Calcium, phosphate & magnesium regulation

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Bone composition

Treated with bleach (hypochlorite) to digest collagen leaves mineral component [hydroxyapatite – Ca$_{10}$(PO$_4$)$_6$(OH)$_2$] intact.

Treated with hydrochloric acid to dissolve mineral leaves organic component [mainly type 1 collagen] intact.

Collagen shrinkage on drying.
Ca\textsuperscript{2+} regulation

- Almost all of the Ca\textsuperscript{2+} in the body (>99%) exists as mineral deposits in the skeleton and teeth
- In man, plasma Ca\textsuperscript{2+} is tightly maintained at ~2.5mM
- Local concentrations of Ca\textsuperscript{2+} in extracellular fluid may vary more
- About 50% of plasma Ca\textsuperscript{2+} is ionised and diffusible
Ca$^{2+}$ regulation

Why so precise?

- Ca$^{2+}$ is used as a vital second messenger within cells
- Ca$^{2+}$ is necessary for normal blood coagulation, muscle contraction and nerve function; hypocalcaemia results in excitation of nerve and muscle cells leading to spasms, tetany and asphyxia
- Solubility of many Ca$^{2+}$ salts (phosphate, carbonate, sulphate, oxalate) is low...
  ...increases in Ca$^{2+}$ could lead to inappropriate precipitation (tissue mineralisation)
Ca$^{2+}$ regulation

Major Ca$^{2+}$ regulating hormones

- Parathyroid hormone (PTH)
- 1,25-dihydroxyvitamin D$_3$
- Calcitonin
Parathyroid hormone

- Raises plasma Ca\(^{2+}\) - *key minute-to-minute regulator*

- 84 amino acid polypeptide secreted by chief cells of parathyroid glands (amphibia upwards) into bloodstream in response to small falls in local Ca\(^{2+}\) concentration

- Pulsatile secretion

- Normal plasma concentration \(\leq 10-50\) pg/ml; plasma half life \(\leq 10\) min

- *(synthetic 1-34 peptide has full biological activity)*

- Actions mediated through PTH / PTHrP receptor - coupled to adenylate cyclase through Gs & to phospholipase C via Gq signalling proteins
Parathyroid hormone

*Increases plasma Ca$^{2+}$ by:*

- ↑ osteoclast formation (↑ RANKL) and resorptive activity *(direct & indirect actions)*
- ↑ Ca$^{2+}$ reabsorption in distal renal tubules
- ↑ 1,25(OH)$_2$D$_3$ production by stimulating the activity of the critical 1α- hydroxylase enzyme in kidney

- *PTH is also anabolic for bone when administered intermittently…*  
  … thought to involve suppression of sclerostin production by osteocytes  
  … not fully understood – *PTH does not directly promote bone formation by cultured osteoblasts*
PTH production is very sensitive to plasma $[Ca^{2+}]$.
Ca$^{2+}$ sensing receptor (CaSR)

- CaSR is a G-protein coupled receptor (GPCR)
- Molecular mechanism underlying Ca$^{2+}$ sensing by parathyroid chief cells and renal tubules
- CaSR acts as the body’s thermostat for Ca$^{2+}$ or ‘calciostat’
- Detects perturbations in the ionized Ca$^{2+}$ of only a few percent, leading to alterations in parathyroid function & PTH secretion that are designed to normalise plasma Ca$^{2+}$
Calcimimetics

- Synthetic agonist which activates Ca$^{2+}$ sensing receptor
- Treatment of hyperparathyroidism / hypercalcaemia
PTH directly stimulates resorptive function of human osteoclasts

PTH1R highly expressed on mature osteoclasts

• Parathyroid hormone – related protein

• Action very similar to PTH at receptor level (high homology in 1-34 region)

• Paracrine agent expressed by many normal cell types (not controlled by Ca\textsuperscript{2+} & CaSR)

• Also expressed by tumour cells – contributes to hypercalcaemia of malignancy

• Important developmental actions: required for cartilage differentiation, tooth eruption, normal development of mammary glands… etc
Vitamin D

- Vitamin D (cholecalciferol), a ‘seco-steroid’ has no biological activity

- 25-hydroxy vitamin D is main circulating metabolite (nanomolar range) - very low biological activity

- 1,25-dihydroxy vitamin D is active metabolite – circulating concentration \( \approx 10-50 \text{ pM} \)

- 1,25-dihydroxy vitamin D is classed as a steroid hormone – acts via nuclear receptor (VDR)

- Vitamin D metabolites are fat soluble – slower action than peptide hormones – not involved in minute-to-minute regulation of plasma \( \text{Ca}^{2+} \)
$1,25(OH)_2D$ - *actions*

- ↑ Gut $\text{Ca}^{2+}$ uptake
- ↑ Plasma $\text{Ca}^{2+}$
- ↑ OC recruitment, activity (↑ RANKL)
- ↓ OB proliferation; ↑ OB (and skin cell) differentiation

Required for normal matrix mineralisation. Acts mainly via promoting $\text{Ca}^{2+}$ uptake - and thus ensuring adequate local $\text{Ca}^{2+}$ supply; not much evidence for a direct effect on mineral deposition by normal osteoblasts

- deficiency → osteomalacia, rickets…
Ca\textsuperscript{2+} uptake / transport

- Ca\textsuperscript{2+} uptake from the intestine now thought to occur primarily via the epithelial TRPV6 Ca\textsuperscript{2+} transport channel (which is strongly upregulated by 1,25(OH)\textsubscript{2}D)

- Ca\textsuperscript{2+} reabsorption in the kidney occurs primarily via the epithelial TRPV5 Ca\textsuperscript{2+} transport channel (upregulated by 1,25(OH)\textsubscript{2}D and PTH)

- TRPV5 also expressed in osteoclast ruffled border


- 1,25(OH)\textsubscript{2}D also increases expression of intracellular Ca\textsuperscript{2+} binding proteins, esp. calbindin D 9K (old mechanism!)
Calcitonin

- 32 amino acid peptide hormone secreted by parafollicular ‘C’ cells in thyroid / ultimobranchial gland (elasmobranchs upwards)
- ↓ plasma Ca\(^{2+}\) in young / hypercalcaemic animals
- ↓↓ osteoclast resorptive function & recruitment
- Inhibits Ca\(^{2+}\) and PO\(_{4}^{3-}\) reabsorption by the kidney tubules
- ‘emergency’ hormone; not much effect in normal adults

Time-lapse sequence showing rapid inhibition of rat osteoclast motility in response to salmon calcitonin (Arnett & Dempster, Endocrinology, 1987)
PO$_4^{3-}$ regulation

- Plasma PO$_4^{3-}$ is less tightly regulated than Ca$^{2+}$ - normal range is ~0.8-1.5mM in adult humans

- PTH decreases PO$_4^{3-}$ reabsorption from proximal tubules of kidney – but this is compensated by enhanced 1,25-(OH)$_2$ vitamin D-mediated PO$_4^{3-}$ uptake from intestine

- FGF23 is the key circulating PO$_4^{3-}$-lowering hormone. Reduces PO$_4^{3-}$ reabsorption in proximal renal tubules and also decreases PO$_4^{3-}$ absorption from the intestine

- Calcitonin inhibits PO$_4^{3-}$ reabsorption in renal tubules

- PO$_4^{3-}$ stimulates PTH secretion from the parathyroids... mechanism not understood

- Is there a PO$_4^{3-}$ receptor (analogous to Ca$^{2+}$-sensing receptor)? … no real evidence at present
FGF-23

- Phosphaturic hormone; 251 amino acid protein, produced by bone (osteocytes)
- Identified in 2000 via gain-of-function mutations associated with autosomal dominant hypophosphatemic rickets
- Prior to discovery, it was hypothesised that a protein existed which performed the function of FGF23. This protein was known as phosphatonin
- Acts through several FGF receptor subtypes in concert with Klotho, a co-receptor for FGF23,

Review: Kuro-O M 2008 Trends Endocrinol Metab 19: 239-245
Klotho

- Klotho identified in 1997: affected / deficient mice show premature ageing and altered mineral homeostasis resulting in osteopenia
- Klotho is a type I transmembrane protein with β-glucuronidase activity in extracellular domain; expressed in kidney and parathyroids, co-localises with TRPV5
- Klotho is required for normal $\text{PO}_4^{3-}$ elimination
- Lowering blood phosphate levels by restricting dietary $\text{PO}_4^{3-}$ intake or by blocking vitamin D function in Klotho / FGF23-deficient mice rescues not only hyperphosphatemia but also many ageing-like phenotypes

*ie*, $\text{PO}_4^{3-}$ retention is toxic – and responsible for accelerated ageing…

Regulation of bone cell function by $\text{Ca}^{2+}$ & $\text{PO}_4^{3-}$

- $\text{Ca}^{2+}$ has a minor inhibitory action on osteoclasts at concentrations $>10$ mM (ie, 4 x higher than physiological)

- $\text{PO}_4^{3-}$ exerts a strong, reversible inhibitory action on osteoclast function at concentrations $>2$ mM (ie, close to physiological) \( (Yates \ et \ al, \ JBMR \ 1991) \)

- $\text{Ca}^{2+}$ & $\text{PO}_4^{3-}$ both promote mineral deposition by differentiated osteoblasts
Mineralisation

- $\text{Ca}^{2+}$ and $\text{PO}_4^{3-}$ regulation must be understood in relation to mineralisation

- Total body Ca content in adults is about 1000 g, of which 99% exists as hydroxyapatite [$\text{Ca}_{10}(\text{PO}_4)_6 (\text{OH})_2$] in the mineral phase of bone

- Mineralisation is a physicochemical process – but ‘managed’ by osteoblasts and osteocytes

- Release of mineral from bone largely under cellular control (osteoclasts)
TRAP stained calvarial bone
3 day culture - *control*
Resorption of calvarial bone stimulated by acidosis (pH 7.01)
Ca$^{2+}$ release stimulated by acidosis is blocked by salmon calcitonin.
Mineralisation

• Major function of 1,25(OH)$_2$D and PTH for the bone mineralisation process is to maintain [Ca$^{2+}$] x [PO$_4$$^{3-}$] solubility product in circulation / ECF in a supersaturated state (<4.5), resulting in passive mineralisation of the collagen matrix (osteoid) laid down by osteoblasts.

• **So why don’t all tissues mineralise?**

• ‘Vascular calcification - a passive process in need of inhibitors’ (Schinke & Karsenty, 2000)

• **Matrix Gla protein:** MGP $^{-/-}$ mice exhibit lethal arterial calcification and inappropriate calcification of cartilage (in mice); MGP expressed by vascular smooth muscle cells but not bone cells.

• **ASARM peptides** (Clemens Löwik)

• **Pyrophosphate**

• ATP & other nucleotide triphosphates...
ATP and UTP selectively inhibit bone mineralisation

- Control
- Mineral stained with Alizarin red
- Un-mineralised collagenous matrix

- 10 μM ATP / UTP
- Un-mineralised collagenous matrix only
- Organic matrix deposition unaffected
Differentiated osteoblasts express high levels of alkaline phosphatase (ALP) (which is selectively inhibited by ATP / UTP… evidence for P2Y<sub>2</sub> receptor involvement)
Pyrophosphate and alkaline phosphatase

Major function of ALP in bone is to hydrolyse pyrophosphate (PPI), a key inhibitor of mineralisation.

\[
\text{Pyrophosphate} \xrightarrow{\text{ALP}} \text{phosphate} + \text{phosphate} \rightarrow \text{enables mineralisation}
\]
Pyrophosphate: a potent inhibitor of mineralisation

- Inorganic pyrophosphate (PPi) in the low micromolar range inhibits mineralisation (Fleisch 1962 & 1966)
Pyrophosphate: a potent inhibitor of mineralisation

- PPi is generated from many phosphate-containing molecules, including ATP
- Members of the ecto-phosphodiesterase / pyrophosphatase (ENPP) family hydrolyse:
  \[ \text{NTP} \rightarrow \text{NMP} + \text{PPi} \]

Could PPi generated by hydrolysis of ATP & UTP contribute to inhibition of bone mineralisation?
Dual inhibitory action of ATP / UTP on bone mineralisation

1. Via P2Y₂ receptor
2. Via PPI generated by ENPPs

Mg\(^{2+}\) homeostasis

- Total adult body content of Mg\(^{2+}\) is \(~25\)g
- Mg\(^{2+}\) salts have similar solubility profiles to those of Ca\(^{2+}\)
- \(~66\)% of Mg\(^{2+}\) located in the skeleton as mineral deposits (phosphate, carbonate)
- \(~33\)% of Mg\(^{2+}\) in body is intracellular.... Mg\(^{2+}\) required for \(\geq 300\) biochemical reactions in the body
- \(\leq 1\)% of Mg\(^{2+}\) in the body exists in soluble form in the extracellular fluid
- Plasma concentration of magnesium (Mg\(^{2+}\)) also closely regulated (to about 0.85 mM); mechanisms involved still not well understood...
Mg$^{2+}$ homeostasis

- Ca$^{2+}$-sensing receptor (in parathyroids) also senses Mg$^{2+}$

- Mg$^{2+}$ involved in regulation of cardiovascular function; deficiency can cause cardiac arrhythmia and impaired control of blood pressure (and also impaired PTH secretion)

- TRPM6 channels (expressed in renal tubules) thought to be important for transport of Mg$^{2+}$

- The membrane protein GPCR6A is activated by Mg$^{2+}$ as well as other divalent cations (eg, Ca$^{2+}$, Sr$^{2+}$)

- Mg$^{2+}$ uptake from intestine not regulated by 1,25(OH)$_2$D

(see: Hoenderop & Bindels, Physiology 23: 32-40; 2008)
Key reading

  - Ch 1-7: Morphogenesis, structure & cell biology of bone
  - Ch 8-12: Skeletal physiology
  - Ch 13-18: Mineral homeostasis
  - Ch 19-25: Clinical evaluation of bone & mineral disorders
  - Ch 26-41: Disorders of serum minerals
  - Ch 42-58: Osteoporosis
  - Ch 59-66: Metabolic bone diseases
  - Ch 67-70: Cancer & bone
  - Ch 71-74: Genetic & developmental disorders
  - Ch 75-76: Acquired disorders
  - Ch 77-80: Extraskeletal calcification & ossification
  - Ch 81-82: Hypercalciuria & kidney stones
  - Ch 83-87: Dental biology
