl sulfate and p-cresyl sulfate may contribute to or participate in downregulation of renal Klotho. Furthermore, high serum phosphate and FGF23 as well as low serum 1,25-Vit.D3 inhibit renal Klotho expression. Low serum 1,25-Vit.D3 not only reduces Klotho expression, but also stimulates renin-aldosterone-angiotensin (RAA) system which further suppresses Klotho production. Middle panel: Reduced Klotho expression in the kidney would lead to endocrine Klotho deficiency in CKD. Low soluble Klotho promotes CKD progression to ESRD through impaired normal renal repair process and induction of maladaptive repair process. Right panel: Supplementation of soluble Klotho protein retards CKD progression through multiple biologic actions: (1) cytoprotection via anti-oxidation, reduction of cell senescence and apoptosis, and upregulation of autophagy, hence accelerating renal tubule regeneration; (2) correction of high serum phosphate and FGF23; (3) maintenance of peritubular capillary formation and function; and (4) inhibition of tubulointerstitial fibrosis.

Abstract The extracellular domain of transmembrane alpha-Klotho (αKlotho, hereinafter simply called Klotho) is cleaved by secretases and released into the circulation as soluble Klotho. Soluble Klotho in the circulation starts to decline

early in chronic kidney disease (CKD) stage 2 and urinary Klotho possibly even earlier in CKD stage 1. Therefore soluble Klotho could serve as an early and sensitive marker of kidney function decline. Moreover, preclinical animal data support Klotho deficiency is not just merely a biomarker, but a pathogenic factor for CKD progression and extrarenal CKD complications including cardiovascular disease and disturbed mineral metabolism. Prevention of Klotho decline, re-activation of endogenous Klotho production or supplementation of exogenous Klotho are all associated with attenuation of renal fibrosis, retardation of CKD progression, improvement of mineral metabolism, amelioration of cardiomyopathy, and alleviation of vascular calcification in CKD. Therefore Klotho is not only a diagnostic and/or prognostic marker for CKD, but the treatment of Klotho deficiency may be a promising strategy to prevent, retard, and decrease the burden of comorbidity in CKD.



Mitochondria, calcium-dependent neuronal death and neurodegenerative disease. Eur J Physiol 464:111–121, 2012. Michael R. Duchen

Fig. 3 Scheme of pathways involved in glutamate-induced excitotoxicity. Calcium influx through voltage-gated or NMDAR-gated channels is followed by mitochondrial Ca2+ influx through the mitochondrial calcium uniporter (MCU). While the physiological consequence of raised intra-mitochondrial [Ca2+] is an increased activity of the three rate limiting enzymes of the TCA cycle, pathological and prolonged Ca2+ influx leads to mitochondrial Ca2+overload. NMDAR mediated Ca2+ influx is closely coupled to the generation of NO by nNOS; raised Ca2+ may activate the NADPH oxidase (Nox), while mitochondrial Ca2+ overload may also increase generation of superoxide by the electron transport chain (ETC). Nitrosative or oxidative stress arising either from the ETC or from Nox activation may cause over activation of PARP. PARP consumes NAD+ to form PAR polymers, causing depletion of NAD+, failure of glycolysis and so failure of mitochondrial substrate supply. This culminates in the loss of $\Delta\psi m$, ATP depletion and cell death. The PAR polymers generated by PARP may also cause release of AIF which amplifies cell death following its translocation to the nucleus.

Abstract Understanding the mechanisms of neuronal dysfunction and death represents a major frontier in contemporary medicine, involving the acute cell death

in stroke, and the attrition of the major neurodegenerative diseases, including Parkinson's, Alzheimer's, Huntington's and Motoneuron diseases. A growing body of evidence implicates mitochondrial dysfunction as a key step in the pathogenesis of all these diseases, with the promise that mitochondrial processes represent valuable potential therapeutic targets. Each disease is characterized by the loss of a specific vulnerable population of cells--dopaminergic neurons in Parkinson's disease, spinal motor neurons in Motor neuron disease, for example. We discuss the possible roles of cell type-specific calcium signalling mechanisms in defining the pathological phenotype of each of these major diseases and review central mechanisms of calcium-dependent mitochondrial-mediated cell death.

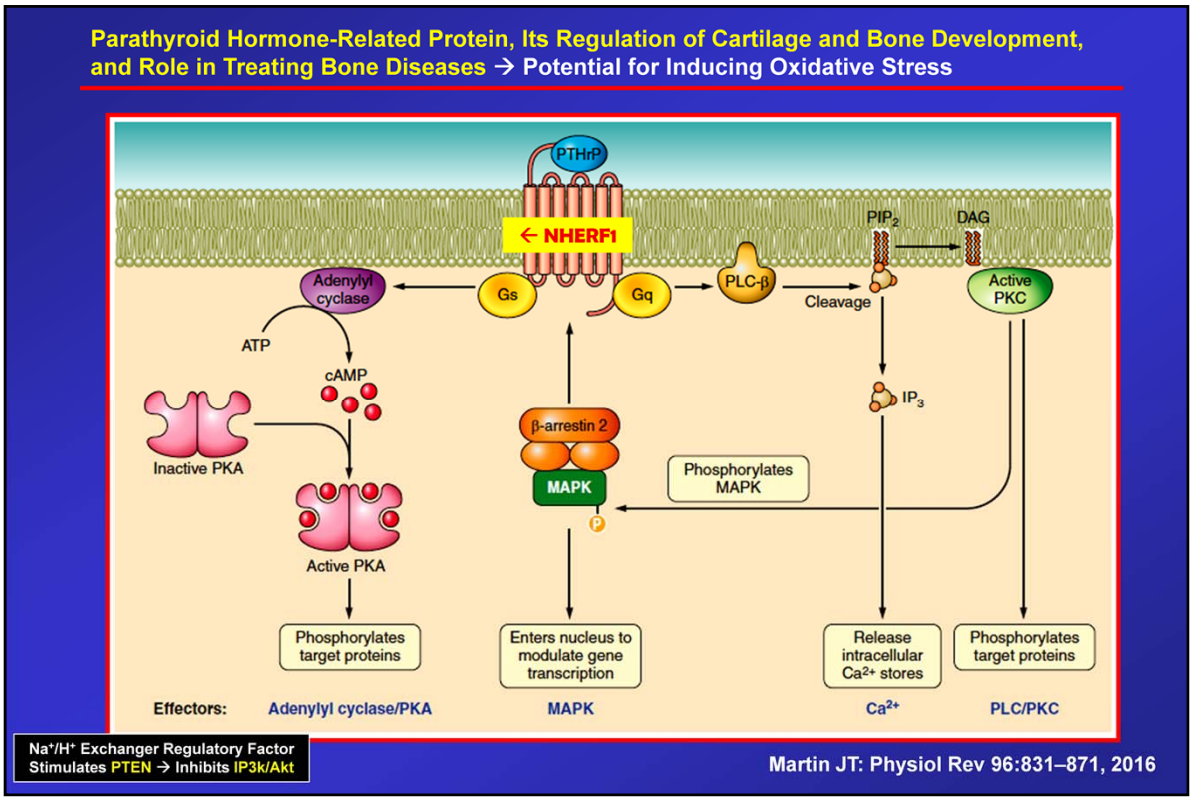


FIGURE 5. Signal transduction pathways in ligand-induced activation of PTHR1. Ligand binding leads to association with Gs subunit and adenylyl cyclase activation, or with Gq that activates phospholipase C-(PLC-). MAPK can be involved through interaction of PTHR1 with the MAPK scaffolding protein-arrestin 2.

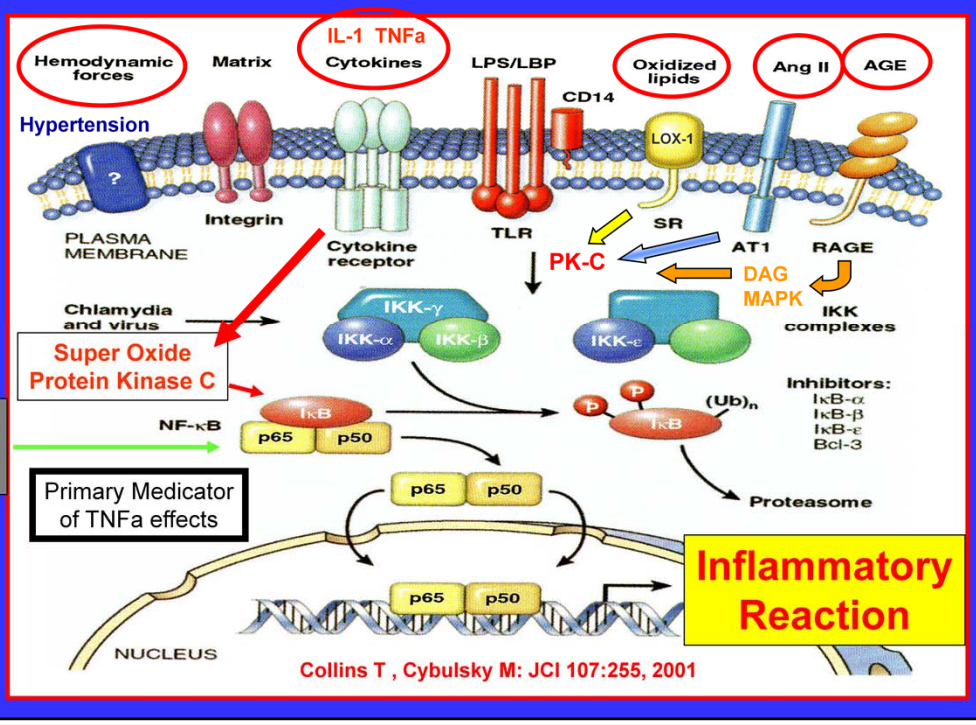
Abstract Although parathyroid hormone-related protein (PTHrP) was discovered as a cancer-derived hormone, it has been revealed as an important paracrine/autocrine regulator in many tissues, where its effects are context dependent. Thus its location and action in the vasculature explained decades-long observations that injection of PTH into animals rapidly lowered blood pressure by producing vasodilatation. Its roles have been specified in development and maturity in cartilage and bone as a crucial regulator of endochondral bone formation and bone remodeling, respectively. Although it shares actions with parathyroid hormone (PTH) through the use of their common receptor, PTHR1, PTHrP has other actions mediated by regions within the molecule beyond the amino-terminal sequence that resembles PTH, including the ability to promote placental transfer of calcium from mother to fetus. A striking feature of the physiology of PTHrP is that it possesses structural features that equip it to be transported in and out of the nucleus, and makes use of a specific nuclear import mechanism to do so. Evidence from mouse genetic experiments shows that PTHrP generated locally in bone is essential for normal bone remodeling. Whereas the main physiological function of PTH is the hormonal regulation of calcium metabolism, locally generated PTHrP is the

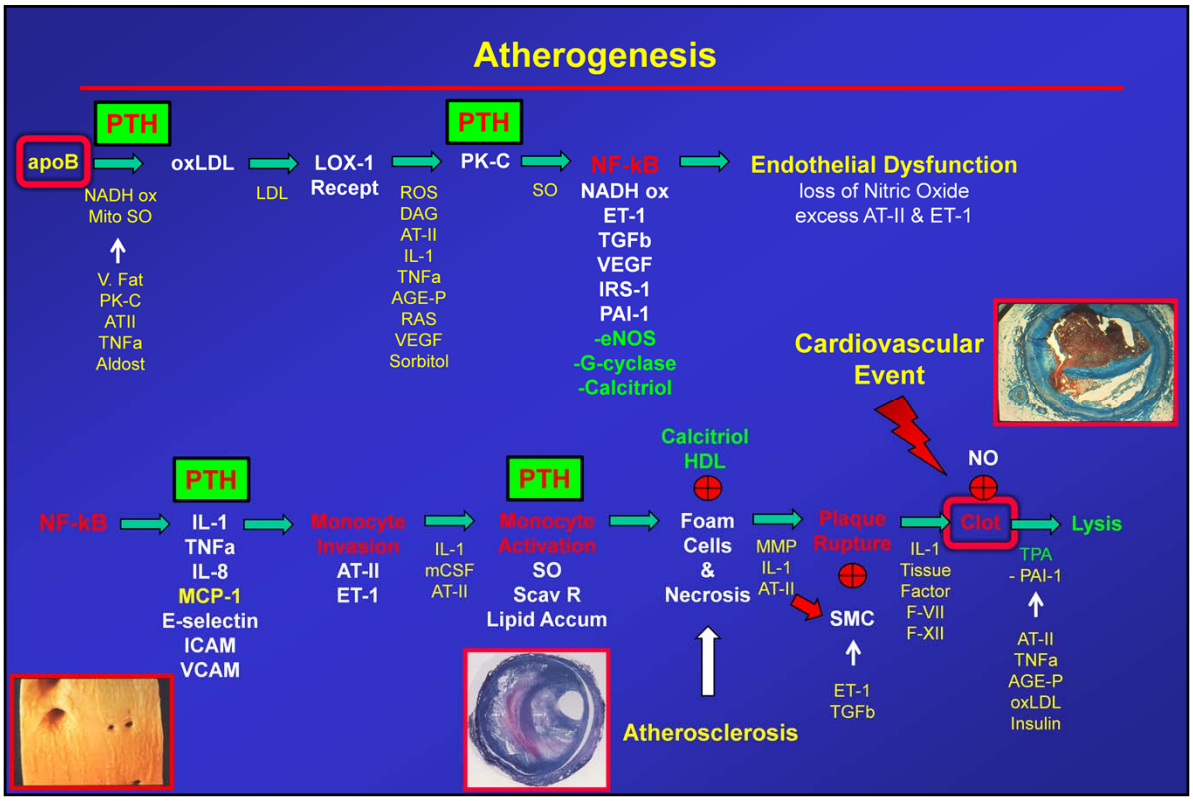
important physiological mediator of bone remodeling postnatally. Thus the use of intermittent injection of PTH as an anabolic therapy for bone appears to be a pharmacological application of the physiological function of PTHrP. There is much current interest in the possibility of developing PTHrP analogs that might enhance the therapeutic anabolic effects.

N Engl J Med 359:1128-1135, 2008:

Impaired renal phosphate reabsorption, as measured by dividing the tubular maximal reabsorption of phosphate by the glomerular filtration rate (TmP/GFR), increases the risks of nephrolithiasis and bone demineralization. Data from animal models suggest that sodium–hydrogen exchanger regulatory factor 1 (NHERF1) controls renal phosphate transport. We sequenced the *NHERF1* gene in 158 patients, 94 of whom had either nephrolithiasis or bone demineralization. We identified three distinct mutations in seven patients with a low TmP/GFR value. No patients with normal TmP/GFR values had mutations. The mutants expressed in cultured renal cells increased the generation of cyclic AMP (cAMP) by parathyroid hormone (PTH) and inhibited phosphate transport. These NHERF1 mutations suggest a previously unrecognized cause of renal phosphate loss in humans.

Nuclear Factor kB (NF-kB) Activation




Atherogenesis
PTH
apoB
NADH ox
Mito SO
V. Fat
PK-C
ATII
TNFa
Aldost
oxLDL
LDL
LOX-1
Recept
PTH
ROS
DAG
AT-II
IL-1
TNFa
AGE-P
RAS
VEGF
Sorbitol
PK-C
SO
NF-kB
NADH ox
ET-1
TGFb
VEGF
IRS-1
PAI-1
-eNOS
-G-cyclase
-Calcitriol
Endothelial Dysfunction
loss of Nitric Oxide
excess AT-II & ET-1
Cardiovascular Event
NO
Clot
Lysis
TPA
- PAI-1
AT-II
TNFa
AGE-P
oxLDL
Insulin
Calcitriol
HDL
Foam Cells & Necrosis
MMP
IL-1
AT-II
Plaque Rupture
SMC
ET-1
TGFb
IL-1
Tissue Factor
F-VII
F-XII
Atherosclerosis
NF-kB
PTH
IL-1
TNFa
IL-8
MCP-1
E-selectin
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PTH
Monocyte Activation
SO
Scav R
Lipid Accum

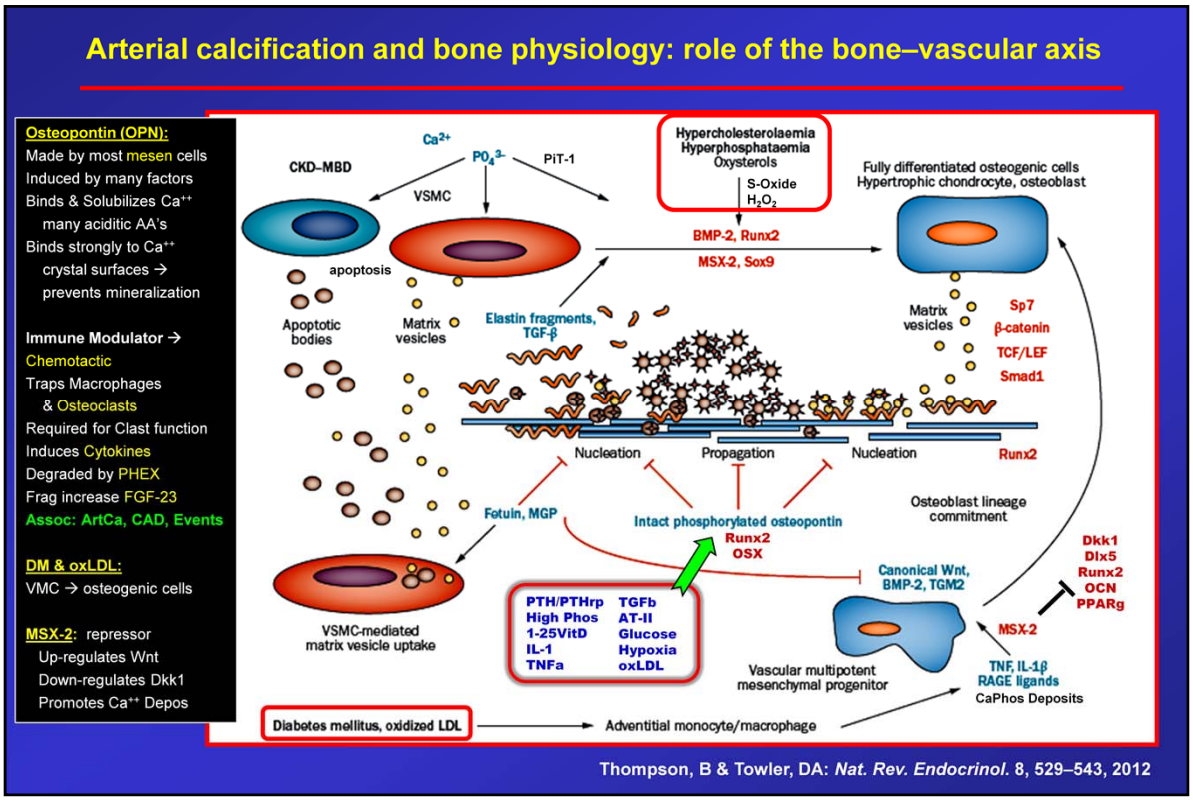

Figure 3 | Vascular osteogenic cell origins, functions and phenotypes in arterial calcification. Vascular mineralization is regulated by processes overlapping yet distinct from those that control skeletal bone formation. Osteogenic progenitors can arise from 'transdifferentiation' of VSMCs, or osteogenic lineage allocation of multipotent mesenchymal progenitors. Healthy VSMCs also have an important role in limiting vascular calcium accrual via fetuin-dependent and MGP-dependent pinocytotic uptake of matrix vesicles. Metabolic and inflammatory insults induce vascular changes that impair normal VSMC function and viability and induce osteogenic differentiation of vascular mesenchymal cells. Not shown are the circulating osteoprogenitors that may contribute to the 'vascular ossification'—true bone formation replete with marrow elements—that can be seen in ~15% of calcified vascular segments. Extracellular factors are blue and intracellular transcriptional regulators are red.

Abstract Bone never forms without vascular interactions. This simple statement of fact does not adequately reflect the physiological and pharmacological implications of the relationship. The vasculature is the conduit for nutrient exchange between bone and the rest of the body. The vasculature provides the sustentacular niche for development of osteoblast progenitors and is the conduit for egress of bone marrow cell products arising, in turn, from the osteoblast-dependent haematopoietic niche. Importantly, the second most calcified structure in humans after the skeleton is the vasculature. Once considered a passive process of dead and dying cells, vascular

calcification has emerged as an actively regulated form of tissue biomineralization. Skeletal morphogens and osteochondrogenic transcription factors are expressed by cells within the vessel wall, which regulates the deposition of vascular calcium. Osteotropic hormones, including parathyroid hormone, regulate both vascular and skeletal mineralization. Cellular, endocrine and metabolic signals that flow bidirectionally between the vasculature and bone are necessary for both bone health and vascular health. Dysmetabolic states including diabetes mellitus, uraemia and hyperlipidaemia perturb the bone-vascular axis, giving rise to devastating vascular and skeletal disease. A detailed understanding of bone-vascular interactions is necessary to address the unmet clinical needs of an increasingly aged and dysmetabolic population.

CKD–MBD: chronic kidney disease mineral and bone disorder

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RAGE ligands: ligands for receptor for advanced glycation end products

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VSMCs: vascular smooth muscle cells

Fetuin-A: (α2-HS-glycoprotein; secreted by liver) was originally discovered to be an inhibitor of vascular calcification in early 1990s. Since then the biologic roles attributed to fetuin-A have increased exponentially. Fetuin-A has been demonstrated to play an important role in free fatty acid induced insulin resistance in the liver. Increased fetuin-A in patients with pre-diabetes is associated with increased progression to diabetes and decreased reversal to normoglycemia. Hence, fetuin-A is a predictor of adverse glycemic outcomes in pre-diabetes. Increased fetuin-A had been also been linked to increased occurrence of non-alcoholic fatty liver disease and cardiovascular events, believed to be due to its pro-inflammatory effects. Fetuin-A in contrast has also been demonstrated to have anti-inflammatory properties. It is a negative acute-phase reactant in sepsis and endotoxemia, promotes wound healing, and is neuroprotective in Alzheimer's disease. Decreased fetuin-A is a predictor of increased disease activity in obstructive lung disease, Crohn's disease, and ulcerative colitis. Differential effects on different toll like receptors in different tissues and organ systems may explain these paradoxical effects in different systems.

Fetuin: Hepatocyte-derived, Ca-binding protein that maintains Ca solubility in serum & interstitial fluid; removed by dialysis, reduced by inflammation.

Osteopontin: OPN is a highly negatively charged, extracellular matrix protein that lacks an extensive secondary structure. Osteopontin is biosynthesized by a variety of tissue types including cardiac fibroblasts, preosteoblasts, osteoblasts, osteocytes, odontoblasts, some bone

marrow cells, hypertrophic chondrocytes, dendritic cells, macrophages, smooth muscle, skeletal muscle myoblasts, endothelial cells, and extra-osseous (non-bone) cells in the inner ear, brain, kidney, deciduum, and placenta. Synthesis of osteopontin is stimulated by calcitriol. OPN expression in bone predominantly occurs by osteoblasts and osteocytes (bone-forming cells) as well as osteoclasts (bone-resorbing cells). Runx2 (aka Cbfa1) and osterix (Osx) transcription factors are required for the expression of OPN. Runx2 and Osx bind promoters of osteoblast-specific genes such as *Col1α1*, *Bsp*, and *Opn* and upregulate transcription. There is a high-specificity vitamin D response element (VDRE) in the OPN gene promoter. Extracellular inorganic phosphate (ePi) has also been identified as a modulator of OPN expression. Stimulation of OPN expression also occurs upon exposure of cells to pro-inflammatory cytokines, classical mediators of acute inflammation (e.g. tumour necrosis factor α [TNFα], infterleukin-1β [IL-1β]), angiotensin II, transforming growth factor β (TGFβ), and parathyroid hormone (PTH) although a detailed mechanistic understanding of these regulatory pathways are not yet known. Hyperglycemia and hypoxia are also known to increase OPN expression.

Role in biomineralization

OPN belongs to a family of secreted acidic proteins whose members have an abundance of negatively charged amino acids such as Asp and Glu. OPN also has a large number of consensus sequence sites for post-translational phosphorylation of Ser residues to form phosphoserine, providing additional negative charge. Contiguous stretches of high negative charge in OPN have been identified and named the polyAsp motif (poly-aspartic acid) and the ASARM motif (acidic serine- and asparate-rich motif), with the latter sequence having multiple phosphorylation sites. This overall negative charge of OPN, along with its specific acidic motifs and the fact that OPN is an intrinsically disordered protein allowing for open and flexible structures, permit OPN to bind strongly to calcium atoms available at crystal surfaces in various biominerals. Such binding of OPN to various types of calcium-based biominerals – such as calcium-phosphate mineral in bones and teeth, calcium-carbonate mineral in inner ear otoconia and avian eggshells, and calcium-oxalate mineral in kidney stones – acts as a mineralization inhibitor to regulate crystal growth.[54]

OPN is a substrate protein for a number of enzymes whose actions may modulate the mineralization-inhibiting function of OPN. PHEX (phosphate-regulating gene with homologies to endopeptidases on the X chromosome) is one such enzyme, which extensively degrades OPN, and whose inactivating gene mutations (in X-linked hypophosphatemia, XLH) lead to altered processing of OPN such that inhibitory OPN cannot be degraded and accumulates in the bone (and tooth) extracellular matrix, likely contributing locally to the osteomalacia (soft hypomineralized bones) characteristic of XLH.

Along with its role in the regulation of normal mineralization within the extracellular matrices of bones and teeth, OPN is also upregulated at sites of pathologic, ectopic calcification – such as for example, in urolithiasis and vascular calcification – presumably at least in part to inhibit debilitating mineralization in these soft tissues.

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Osteopontin has been implicated as an important factor in bone remodeling. Specifically,

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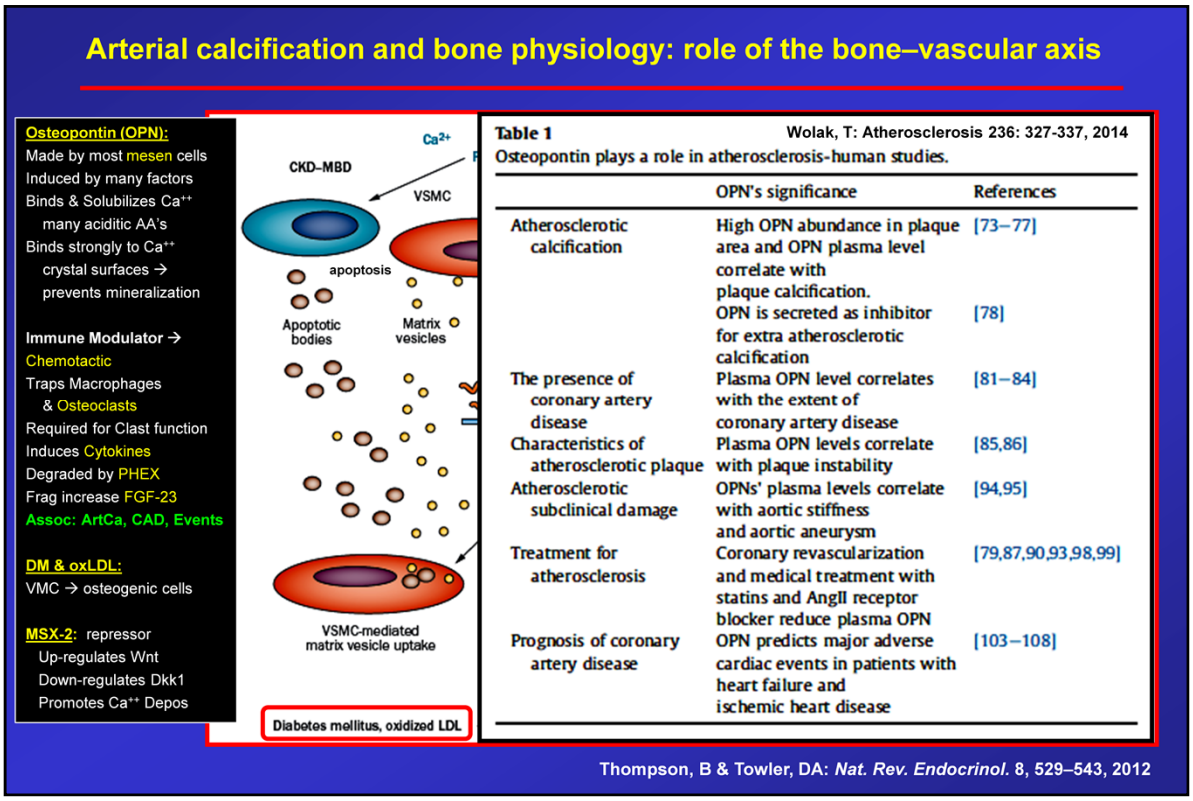

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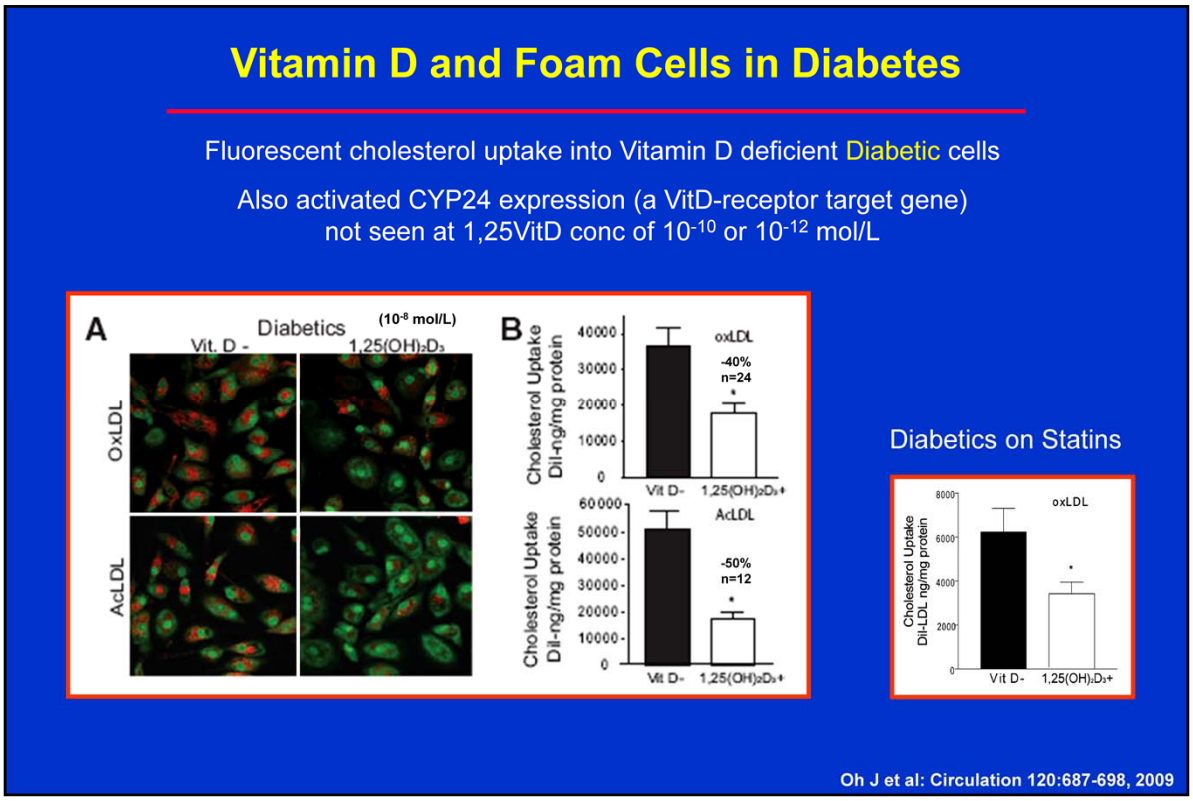

Abstract

BACKGROUND: Cardiovascular disease is the leading cause of death among those with diabetes mellitus. Vitamin D deficiency is associated with an increased risk of cardiovascular disease in this population. To determine the mechanism by which vitamin D deficiency mediates accelerated cardiovascular disease in patients with diabetes mellitus, we investigated the effects of active vitamin D on macrophage cholesterol deposition.

METHODS AND RESULTS: We obtained macrophages from 76 obese, diabetic, hypertensive patients with vitamin D deficiency (25-hydroxyvitamin D <80 nmol/L; group A) and 4 control groups: obese, diabetic, hypertensive patients with normal vitamin D (group B; n=15); obese, nondiabetic, hypertensive patients with vitamin D deficiency (group C; n=25); and nonobese, nondiabetic, nonhypertensive patients with vitamin D deficiency (group D; n=10) or sufficiency (group E; n=10). Macrophages from the same patients in all groups were cultured in vitamin D-deficient or 1,25-dihydroxyvitamin D(3) [1,25(OH)(2)D(3)] -supplemented media and exposed to modified low-density lipoprotein cholesterol. 1,25(OH)(2)D(3) suppressed foam cell formation by reducing acetylated or oxidized low-density lipoprotein cholesterol uptake in diabetic subjects only. Conversely, deletion of the vitamin D receptor in macrophages from diabetic patients accelerated foam cell formation induced by modified LDL. 1,25(OH)(2)D(3) downregulation of c-Jun N-terminal kinase activation reduced peroxisome proliferated-activated receptor-

gamma expression, suppressed CD36 expression, and prevented oxidized low-density lipoprotein-derived cholesterol uptake. In addition, 1,25(OH)(2)D(3) suppression of macrophage endoplasmic reticulum stress improved insulin signaling, downregulated SR-A1 expression, and prevented oxidized and acetylated low-density lipoprotein-derived cholesterol uptake.

CONCLUSIONS: These results identify reduced vitamin D receptor signaling as a potential mechanism underlying increased foam cell formation and accelerated cardiovascular disease in diabetic subjects.

corrected calcium (calciumAlb) in mg/dL to mmol/L, multiply by 0.25; serum phosphorus in mg/dL to mmol/L, multiply by 0.323; PTH levels expressed in pg/mL and ng/L are equivalent.

Abstract BACKGROUND: Abnormalities in serum calcium, phosphorus, and parathyroid hormone (PTH) concentrations are common in patients with chronic kidney disease and have been associated with increased morbidity and mortality. No clinical trials have been conducted to clearly identify categories of calcium, phosphorus, and PTH levels associated with the lowest mortality risk. Current clinical practice guidelines are based largely on expert opinions, and clinically relevant differences exist among guidelines across countries. We sought to describe international trends in calcium, phosphorus, and PTH levels during 10 years and identify mortality risk categories in the Dialysis Outcomes and Practice Patterns Study (DOPPS), an international study of hemodialysis practices and associated outcomes.

STUDY DESIGN: Prospective cohort study.

PARTICIPANTS: 25,588 patients with end-stage renal disease on hemodialysis therapy for longer than 180 days at 925 facilities in DOPPS I (1996-2001), DOPPS II (2002-2004), or DOPPS III (2005-2007).

PREDICTORS: Serum calcium, albumin-corrected calcium (Ca(Alb)), phosphorus, and PTH levels.

OUTCOMES: Adjusted hazard ratios for all-cause and cardiovascular mortality calculated using Cox models.

RESULTS: Distributions of mineral metabolism markers differed across DOPPS countries and phases, with lower calcium and phosphorus levels observed in the most recent phase of DOPPS. Survival models identified categories with the lowest mortality risk for calcium (8.6 to 10.0 mg/dL), Ca(Alb) (7.6 to 9.5 mg/dL), phosphorus (3.6 to 5.0 mg/dL), and PTH (101 to 300 pg/mL). The greatest risk of mortality was found for calcium or Ca(Alb) levels greater than 10.0 mg/dL, phosphorus levels greater than 7.0 mg/dL, and PTH levels greater than 600 pg/mL and in patients with combinations of high-risk categories of calcium, phosphorus, and PTH.

LIMITATIONS: Because of the observational nature of DOPPS, this study can only indicate an association between mineral metabolism categories and mortality.

CONCLUSIONS: Our results provide important information about mineral metabolism trends in hemodialysis patients in 12 countries during a decade. The risk categories identified in the DOPPS cohort may be relevant to efforts at international harmonization of existing clinical guidelines for mineral metabolism.

Arterial Calcification, Bone Physiology, & Renal Function

Summary

Need to consider 3 processes: **Arteriosclerosis, Osteodystrophy, & Nephropathy**

Osteogenesis is a "normal" function of arteries & BMP-2 (PTHrp) is required for this function

All of the modulators for osteogenesis are available in arteries (TGFb, PTHrp, BMP-2, Wnt)

Osteogenesis is triggered by many of the same factors that trigger atherosclerosis

Free Radicals, Inflammation, Oxy-sterols, Hypertension, Hyperglycemia

Phosphate appears to be the dominate promotional mineral

Magnesium may be the dominate inhibiting mineral

As bone becomes "non-responsive" it is less able to buffer changes in Calcium & Phosphate

PTH's primary purpose is to maintain serum [Ca++], not maintain bone integrity & strength

PTHrp & Wnt are the primary mediators maintaining bone integrity

Osteoblasts, BMP, OPN, & excess phosphate are not good things to have in arteries