Endocrinology – calcium

Rudolf Cardinal, 25 Oct 98

Functions of calcium

- **Structural.**
  Calcium hydroxyapatite, Ca_{10}(OH)_{2}(PO_{4})_{6}, is the main structural mineral of bone. Calcium also present in teeth and other connective tissue.

- **Chemical.**
  *Membrane stabilization.* Calcium ions adhere to fixed negative charges on the membranes of axons. Thus high external [Ca^{2+}] reduces the negative charge density on the outer membrane surface, increasing the gradient of the potential field within the membrane and thus raising the membrane potential at which the channels open. (Ask me to draw it, or see Aidley, 1989, p. 75.)
  *Alveolar surfactant.* Calcium ions (+surfactant apoproteins) speed the spread of dipalmitoyl lecithin.

- **Intracellular signalling and modulation of enzyme activity.**
  *Excitation-contraction coupling.* In cardiac muscle, calcium directly excites contraction and causes the plateau in the AP. In skeletal muscle, calcium acts via troponin.
  *Excitation-secretion coupling.* Triggers exocytosis by vesicle fusion: calcium interacts with vesicles to cause fusion to cell membrane. (Example – release of ACh at synapse.) Also responsible for synaptic potentiation causing post-tetanic facilitation.
  *Action potential.* ‘Slow’ calcium channels (voltage-gated Ca/Na channels) prevalent in cardiac & SM
  *Blood flow control.* Calcium causes vasoconstriction by stimulation of vascular SM.
  *Blood clotting.* Calcium required a) for conversion of prothrombin to thrombin; b) for polymerization of fibrin.
  *Ciliary movement.* Calcium necessary (for ciliary ATPase complex?)
  *As an intracellular secondary messenger.* Detected by calmodulin & variants.
  *Pinocytosis.* Requires calcium in ECF, which probably reacts with contractile filaments beneath coated pits to provide force for ‘pinching’ off.
  *Sperm function.* Alters sperm flagellum activity, probably alters acrosome membrane facilitating ovum penetration.

- **Other.**
  *Bile.* Calcium concentrated in bile by gallbladder – function?
  *Nutrition.* In milk.

The movement of calcium between bodily compartments


Controllable: 1. input to the pool by intestinal absorption
2. input to the pool by resorption of Ca^{2+} from the skeleton
3. output from pool by renal loss (the kidney actively reabsorbs Ca^{2+} and can regulate this)
4. output from pool by deposition of calcium in bone.

Plasma calcium

- **Calcium is an extracellular ion.** Cells work hard to exclude calcium, which is part and parcel of its action as an intracellular messenger. (If you didn’t exclude it, its presence wouldn’t convey a message.)

- **Plasma calcium:** total 2.5 mM, of which 44% as free Ca^{2+} ions, 11% complexed with bicarbonate/citrate/phosphate etc. – these two comprise “free” calcium. The remainder is “bound” – 31.5% to albumin, 13.5% to globulins.

- Changes in plasma proteins cause changes in total calcium (more protein, more total calcium) but no change in free [Ca^{2+}], because it is the free calcium that is regulated (just like many hormones).

- Calcium binds to plasma proteins, but so do protons (H^+). As you’d expect from basic equilibrium chemistry, the more protons around, the less calcium can bind to the protein, so the greater the free Ca^{2+} concentration – and vice versa. Therefore acidosis → hypercalcaemia, alkalosis → hypocalcaemia.

Bone

- The main calcium store is in bone.
- Bone is covered in fibrous periosteum.

- **Osteoblasts** in the deeper layers of the periosteum synthesize and release collagen to form an extracellular matrix, and calcium is precipitated onto this matrix in the form of hydroxyapatite, Ca_{10}(OH)_{2}(PO_{4})_{6}. Once the osteoblasts are embedded in the calcified matrix, they are called osteocytes.

- Precipitation of calcium phosphate depends on the product [Ca^{2+}] × [PO_{4}^{3-}]. When this exceeds the local solubility product, precipitation occurs (see Common Misconceptions below). Osteoblasts cause local alkalization (high pH), which favours calcium precipitation.

- **Osteoclasts** are large multinucleate cells that are responsible for bone demineralization. They release acid phosphatase, lowering the pH and favour calcium solubilization from the matrix.

- Bone also serves as a Ca^{2+} reservoir for rapid uptake and release. A layer of osteoblasts is separated from the underlying layers of osteocytes by a bone fluid compartment. Calcium stored here is kept from being transferred to hydroxyapatite crystals, so is readily accessible when required.

- **Bone-lining cells** (of osteoblast lineage) act as barriers separating interstitial fluids from the fluids percolating through the osteocyte and lacunar canalicular system. They can retract to allow osteoclasts access to the bone surface, they produce collagenase to prepare the bone surface for osteoclastic resorption, and they produce an osteoclast-stimulating protein.
**Parathyroid hormone (PTH)**

The parathyroid glands
There are normally four. They are tiny: each weighs 35–40 mg. They are located near the poles of the thyroid. Parathyroidectomy (i.e. lack of PTH) is lethal. They contain two types of cell: chief cells, which make PTH, and oxyphil cells whose function is unknown.

**Nature, synthesis, function, mechanism**
- PTH is an 84-amino acid polypeptide with a plasma half-life of 3–4 minutes.
- **PTH acts to increase plasma Ca\(^{2+}\) concentration.**
- PTH receptors exist in the kidney, on osteoblasts and on circulating lymphocytes (but never mind them). It activates adenylate cyclase.
- **In the kidney,** PTH (1) decreases phosphate reabsorption, promoting phosphate excretion; (2) promotes calcium reabsorption; (3) promotes formation of 1,25-DHCC.
- **On bone,** PTH causes osteoclasts to resorb bone mineral, releasing calcium into the plasma.
- The effects on bone are slightly complex. Osteoclasts do not have PTH receptors, so how are they stimulated to resorb bone? PTH acts on cells of osteoblast lineage (i.e. osteoblasts, osteocytes, bone-lining cells) and causes them to produce a product that stimulates osteoclasts (activating them, causing their precursors to differentiate and stimulating resorption itself). In addition, PTH causes retraction of bone-lining cells, inhibits osteoblastic function acutely and thus enhances the rate of bone turnover.
- In the long term, low concentrations of PTH stimulate both osteoblast and osteoclast differentiation – both destruction and formation of bone are required for *remodelling*. High concentrations of PTH favour osteoclast formation, favouring bone demineralization.

**Control of PTH secretion**
The only control mechanism is *plasma [Ca\(^{2+}\)].* High levels depress PTH secretion, low levels stimulate it. There is a “calcium sensor” on the chief cells (G-protein-coupled receptor).

**Calcitonin (CT)**
Calcitonin is a 32-amino acid peptide hormone made by parafollicular cells (“C cells”) of the thyroid gland.

- **Calcitonin acts to decrease plasma [Ca\(^{2+}\)].**
- **It is not essential.** No diseases are attributable to deficiency or excess. Its role is to “prevent hypercalcaemia” – without calcitonin, experimental animals given a calcium infusion cannot bring calcium levels down to normal as fast as normal animals can.
- **It inhibits osteoclasts,** directly, thus lowering blood [Ca\(^{2+}\)]. Its effects are via the cAMP pathway. Bone is the only important target site.
- Calcitonin is released in response to elevated plasma [Ca\(^{2+}\)], and release is also stimulated by gut hormones (inc. gastrin, CCK) – this may help to lower blood Ca\(^{2+}\) in anticipation of Ca\(^{2+}\) absorption. Calcitonin assists in moving calcium into bone after a meal, preventing postprandial hypercalcaemia.
- It may also be important in protecting the maternal skeleton from excessive demineralization in pregnancy – at least in ruminants!

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1 Also known as parathormone.
2 See Common Misconceptions for why this is important.
3 Despite this, hyperparathyroidism usually increases urinary calcium, simply because the plasma level is so high that the increase in filtered calcium overwhelms the increased reabsorption.
4 Inheritance of one defective gene for this sensor causes *familial hypocalciuric hypercalcaemia (FHH)* – chief cells can’t sense calcium, so behave as if calcium is low, so make too much PTH, so plasma calcium is raised and little calcium is excreted in the urine. Homozygosity is fatal in infants unless the parathyroids are removed.
5 It’s easy to get confused – parathyroid hormone is from the parathyroid gland, while calcitonin is from the parafollicular cells of the thyroid...
1,25-dihydroxycholecalciferol (1,25-DHCC, calcitriol)

Nature
- A secosteroid™ hormone derived from vitamin D.
- Ultraviolet light from sunlight can convert 7-dehydrocholesterol into cholecalciferol (vitamin D₃) in the skin. Vitamin D₃ is also present in some foods.
- Whatever the source, cholecalciferol is converted by a hepatic mitochondrial 25-hydroxylase to 25-hydroxycholecalciferol (25-HCC). This is then converted by a renal mitochondrial 1α-hydroxylase to 1,25-DHCC.
- The hormone binds to nuclear receptors and regulates gene transcription – upregulated proteins include calcium-binding proteins and proteins that regulate osteoblast and osteoclast function.

Functions
- Enhances the intestinal absorption of calcium.
- Deficiency causes rickets.
- In gut, calcium absorption is increased by (1) facilitated calcium binding to the brush border; (2) facilitated calcium pumping into the cell; (3) increased calcium pumping into the ECF.
- In kidney, tubular reabsorption of phosphorus is increased, and perhaps that of calcium.
- In bone, 1,25-DHCC acts to cause demineralisation – slightly unexpected? – perhaps to provide calcium and phosphate for new bone formation (i.e. remodelling).
- In addition, 1,25-DHCC acts synergistically with PTH on bone – PTH is less effective without 1,25-DHCC. A minor point.

Control
- Recall that low calcium stimulates PTH secretion. PTH stimulates the conversion of 25-HCC to 1,25-DHCC in the kidney by the mitochondrial 1α-OHase.

Other influences on calcium metabolism
- Glucocorticoid excess → osteoporosis. Glucocorticoids profoundly inhibit production of osteocalcin, the major non-collagenous bone protein.
- Androgens and oestrogens protect against osteoporosis (so it’s most common in post-menopausal women).
- T₄, T₃ and GH are needed for normal bone growth.
- Calcium is a dietary requirement.
- Dietary vitamin D or sunlight is required, to make 1,25-DHCC, so the dietary calcium can be absorbed.
- Physical stress is required for maintenance of normal bone calcium levels. Prolonged bed rest and weightlessness cause loss of bone mass.
- There are a host of other osteoclast-activating factors, e.g. interleukin-1.

Summary of effects

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<tr>
<th>bone</th>
<th>GI tract</th>
<th>kidney</th>
<th>plasma</th>
<th>plasma</th>
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<tr>
<td>PTH</td>
<td>↑ Ca²⁺ resorption; also remodelling</td>
<td>... acts via 1,25-DHCC...</td>
<td>↓ renal PO₄³⁻ reabsorption</td>
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<td>↑ renal Ca²⁺ reabsorption</td>
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<td>effects secondary to changes in plasma levels</td>
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<td>Calcitonin</td>
<td>↓ Ca²⁺ resorption</td>
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<tr>
<td>1,25-DHCC</td>
<td>remodelling – increased resorption and formation of new bone</td>
<td>↑↑ Ca²⁺ uptake</td>
<td>↑↑ Mg²⁺ and PO₄³⁻ uptake (weaker effect)</td>
<td>↑↑ Ca²⁺ reabsorption</td>
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6 secosteroid = a steroid in which one of the rings has been opened
Calcium pathology

I’m not attempting to list all diseases or go into any detail, merely provide an overview.

Some effects of hypo- and hypercalcaemia

- **Hypocalcaemia** causes increased neuromuscular excitability\(^7\); mental changes (irritability, depression, psychosis); abnormalities of cardiac conduction; lens calcification; intracranial calcification; prolonged cardiac QT interval; cardiac dilatation if severe; change in enzyme activities; increased cell membrane permeability generally; impaired blood clotting… Severe hypocalcaemia causes death by respiratory spasm, so PTH is essential for life.

- **Hypercalcaemia** causes renal damage (calcium phosphate precipitates and causes damage), kidney stones, delayed neuromuscular conduction and muscular paralysis, depression, short decreased heart QT interval, constipation & lack of appetite (probably because of depressed GI muscle contractility), nausea and vomiting, increased gastrin secretion (so more gastric acid, so peptic ulceration)…

Hyperparathyroidism

- Causes hypercalcaemia.
- **Primary hyperparathyroidism** is when a parathyroid lesion causes PTH hypersecretion. Due to an adenoma (75–80%), primary hyperplasia (10–15%) or parathyroid carcinoma (<5%).
- “Humoral hypercalcaemia of malignancy” is when a tumour makes something called PTH-related peptide (PTHrP), which has all the effects of PTH.
- **Secondary hyperparathyroidism** is a slightly misleading term. Plasma calcium is low, so the parathyroids make a lot of PTH. This is perfectly normal. For example, dietary calcium or vitamin D deficiency would cause this.

Hypoparathyroidism

- Causes hypocalcaemia.
- Usually due to surgical excision (thyroidectomy – whoops; thought they were a malignant lymph node – whoops, etc.). Can also be due to congenital absence, autoimmune disease etc.

Pseudohypoparathyroidism

- Extremely rare. Daft name.
- The PTH receptors are abnormal, so neither bone nor kidneys respond to PTH (which is obviously being made aplenty). The effect is hypocalcaemia.

Osteomalacia and rickets

- Due to a lack of vitamin D or a disturbance in its metabolism.
- In children, this causes rickets; in adults osteomalacia. Basically the same thing: osteoid (matrix) is formed but not properly mineralized. The bone is soft. In growing children, epiphyseal cartilage is not mineralized properly either, so characteristic skeletal deformity occurs.
- Particular risk group in Britain: Asian immigrants. Dark-skinned races living in a Northern country with little sunlight [though there’s more to it than this because Afro-Caribbeans have a lower incidence of rickets than Asians]; culturally inclined to cover up skin (esp. women); so rely more on dietary vitamin D; poverty exacerbates the problem of poor nutrition; chapattis contain phytates which reduce calcium absorption.

Osteoporosis

- Defect of matrix production. The bone that is present is normal, but there’s not enough.
- Age, postmenopausal women (oestrogens and androgens are protective against osteoporosis), glucocorticoid excess are perhaps the most important of a long list of causal factors.
- Very significant medical problem. Fractures of the femoral neck in the elderly (esp. women) are common. Up to 20% of orthopaedic beds in the UK are occupied by elderly women with hip fractures. Following a femoral neck fracture, up to 25% of patients are dead 6 months later\(^8\) and the majority are disabled.

\(^7\) Chvostek’s sign – tapping the facial nerve induces contractions of the muscles of the eyes, nose or mouth; Trousseau’s sign – occluding the circulation to the forearm induces carpal spasm. When hypocalcaemia is severe, tetany appears with muscular cramps and spasms, and convulsions.

\(^8\) Intracapsular fractures carry a 50% mortality! (Oxford Handbook of Clinical Specialties, 1995).
Common misconceptions

The relationship between calcium, phosphate and protons

People often get confused between the chemical and the physiological mechanisms that affect Ca\(^{2+}\) and PO\(_{4}^{3-}\) ion concentrations. The following two relationships are chemical:

1. Ca\(^{2+}\) and H\(^{+}\) ions both bind to anionic sites on proteins. Therefore increasing the concentration of one displaces the other from the protein into the free form. For example, adding acid will raise the free [Ca\(^{2+}\)].

2. Calcium and phosphate ions may combine to form calcium phosphate (a salt). In general, when a saturated solution of an electrolyte is in contact with undissolved electrolyte, the following equilibrium exists:

\[ AB_{\text{solid}} \leftrightarrow AB_{\text{dissolved}} \leftrightarrow A^{+} + B^{-} \]

and the equilibrium constant is \( K = [A^{+}][B^{-}]/[AB_{\text{dissolved}}] \). Since there is also an equilibrium between solid and dissolved undissociated \( AB \), the concentration of the latter may be considered to remain constant, so

\[ [A^{+}][B^{-}] = K_{sp} \]

where \( K_{sp} \) is the solubility product of the electrolyte. It can be seen that if an excess of \( A^{-} \) or \( B^{+} \) ions are added to the solution, the \( K_{sp} \) is exceeded and the salt will be precipitated to restore the equilibrium. Remember that this equilibrium only holds when the solution is saturated.

Now, it happens that extracellular calcium phosphate is normally near its solubility limit (i.e. is almost a saturated solution). The solubility product is obviously \([Ca^{2+}][PO_{4}^{3-}]\). Since this is the case, a rise in phosphate favours precipitation of calcium phosphate – deposition in bones and precipitation as crystals in soft tissues – which will "use up" calcium and depress free calcium levels.\(^9\)

Note also that renal excretion of phosphate lowers the solubility product \([Ca^{2+}][PO_{4}^{3-}]\), which enhances the ionization of calcium (i.e. removing \( B^{-} \) leads to more salt being dissolved to restore the equilibrium, which leads to increased \( A^{-} \)). This should make sense in the context of the effects of PTH.

3. Acidity increases the solubility of calcium. This is why osteoblasts alkalinize their environment to precipitate calcium, and osteoclasts acidify it to dissolve the mineral.

The other relationships are physiological. The most important to remember is the following:

4. Low [Ca\(^{2+}\)] stimulates PTH production, causing phosphaturia and a tendency to hyperphosphataemia due to the action of PTH on bone. The phosphaturia is the more powerful effect, so PTH tends to lower phosphate. Therefore calcium and phosphate tend to vary in the same direction. (This is only untrue in two cases. The first is when there is inappropriate excess or deficiency of PTH, when they vary in opposite directions: for example, rather than low calcium \( \rightarrow \) PTH secretion \( \rightarrow \) low phosphate, you get excess PTH secretion \( \rightarrow \) high calcium and low phosphate. The second is when kidney function is impaired enough to impair the phosphaturic effect of PTH.)

Osteomalacia and osteoporosis

- Osteomalacia is a disease of bone mineralization. The matrix is present, but calcium hydroxyapatite is not deposited on it. The bone is soft. Caused by vitamin D deficiency. In children, it is called rickets and causes limb deformity.
- Osteoporosis is a disease of bone matrix. The matrix is properly mineralized, but there isn’t enough of it. The bone is weak and brittle. Seen especially in post-menopausal women (hip fractures…) and patients given glucocorticoids.

\(^9\) The Penguin Dictionary of Chemistry and Hladky & Rink (1986), Body Fluid and Kidney Physiology, contributed to this explanation! The principles apply to other salts, not just calcium phosphate – you will encounter precipitation again when you come to look at kidney stones and gallstones, so it’s worth understanding it now.