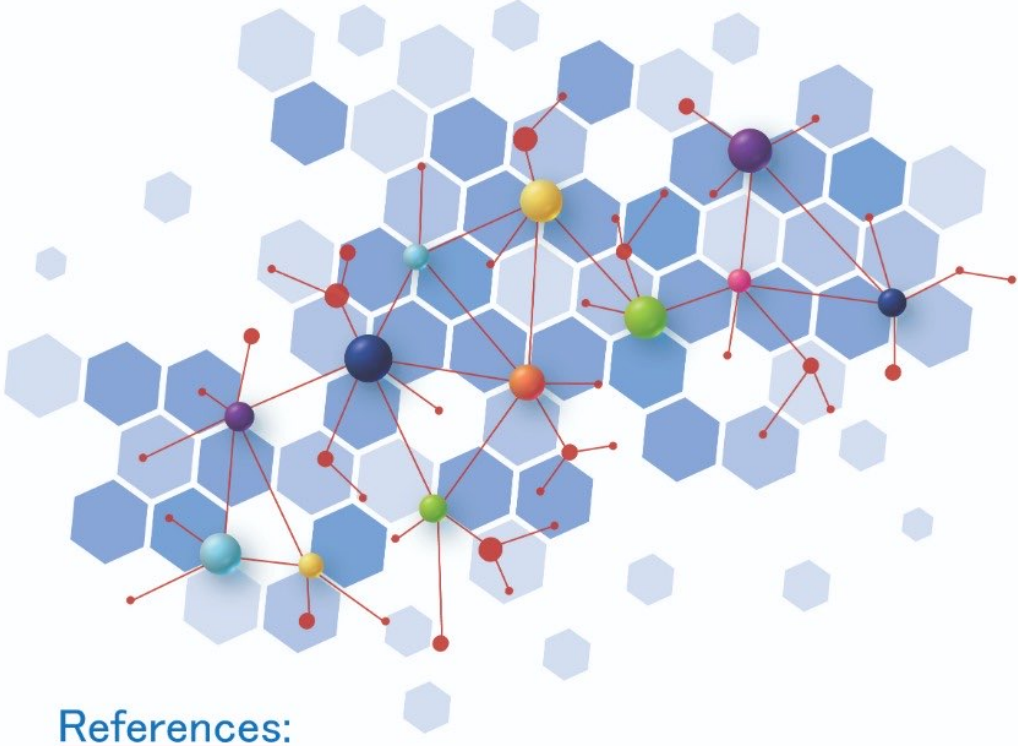
Clinical biochemistry

Done by your colleague: Mo'ath E Bani Salem



References:

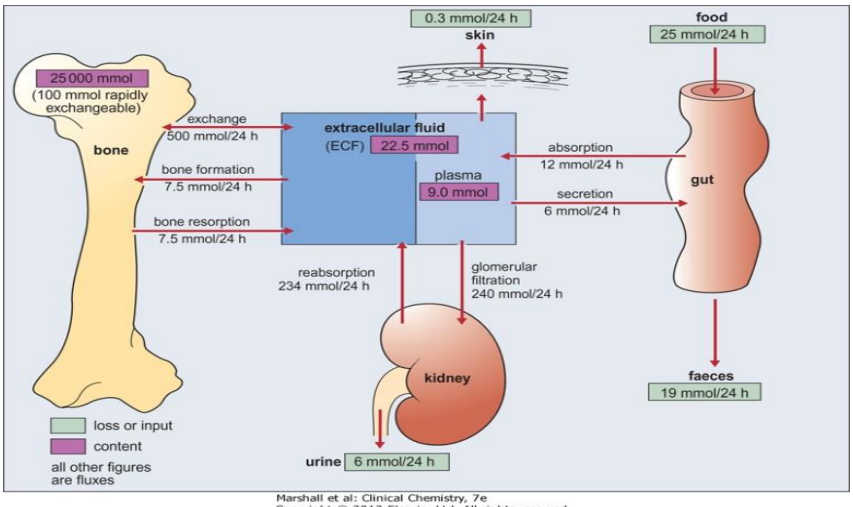
- Clinical biochemistry book 13th edition.
- Doctor's slides and records.
- Past years questions.

Version: 1

Lecture #: #9: calcium, phosphate & magnesium

Calcium, phosphate & magnesium

- Ca^{2+} is the most abundant mineral in the body
- 99% is present in the bone.
- Important for muscle contraction → hypocalcemia can cause tetany



❖ Ca^{2+} homeostasis:

- Bone isn't metabolically inert
- Most Ca^{2+} in bones are stable
- About 500 mmol/dl of Ca is movable → moves between bone & ECF to support calcium homeostasis
- About 7.5 mmol/dl moves between the stable pool(bone) and ECF → this process is called **bone remodeling**
- **bone remodeling** involves 2 processes:
  - bone formation**:** Ca^{2+}contribute to build bones by osteoblasts cells
  - bone resorption: Ca^{2+} is resorbed from bone to plasma by osteoclasts
- **note:** the active form of Ca^{2+} (used by the body) is the plasma component which is free (unbound with other molecules) → usually 9 mmol/dl
- **external Ca^{2+} balance:**
  - 25 mmol daily is taken from diet (absorped from Gi tract)
  - 6 mmol is secreted to Gi tract
  - 6 mmol is excreted in renal system
  - Very minor amount of Ca^{2+} is eliminated from the skin

❖ function of Ca^{2+}:

- Bone and teeth formation
- Neuromuscular excitably:
  - Contraction of skeletal muscles by motor neurons through secretion of neurotransmitters
  - **hypocalcemia** can cause **tetany**
- Enzymatic activities
- Signaling inside the cells (part of second messenger system)
- Activates multiple coagulation factors

Functions of calcium

Function	Example
structural	bone teeth
neuromuscular	control of excitability release of neurotransmitters initiation of muscle contraction
enzymic	coenzyme for coagulation factors
signalling	intracellular second messenger

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❖ Bone:

- Consists of:
  - **osteoid** (collagenous organic matrix)
  - **hydroxyapatites**: complex inorganic hydrated Ca^{2+} salts **($Ca_{10}(PO_4)_6(OH)_2$)**
- biologically active with **continuous** turnover or **remodeling**
- **Remodeling** is controlled by hormones, growth factors & cytokines

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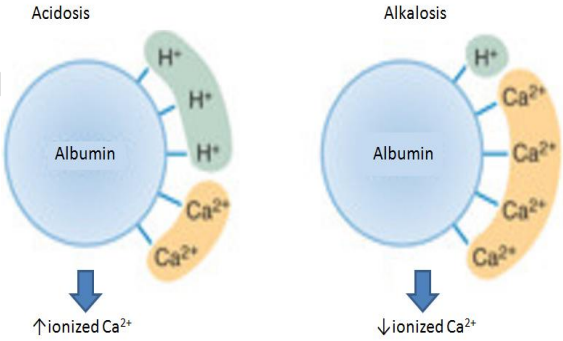
- **osteoclasts** cause bone **resorption**
- **osteoblasts** cause bone **formation**
- osteoblasts require adequate **Ca^{2+}** and **phosphate** for **hydroxyapatites** and **osteoid synthesis**
- once osteoid is formed → hydroxyapatites are added n a process called **mineralization**
- **Alkaline phosphatase**:
  - secreted by **osteoblasts**
  - essential for **bone formation**
  - works by releasing phosphate from pyrophosphate in alkaline media**.**
  - Maybe elevated in children (period of bone growth)
- Defective mineralization of **osteoid** results in diseases with low bone density**:**
  - **rickets** disease: if occurred in children
  - **Osteomalacia**: in adults

- bone is an important **reservoir** of Calcium & phosphate.

❖ plasma Ca^{2+}

- normal **plasma Ca^{2+}** levels: 2.25-2.6 mmol/L
- **three forms of Ca^{2+} in the plasma:**

1) non-diffusible (46%):
   a. bound to protein (mainly albumin).
2) Diffusible:
   a. Complexed with citrate & phosphate (7%).
   b. **Free ions (Ca^{2+})**: 47%
      i. the **physiologically active form**
      ii. **the amount available for use by cells…etc.**
      iii. **when decreased → hypocalcemia**
      iv. **when increased → hypercalcemia**
      v. is the concentration that is **maintained by homeostatic mechanisms.**



❖ Effect of **alkalosis** & **acidosis** on ionized Ca^{2+}:

- Albumin protein can bind both Ca^{2+} & H^+
- When there is **acidosis→ more H^+** in the blood → more H^+ binds to albumin instead of Ca^{2+}→ Ca^{2+} detaches from albumin → **increase free Ca^{2+}** concentration → **HYPERCALCEMA**!
- When there is **alkalosis→ less H^+** in the blood → less H^+ binds to albumin instead of Ca^{2+}→ more Ca^{2+} binds to albumin → **decrease free Ca^{2+}** concentration → **HYPOCALCEMA**!

❖ Measurement of Ca^{2+}:

- **total calcium:**
  a. most frequently used
  b. satisfactory for most clinical purposes.
- Plasma **ionized calcium:**
  a. measured using an ion-selective electrode but is **inconvenient** method
  b. Most beneficial to measure

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- Changes in **plasma albumin concentration** → affect total calcium concentration **independently** from ionized calcium concentration leading to **mis-interpretation** of results in both hypoproteinemic & hyperproteinemic states.
  - When there is **hyperproteinemia**:
    - More albumin bind with Ca → less free Ca and more Ca bound to albumin
    - Less free Ca → hypocalcemia →parathyroid hormone increases → more reabsorption of Ca from Gi / kidneys → normal free Ca levels but **increased total Ca levels**!
  - When there is hypoproteinemia:
    - Normal free Ca with low total Ca levels

Plasma concentration	Normal [albumin]	Low [albumin]	High [albumin]
[total calcium]	Normal	Low	High
[free calcium]	Normal	Normal	Norma
[albumin-bound calcium]	Normal	Low	High

- **formula** to correct calcium concentration if there is high or low plasma albumin concentration (see the table):
- when albumin concentration is less than 40→ corrected Ca levels:
  - corrected Ca= Ca + .02 * (40- albumin)
- when albumin concentration is more than 45→ corrected Ca levels:
  - corrected Ca= Ca- .02* (albumin -45)

Calculation of 'corrected' plasma calcium concentration

If plasma albumin concentration is [alb] g/L and measured total calcium is [Ca] mmol/L

for [alb] <40, corrected calcium =
[Ca] + 0.02 × {40 – [alb]} mmol/L

for [alb] >45, corrected calcium =
[Ca] – 0.02 × {[alb] – 45} mmol/L

for example: [Ca] = 1.82 mmol/L
[alb] = 28 g/L
'corrected' calcium = 1.82 + 0.02 x (40-28) = 2.06 mmol/L

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❖ **causes of total Ca misinterpretation:**

- hypo/ hyper - proteinemia
- **appearent hyperproteinemia:**
  - increase proteins concentration without increasing the amount
  - causes by **venous stasis** → stasis of blood → fluids gets out of vessels → increase concentration of proteins → increase total calcium
- **Multiple Myeloma:**
  - Cancer of plasma cells → secrete huge amounts of **γ-globulin** proteins
  - increase total calcium concentration.
  - Note: globulins bind calcium less than albumin

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Calcium-regulating hormones:

- Calcium concentration in the ECF is normally maintained **within narrow limits** by a control system
- Hormones affecting Calcium:
  1. **Parathyroid hormone** (**PTH**)
  2. **Calcitriol** (1,25-dihydroxycholecalciferol): active form of vitamin D
  3. **Calcitonin** (only a minor role)

1) Parathyroid hormone (PTH):

- Secreted by the parathyroid glands in response to **a fall in plasma ionized (free) calcium** concentration.
- **Hypocalcemia increases PTH secretion**
- **Hypercalcemia inhibits PTH secretion.**

❖ **PTH functions:**

- PTH **increases plasma [calcium]** & **reduces plasma [phosphate]:**
- PTH **mobilizes Ca^{2+} from the bone** in 2 phases:
  - **Rapid** phase involves exciting **osteocytes**.
  - **Longer-term** phase involves exciting **osteoclasts**.
    - **Bone resorption → increase plasma Ca^{2+}**
- **Effects on kidneys:**
  - Increase Ca reabsorption
  - promotes **phosphaturia (**phosphate excretion**)** by decreasing the reabsorption of filtered phosphate in the kidneys
  - stimulates formation of **calcitriol** (active Vitamin D) by activating the enzyme (**1 alpha hydroxylase**) in kidneys responsible for activating vitamin D
    - **1 alpha hydroxylase** converts 25-hydroxycholecalcferol into 1,25-dihydroxycholecalciferol
    - **Vitamin D** increases the reabsorption of calcium and phosphate from GI tract
- **Decrease bicarbonates reabsorption** → may cause **metabolic acidosis**

❖ Notes about PTH:

- changes in **phosphate** concentration **do not** affect PTH secretion unless free Ca changed
- **Mild hypomagnesaemia** stimulates PTH secretion because Mg^{2+} is similar to Ca^{2+}
- more **severe** hypomagnesaemia reduces PTH because the secretion of PTH depends on **Mg^{2+}**.
- Patients with **primary hyperparathyroidism**:
  - Very high levels of parathyroid hormones → hypercalcemia
  - Hypercalcemia→ more filtration → hypercalciuria despites elevated PTH
  - Hyperphosphaturia
  - Bone pain due to excessive bone resorption

Actions of parathyroid hormone

Target organ	Action	Effect
bone	rapid release of calcium ↑ osteoclastic resorption	↑ plasma [Ca^+]
kidney	↑ calcium reabsorption ↓ phosphate reabsorption ↑ 1α-hydroxylation of 25-hydroxycholecalciferol	↑ plasma [Ca^+] ↓ plasma [Pi] ↑ calcium and phosphate absorption from gut
	↓ bicarbonate reabsorption	acidosis

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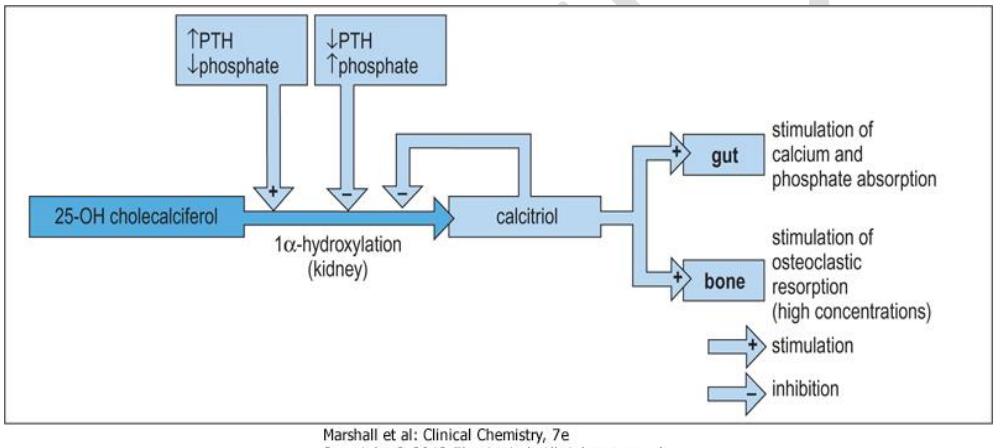
2) Calcitriol (active vitamin D):

- Calcitriol inhibits PTH secretion.
- Synthesized from cholesterol → UV light from the sun causes conversion to cholecalciferol→25-hydroxylation occurs in the liver → 25-hydroxy-cholecalciferol reaches the kidneys for activation by the enzyme 1-hydroxylase → **Calcitriol** (1,25-dihydroxycholecalciferol) is produced
- Important for bone health and mineralization by increasing reabsorption of **Ca and phosphate** from intestines (gi system)
- In very high concentrations → vitamin D activates osteoclasts → bone resorption!
- In low concentration → inhibits osteoclasts
- vitamin D binds with osteoblasts and increase the levels of **alkaline phosphatase & osteocalcin** (Ca-binding protein)
    - when plasma levels of alkaline phosphatase & osteocalcin are **elevated** → this indicate more **bone formation (active osteoblasts)**

❖ Notes:

- **PTH activate** 1 alpha hydroxylase in kidneys
- **Phosphate inhibits** 1 alpha hydroxylase
- **hypophosphatemia** → calcitriol secretion is increased



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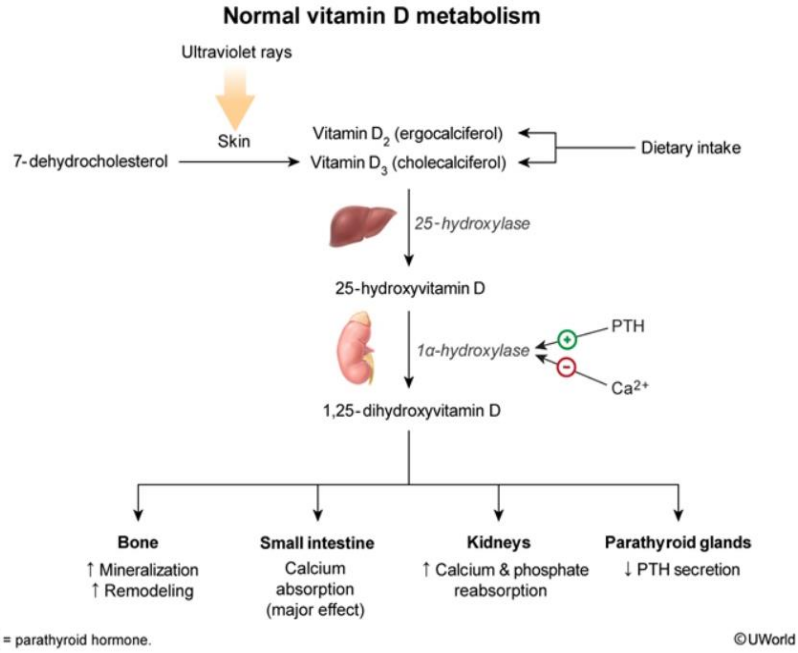

3) **Calcitonin:**

- Secreted from **thyroid** gland in response to hypercalcemia.
- Reduces the levels of calcium
- inhibits osteoclast activity → decreases bone resorption.
- decreases renal reabsorption of calcium & phosphate.
- Weak hormone but opposes PTH
- Calcitriol has much smaller effect on renal Ca^{2+} reabsorption than PTH.
- In case of **hypophosphatemia**:
    - Low phosphate → increase the activity of 1 hydroxylase → more vitamin D activation
    - Vitamin D reabsorbs Ca &phosphate from G.tract
    - This results in normal phosphate levels but hypercalcemia
    - Hypercalcemia inhibits PTH secretion → increase Ca excretion in urine → normal Ca levels

❖ **Hypercalcemia**:

- often clinically silent & discovered incidentally when calcium is measured as part of biochemical profile.
- 90% of the cases are caused by **primary hyperparathyroidism** & **malignancy**.

Malignant disease:

- Very common cause of hypercalcemia.
- Ways of causing hypercalcemia:
    - with **metastasis to bone**
        - no relationship between extent of metastasis & severity of hypercalcemia.
    - without metastasis to bone but through secretion of **PTH-related peptide (PTH-rP)** from the tumor (**ectopic**).
        - Called **paraneoplastic syndrome**

Hypercalcaemia

Causes	Clinical features
Common	weakness, tiredness, lassitude, weight loss and muscle weakness
malignant disease, with or without metastasis to bone	mental changes (impaired concentration, drowsiness, personality changes, coma)
primary hyperparathyroidism	anorexia, nausea, vomiting and constipation
Less common	abdominal pain (rarely peptic ulceration and pancreatitis)
thyrotoxicosis	polyuria, dehydration and renal failure
vitamin D intoxication	renal calculi and nephrocalcinosis (mainly associated with primary hyperparathyroidism)
thiazide diuretics	short QT interval on ECG
sarcoidosis	cardiac arrhythmias and hypertension
familial hypocalciuric hypercalcaemia	corneal calcification and vascular calcification
renal transplantation (tertiary hyperparathyroidism)	there may also be features of the underlying disorder, such as bone pain in malignant disease and hyperparathyroidism
Uncommon	
milk–alkali syndrome	
lithium treatment	
tuberculosis	
immobilization (especially in Paget's disease)	
acute adrenal failure	
idiopathic hypercalcaemia of infancy	
diuretic phase of acute renal failure	

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- **Primary hyperparathyroidism:**
  - Prevalence is 1:1000 person.
  - due to **parathyroid adenoma**.
  - **findings:**
    - ↑PTH
    - ↑plasma [Ca^{2+}]
    - ↑plasma [H^{+}] because:
      - decrease bicarbonate reabsorption
      - more calcium binds with albumin instead to H → increase H → metabolic acidosis
    - ↑alkaline phosphatase
    - ↓ plasma [phosphate].
  - **Symptoms:**
    - Weakness // fatigue // nausea &vomiting
    - Abdominal pain
    - Polyuria
    - Post-renal ARF due to renal calculi
    - Arrhythmias
  - **Treatment:**
    - Surgery to remove the adenoma when
      1. Plasma [Ca^{2+}] > 3 mmol/L.
      2. Marked hypercalciuria → stone formation
      3. Osteoporosis
      4. Age less than 50 years
    - High fluid intake to discourage renal calculus formation
  - **Complications:**
    - calculus formation
    - osteoporosis
    - renal impairment

Note: Although PTH increases Ca^{2+} reabsorption in kidneys, there is **hypercalciuria** because there is a huge amount of Ca^{2+} in blood→ increased filtered Ca^{2+} → kidneys cant reabsorb all Ca^{2+} → calcium appears in urine → **hypercalciuria**

- **Secondary hyperparathyroidism:**
  - Occurs secondary to other causes that decrease blood **free** calcium concentration
  - **Chronic renal failure & vitamin D deficiency** → decreased synthesis of calcitriol → **hypocalcemia** →increase in PTH
  - The increase in PTH may not normalize plasma Ca^{2+} because in the absence of calcitriol, there is resistance to the Calcium-mobilizing effect of PTH on bone.
  - Treatment:
    - Vitamin D and calcium supplements

- **Tertiary hyperparathyroidism:**
  - excessive secretion of PTH after a long period of secondary hyperparathyroidism due to end stage kidney disease
  - tertiary hyperparathyroidism usually causes **hypercalcemia**!
  - **autonomous** (unregulated) parathyroid function following a period of persistent increase in parathyroid stimulation.

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- So; patients with **end-stage renal disease** become hypercalcemic because of the development of autonomous PTH secretion resulting from the prolonged hypocalcemic stimulus.

- **↑plasma [phosphate]** due to **end-stage renal disease** (no filtration at all)

❖ **Case scenario:**

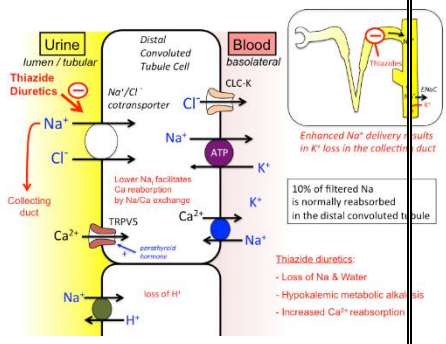
- A 51- year old woman was investigated after 2 episodes of ureteric colic. The stones were composed of calcium. She complains of constipation.
- Labs: adjusted Ca: 2.95 (high) // phosphate: 0.7 (low) // HCO3-: 19 (low) // PTH: 18 (high) // 24 urine calcium: 10.8 (high)
- Explanation:
  - The high concentration of Ca and PTH with suggests **primary hyperparathyroidism** that caused the abnormal labs findings

Other causes of hypercalcemia:

❖ Thyrotoxicosis:
  - Excess thyroid hormones → increase osteoclastic activity → more Ca release from bones

❖ Milk alkali syndrome:
  - Excess ingestion of milk and antacids for dyspepsia (peptic ulcer disease) → increase Ca reabsorption vie unknown mechanisms

❖ **Thiazides diuretics:**
  - Increase Ca reabsorption from distal renal tubules → mild hypercalcemia
  - Note: the image here not for memorization



❖ Chronic lithium therapy:
  - Increase PTH secretion

❖ Sarcoidosis:
  - A granulomatous disease that results in granuloma formation (type of chronic inflammations)
  - Granuloma contains activated macrophages that contain excessive levels of 1 alpha hydroxylase enzyme→ increase activation of vitamin D outside the kidneys→ hypercalcemia

❖ Immobilization:
  - Decrease stimulus for bone formation → bone resorption predominates → hypercalcemia

Treatment of hypercalcemia

- Always treat the underlying cause of hypercalcemia
- Lower Ca levels especially when patients are symptomatic
- If patients are dehydrated → iv saline to prevent renal stones and decrease Ca concentraton
- **Bisphosphonates**:
  - Decrease the activity of osteoclasts
  - Decrease the risk for osteoporosis
  - Decrease Ca levels
- If Bisphosphonates aren't effective:
  - Calcitonin
  - Steroids
- If life threatening hypercalcemia (>3.4 mmol/L) or severe symptoms → **dialysis**
- If the cause is primary hyperparathyroidism → **surgery** (parathyroidectomy)
- **Calcimimetic drugs** (**cinacalcet**):
  - These drugs are agonists at Ca sensing receptors on parathyroid gland→ suppress PTH secretion.

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