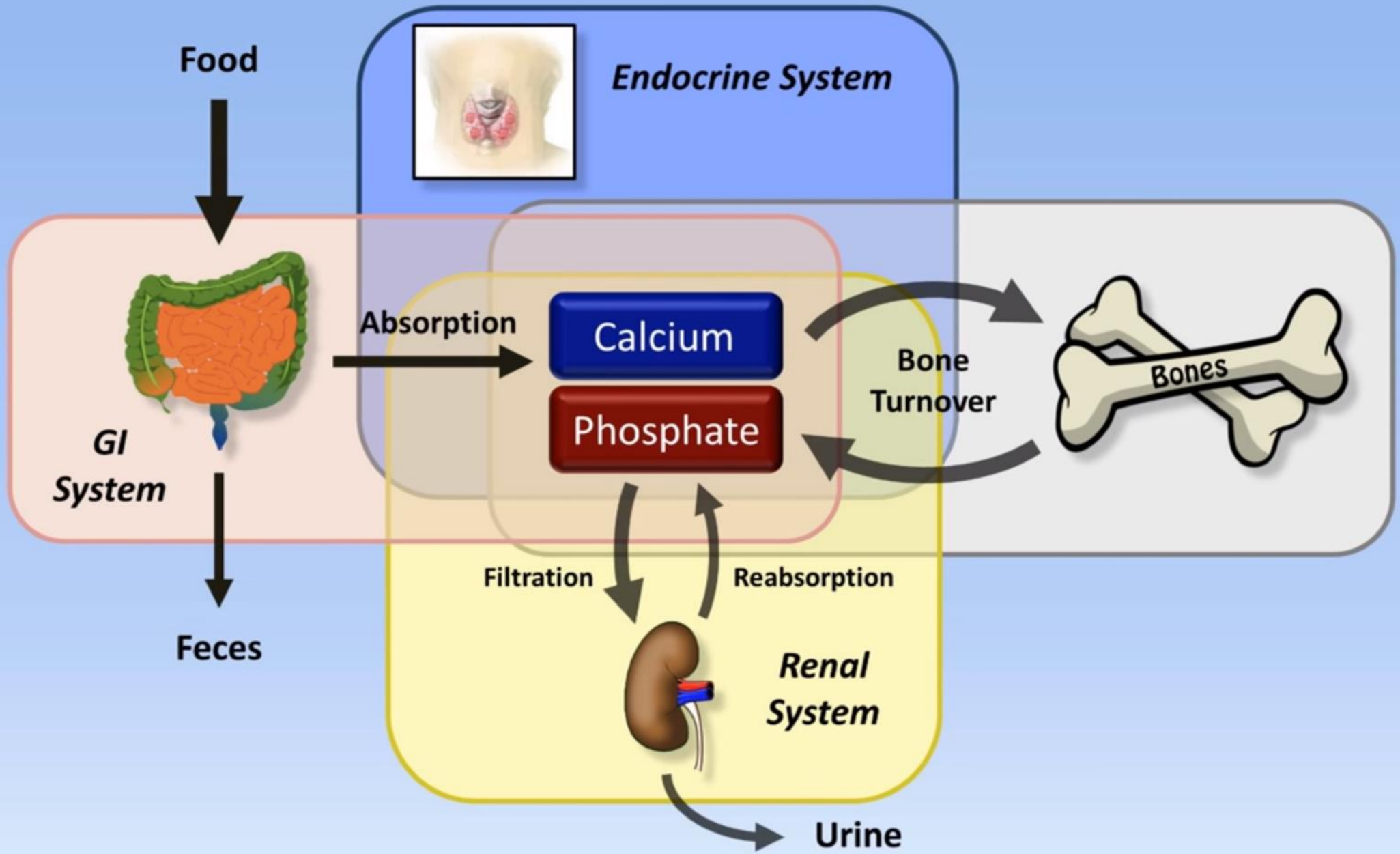
Calcium and Phosphate Disorders

Normal Physiology

Calcium Phosphate Homeostasis



Different Forms of Calcium

- Most of the calcium in the body is stored in the bones as hydroxyapatite.

$Ca_{10}(PO_4)_6(OH)_2$

- Calcium in the plasma:
  - 45% Free ionized form ← Physiologically active
  - 45% Bound to proteins (predominantly albumin)
  - 10% Complexed with anions (e.g. citrate, sulfate, phosphate)

} Typically measured in routine blood tests.

$$[\text{Calcium}]_{\text{corrected}} = [\text{Calcium}]_{\text{measured}} + \left(0.8\,(\,4 - [\text{albumin}])\right)$$

Correcting total serum calcium for hypoalbuminemia

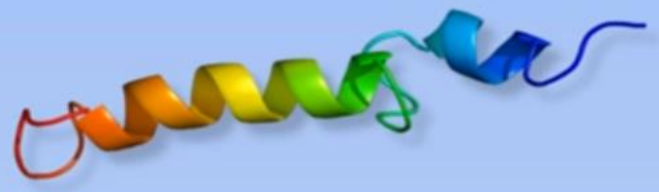
Different Forms of Phosphate

- Most of the phosphate in the body is also stored in the bones as hydroxyapatite.
- Most of the remainder of the body's phosphate is intracellular, as a component of phospholipids in cell membranes, DNA and RNA, and ATP and ADP.
- The small fraction of phosphate that is in the serum exists as circulating phospholipids, and inorganic phosphate.
- Inorganic phosphate consists of HPO_4^{2-} and $H_2PO_4^-$ in a 4:1 ratio at pH 7.40.

Physiologically active ***and*** what is typically measured in routine blood tests.

Major Mediators of Calcium and Phosphate Balance

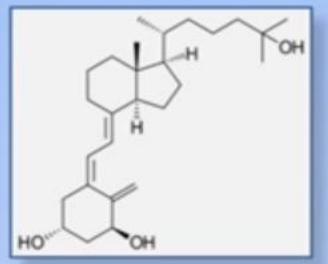
Parathyroid Hormone (PTH)



- 84 amino acid polypeptide
- Produced by parathyroid glands
- Net effect: ↑ serum calcium
  ↓ serum phosphate

Calcitriol (active vitamin D)

- From diet or a cholesterol-derived precursor with help from UV light.



- Requires enzymatic steps in the liver and kidney to become active.
- Net effect: ↑ serum calcium
  ↑ serum phosphate

Actions of Hormones Involved in Calcium Phosphate Homeostasis

GI Tract

Calcium, Phosphate

Calcitriol

+

Blood

Calcium, Phosphate

PTH

-

Calcium, Phosphate

PTH, Calcitriol

+

(Calcitonin)

-

Bones

Calcium, Phosphate

(Glomerular Filtration)

PTH

+

-

Calcitriol

+

+

-

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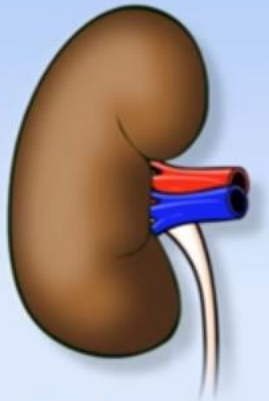
Calcium

Phosphate



Kidneys

Net Effect

PTH: ↑ calcium, ↓ phosphate

Calcitriol: ↑ calcium, ↑ phosphate

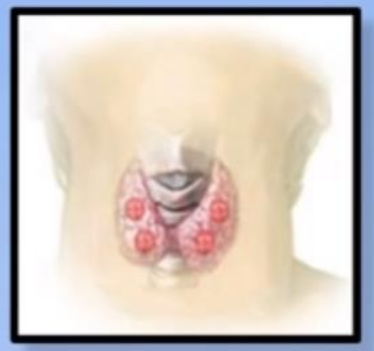
Electrolytes

Hormones

Physiologic Effects

Synthesis and Regulation of PTH

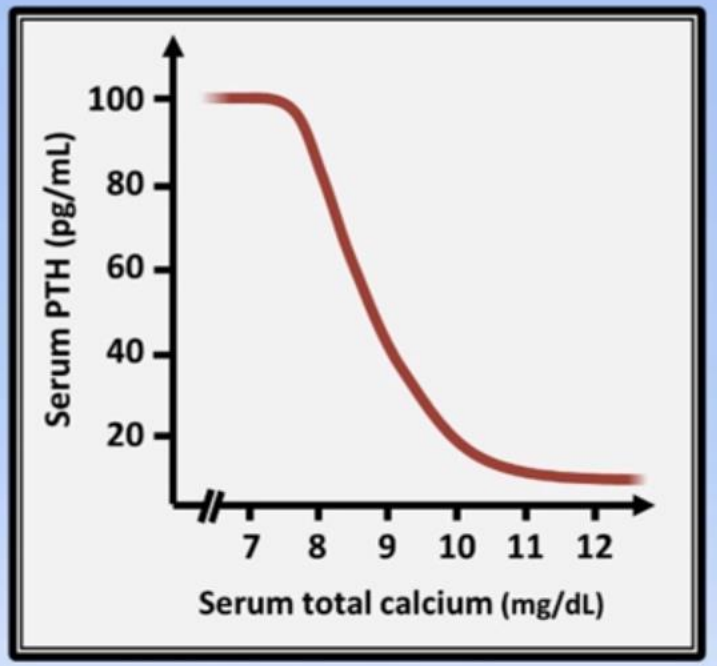
- PTH is synthesized and secreted by the chief cells of the parathyroid gland.



- PTH secretion is primarily regulated by serum calcium:

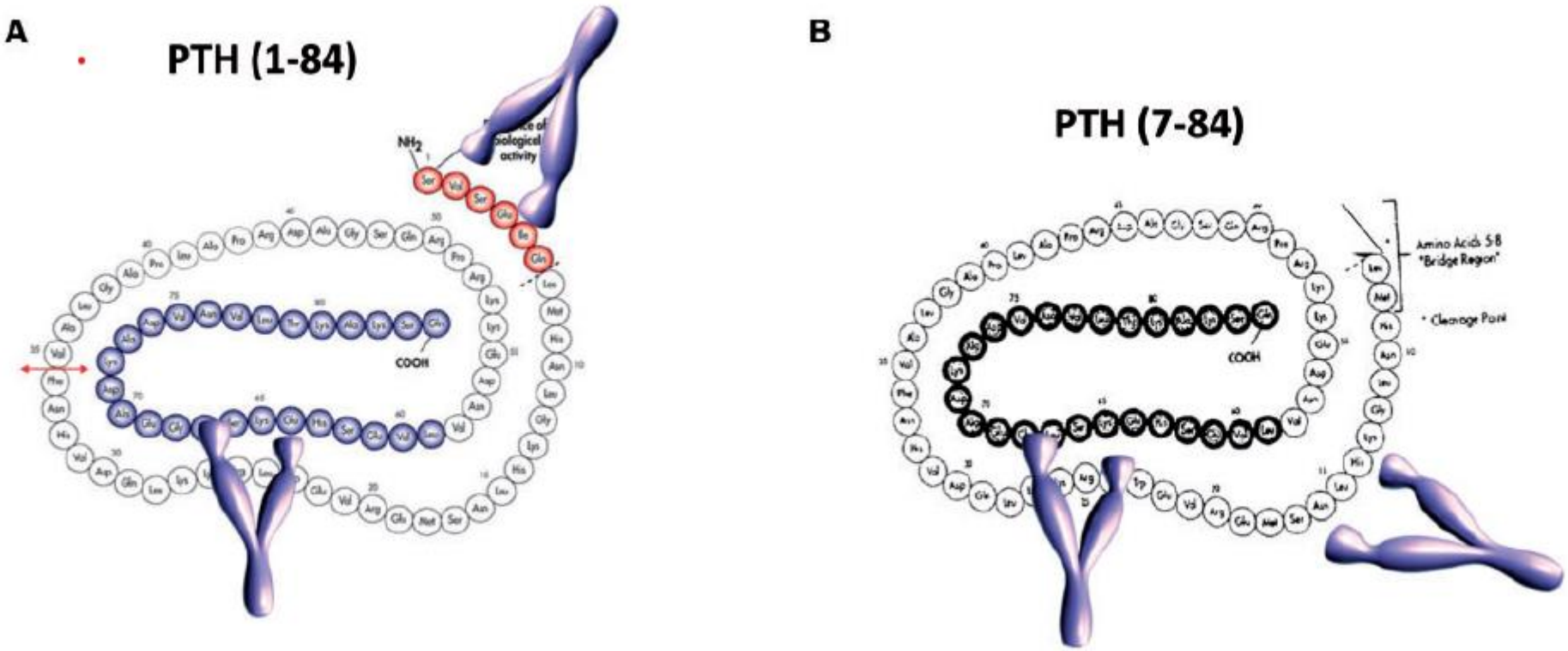
↑ calcium ⟶ ↓ PTH

↓ calcium ⟶ ↑ PTH

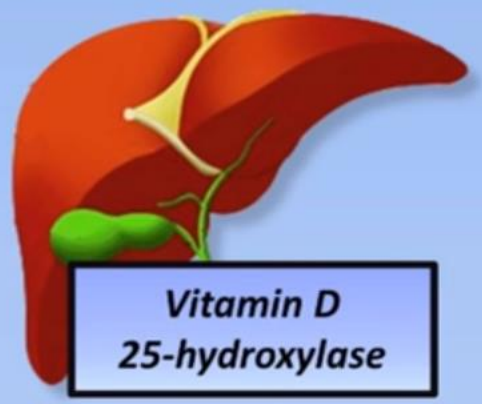


- In normal physiologic conditions, magnesium plays a parallel role to calcium. However, with significant magnesium depletion, there is ↓ PTH secretion and PTH resistance, leading to hypocalcemia.

PTH assays


A
PTH (1-84)
B
PTH (7-84)
COOH
Amino Acids 5-8
"Bridge Region"
Cleavage Point

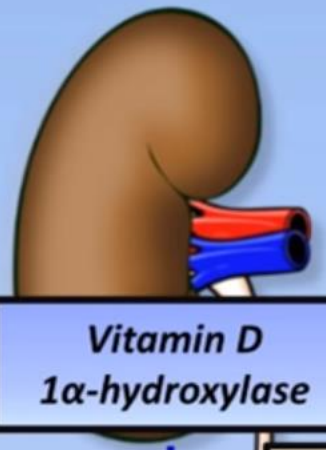
Synthesis and Regulation of Calcitriol



Vitamin D 25-hydroxylase

↓ Serum phosphate

PTH



Vitamin D 1α-hydroxylase

↑ Serum phosphate

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7-Dehydrocholesterol

Cholecalciferol (Vitamin D_3)

25(OH) Cholecalciferol (Calcidiol)

1,25$(OH)_2$ Cholecalciferol (Calcitriol)

Active Vitamin D

Natural sources (e.g. fish)

Supplementation (In US)

Ergocalciferol (Vitamin D_2)

Dietary Sources (few)

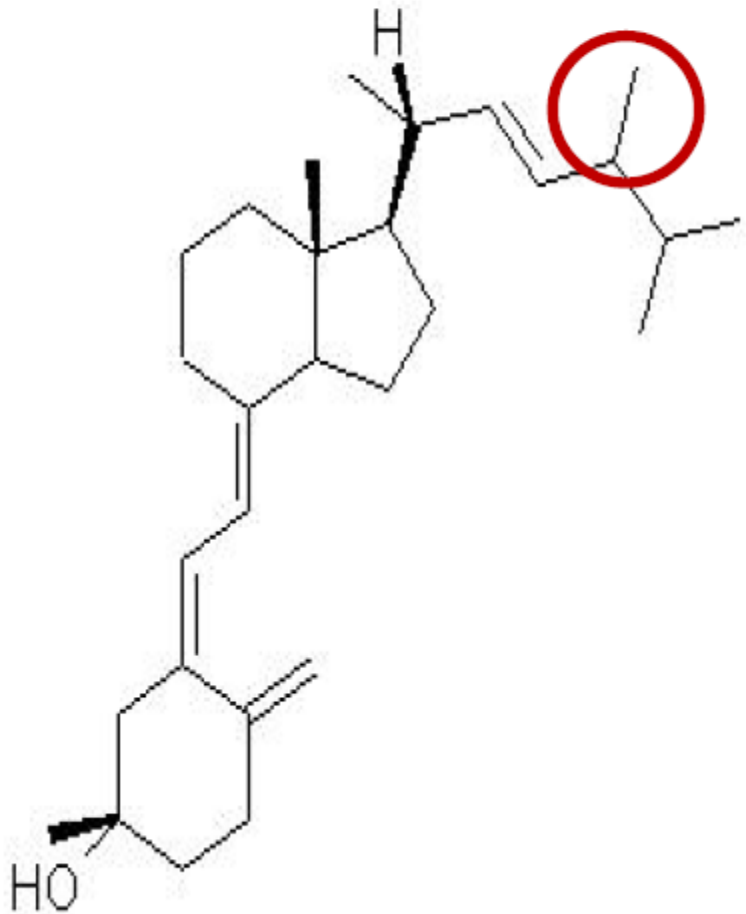
Physiologic Effects

Enzymes

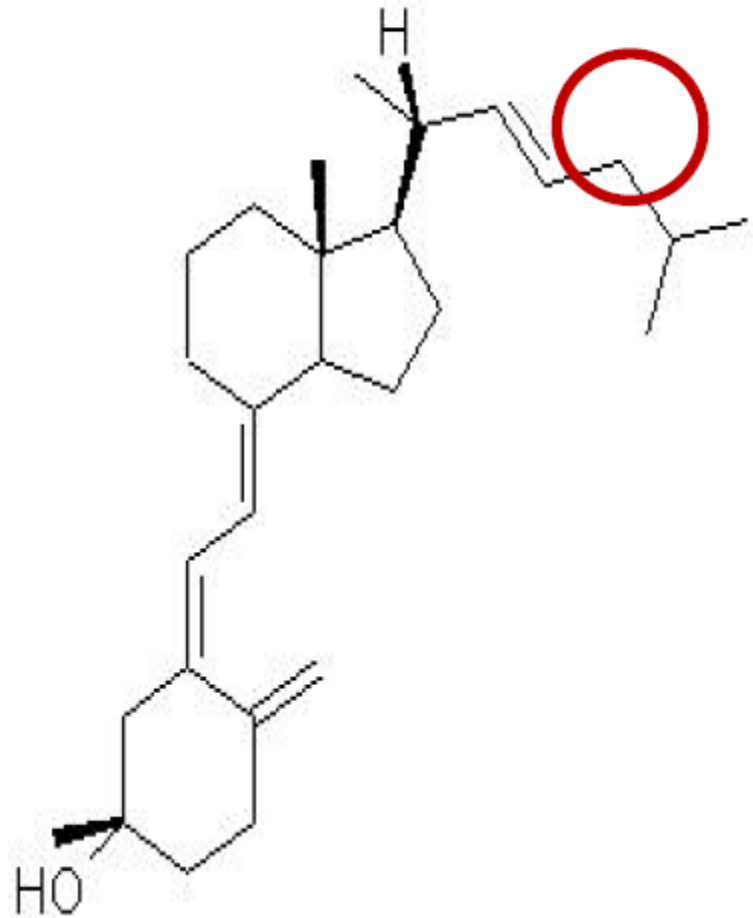
Hormones / Prehormones

Vitamin D_2 and D_3

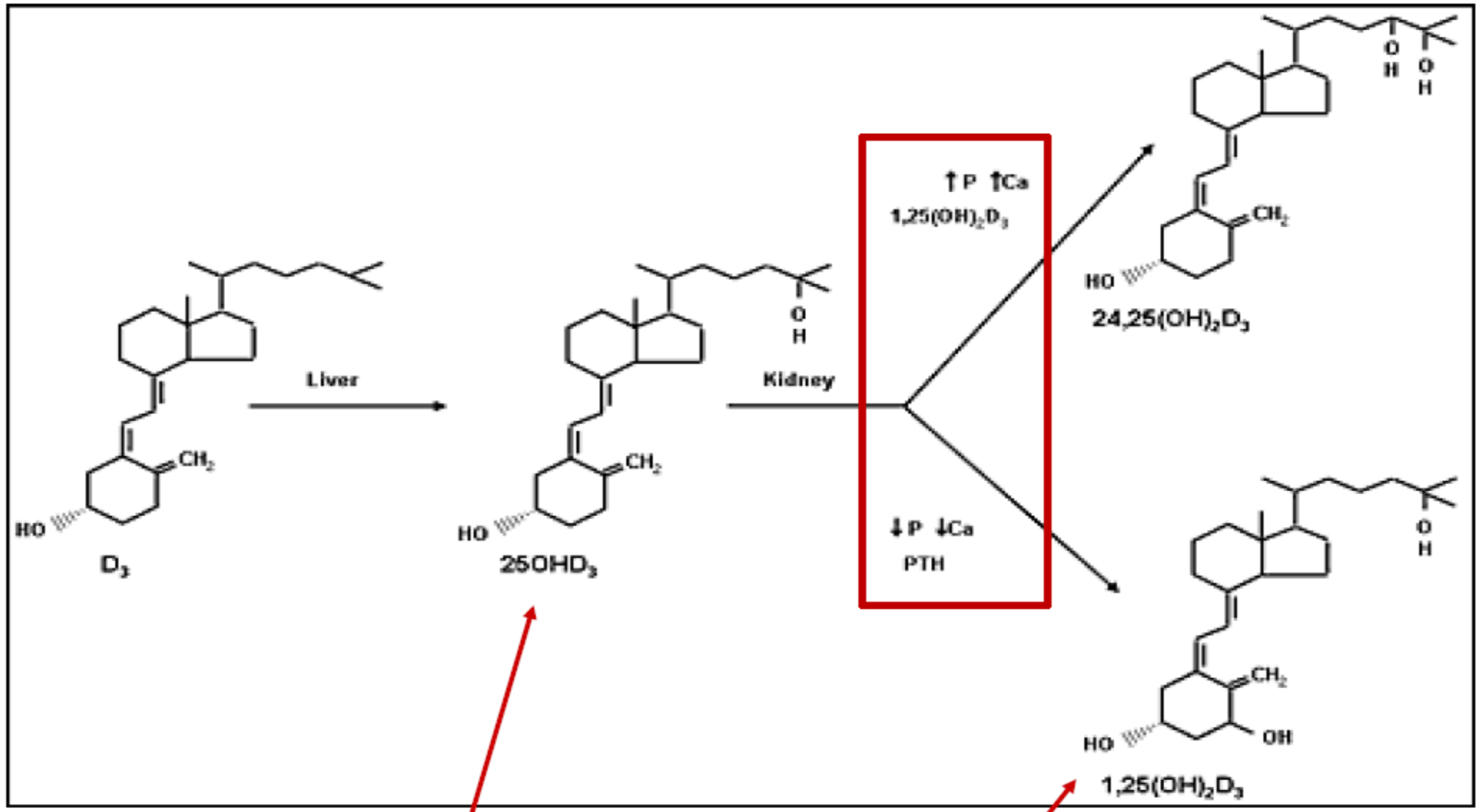
Vitamin D_2 (Ergocalciferol)



Vitamin D_3 (Cholecalciferol)



Vitamin D metabolism



2 – 3 week half-life

4 – 6 hour half-life

Professional Practice

Laboratory testing of Vitamin D

- Vitamin D
  - 25-OH-D - main circulating form
    - best measurement for determining nutritional status and body stores
  - 1,25-diOH-D – biologically active
    - differentiating HPT from HCM
    - D-dependent from D-resistant rickets
    - Monitoring D status in chronic renal failure
    - Assessing D therapy

Laboratory testing of Vitamin D

- Vitamin D
  - Serum sample
  - 25-OH-D – immunoassay (RIA, EIA, ICMA) or LC-MS/MS (D2 and D3 and D3 epimer)
  - 1,25-diOH-D – extraction, chromatography, RIA

Normal values of 25(OH)vitamin D

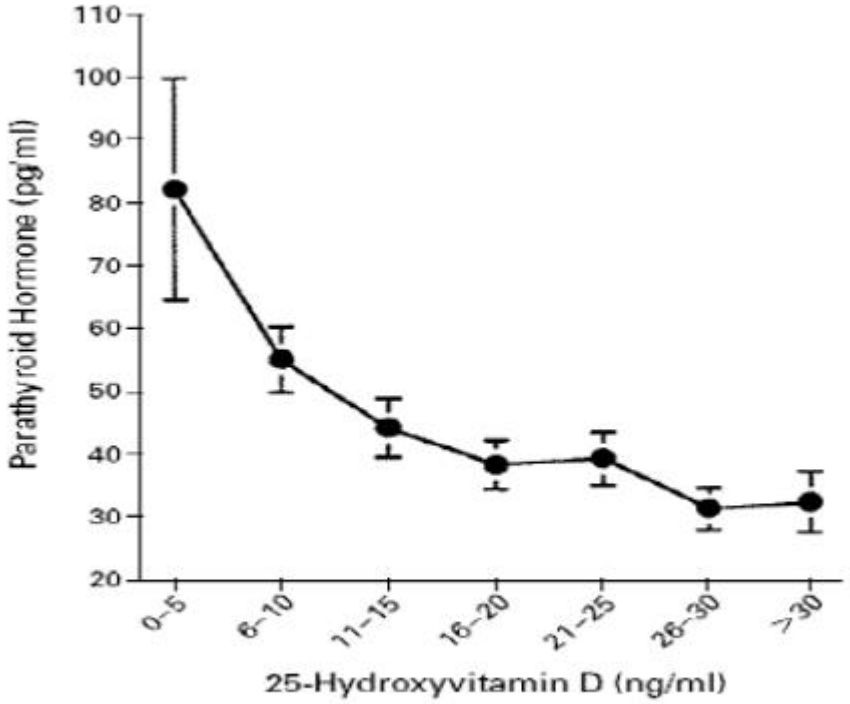
<10 ng/mL (severe deficiency)

10-19 ng/mL (mild to moderate deficiency)

20-50 ng/mL (optimum levels)

51-80 ng/mL (increased risk of hypercalciuria)

>80 ng/mL (toxicity possible)



Calcium and Phosphate Disorders

Hypercalcemia

Hypercalcemia **is defined as total serum calcium > 10.2 mg/dl (>2.5 mmol/L)**
or ionized serum calcium > **5.6 mg/dl (>1.4 m** mol/L)

Severe hypercalemia **is defined as total serum** calcium **> 14 mg/dl (> 3.5 mmol/L)**

Hypercalcemic crisis **is present when severe neurological symptoms or cardiac arrhythmias** are present in a patient with a serum calcium > 14 mg/dl (> 3.5 mmol/L).

Clinical Manifestations

“Stones, bones, groans, and psychiatric overtones”

Stones	→	**Nephrolithiasis**
Bones	→	**Bone pain**
Groans	→	**Abdominal pain**
Psychiatric overtones	→	**Depression, anxiety, confusion**

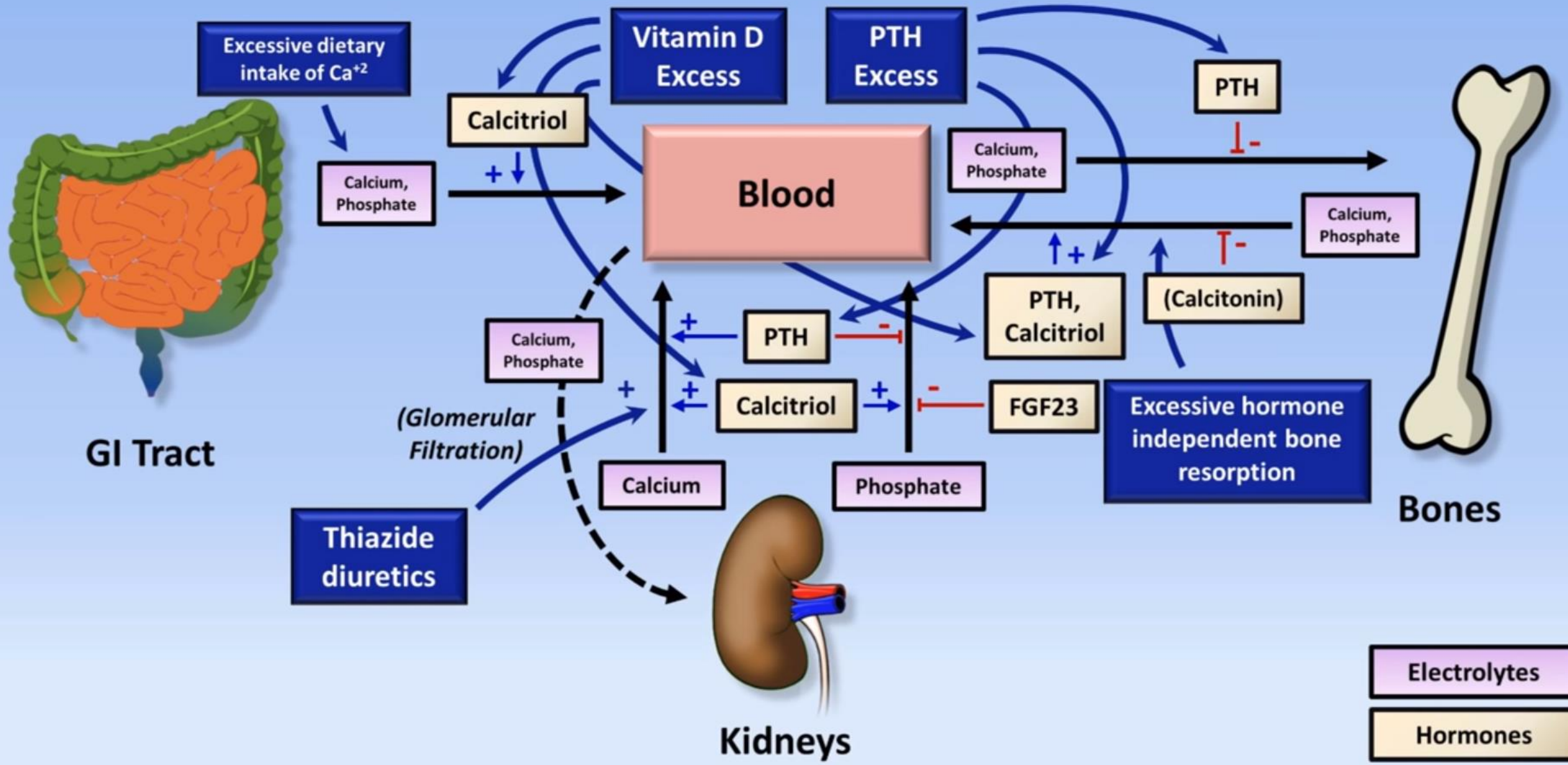
Clinical Manifestations

Other symptoms include:

- **Constipation**
- **Anorexia**
- **Nausea**
- **Weakness**
- **Lethargy**

There are no reliable physical findings of hypercalcemia.

Actions of Hormones Involved in Calcium/Phosphate Homeostasis

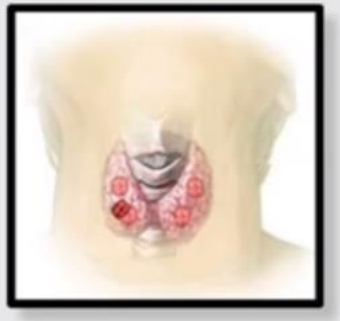
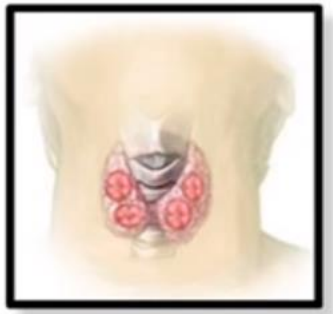
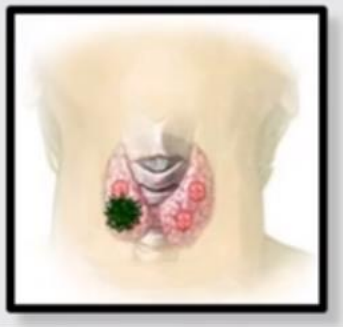


Etiologies by Mechanism

PTH Excess	Hormone-independent bone resorption	Vitamin D Excess	Excessive dietary intake of Ca^{+2}	Thiazides	Rare Miscellaneous Mechanisms
Hyperparathyroidism (1° or 3°) PTHrP-secreting malignancy Lithium Familial hypocalciuric hypercalcemia	Osteolytic bone metastases Paget's disease of bone Hyperthyroidism Immobilization	↑ Intake of vit. D Ectopic calcitriol production (i.e. granulomatous disorders, lymphoma)	Milk alkali syndrome (↑ intake of $CaCO_3$)	NA	Adrenal crisis Severe rhabdo. complicated by AKI Theophylline toxicity

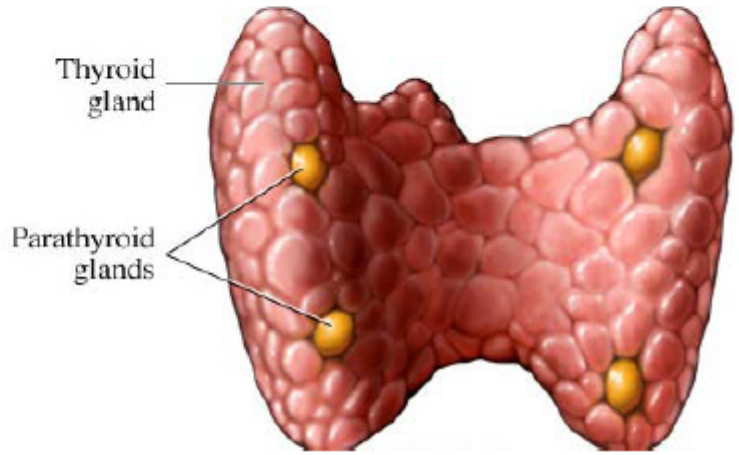
The combination of 1° hyperparathyroidism, PTHrP-secreting malignancy, and osteolytic bone metastases, are collectively responsible for ~90% of all clinically relevant cases of hypercalcemia.

Etiologies of Primary Hyperparathyroidism

Pathology	Share of cases
Adenoma 	90%
Diffuse hyperplasia 	5-10%
Carcinoma 	1-5%

Laboratory testing of PTH

- PTH
  - Intra-operative PTH
    - Parathyroid adenoma excision
    - Baseline PTH – remove gland, wait 5 minutes & re-measure PTH
    - Correct gland removed – PTH will drop >50% in those 5 minutes (short half life!)
    - Rapid TAT is critical! – patient on table



Mechanisms of Hypercalcemia of Malignancy

Mechanism	Typical associated cancers	Share of hypercalcemia in malignancy
Production of PTHrP (Humoral hypercalcemia of malignancy)	Lung, breast, ovarian, and renal	80%
Osteolytic metastases	Almost all (Breast, lung, & multiple myeloma are highest risk)	20%
Ectopic production of calcitriol	Lymphoma (Hodgkin > non-Hodgkin)	~ 1%

Poor prognostic factor
(~ 50% survival at 1 month)

Diagnostic Evaluation

Step 1: Correct calcium for low albumin

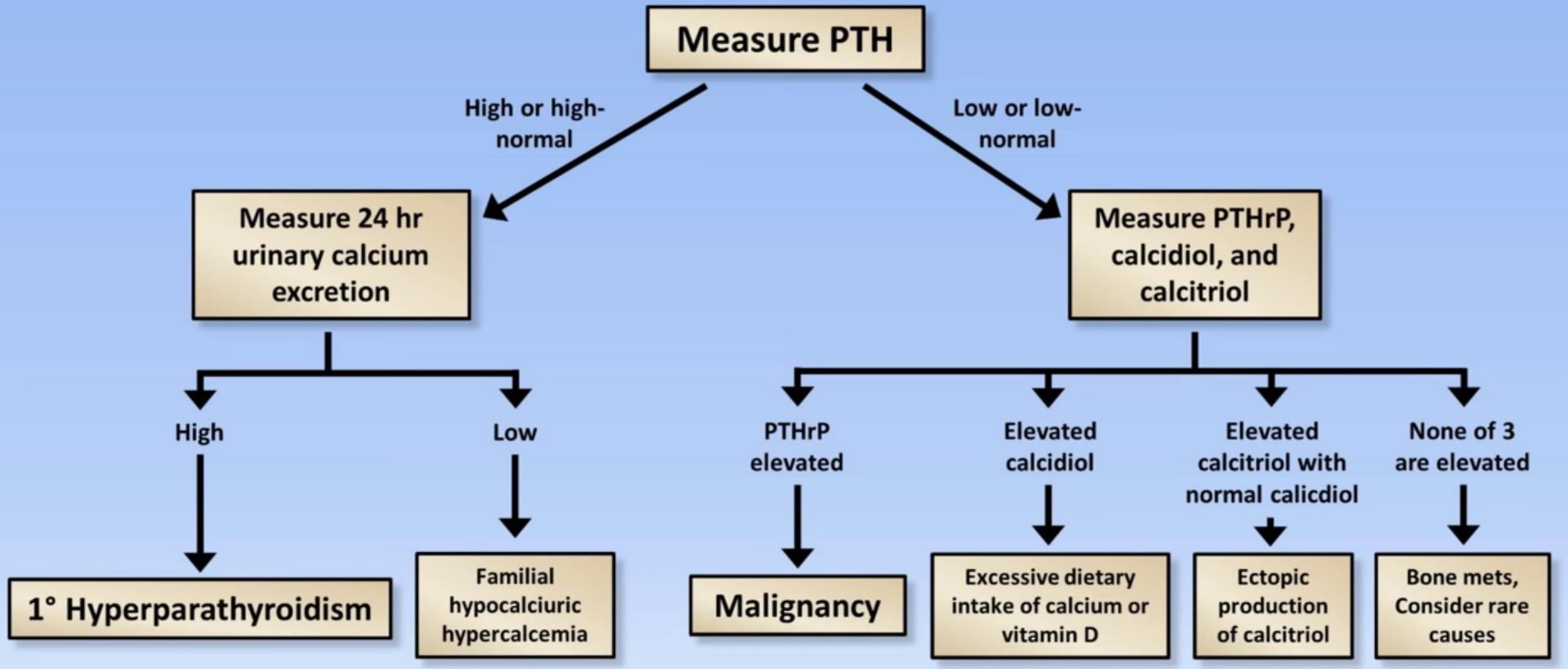
$$[\text{Calcium}]_{\text{corrected}} = [\text{Calcium}]_{\text{measured}} + \left(0.8\,(\,4 - [\text{albumin}])\right)$$

Correcting total serum calcium for hypoalbuminemia

Step 2: Perform thorough physical exam and obtain chest X-ray

Step 3: Measure PTH

Diagnostic Algorithm



Malignancy vs. Primary Hyperparathroidism

	Malignancy	Primary Hyperparathyroidism
Symptom severity	Mild or severe	Usually mild
Acuity of onset	Over days to weeks	Over weeks to months
Physical exam	Usually abnormal	Usually normal
Prevalence	Most common etiology among inpatients	Most common etiology among outpatients
Serum calcium at time of presentation	Usually >12.5 mg/dL	Usually < 12.5 mg/dL

24 hydroxylase deficiency: low vitamin D breakdown

inactivating *CYP24A1* mutations (PTH-independent hypercalcemia)

→reduced breakdown of 25 vit D

→nephrocalcinosis

25 vit D/ 24,25 vit D ratio

< 25: normal

between 25-80: heterozygous CYP24A1 mutations

> 80: biallelic CYP24A1 mutation or deletion

Calcium and Phosphate Disorders

Hypocalcemia

Clinical Manifestations

- The primary physical manifestations of hypocalcemia are various forms of tetany.

- Tetany – Repetitive discharge of peripheral nerves after a single stimulus.

Clinical Manifestations

Symptoms

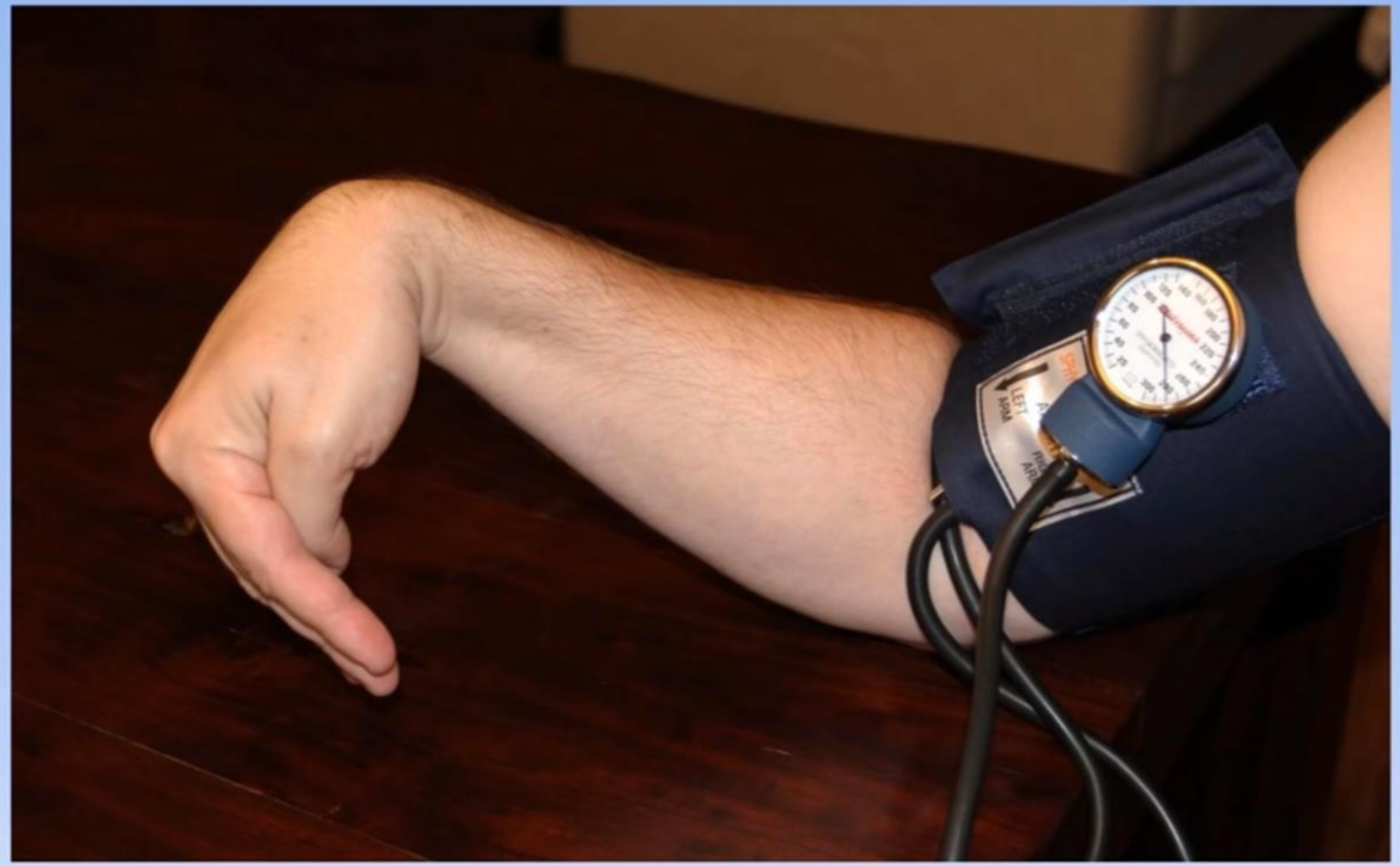
- Perioral paresthesias
- Muscle stiffness, spasms, and cramps
- Shortness of breath (diaphragmatic spasms)
- Diaphoresis

Signs

- *Chvostek's sign* – Facial spasm elicited by tapping on the ipsilateral facial nerve anterior to the ear.
- *Trousseau's sign* – Carpopedal spasm induced by inflation of a BP cuff above systolic BP for 3 minutes.

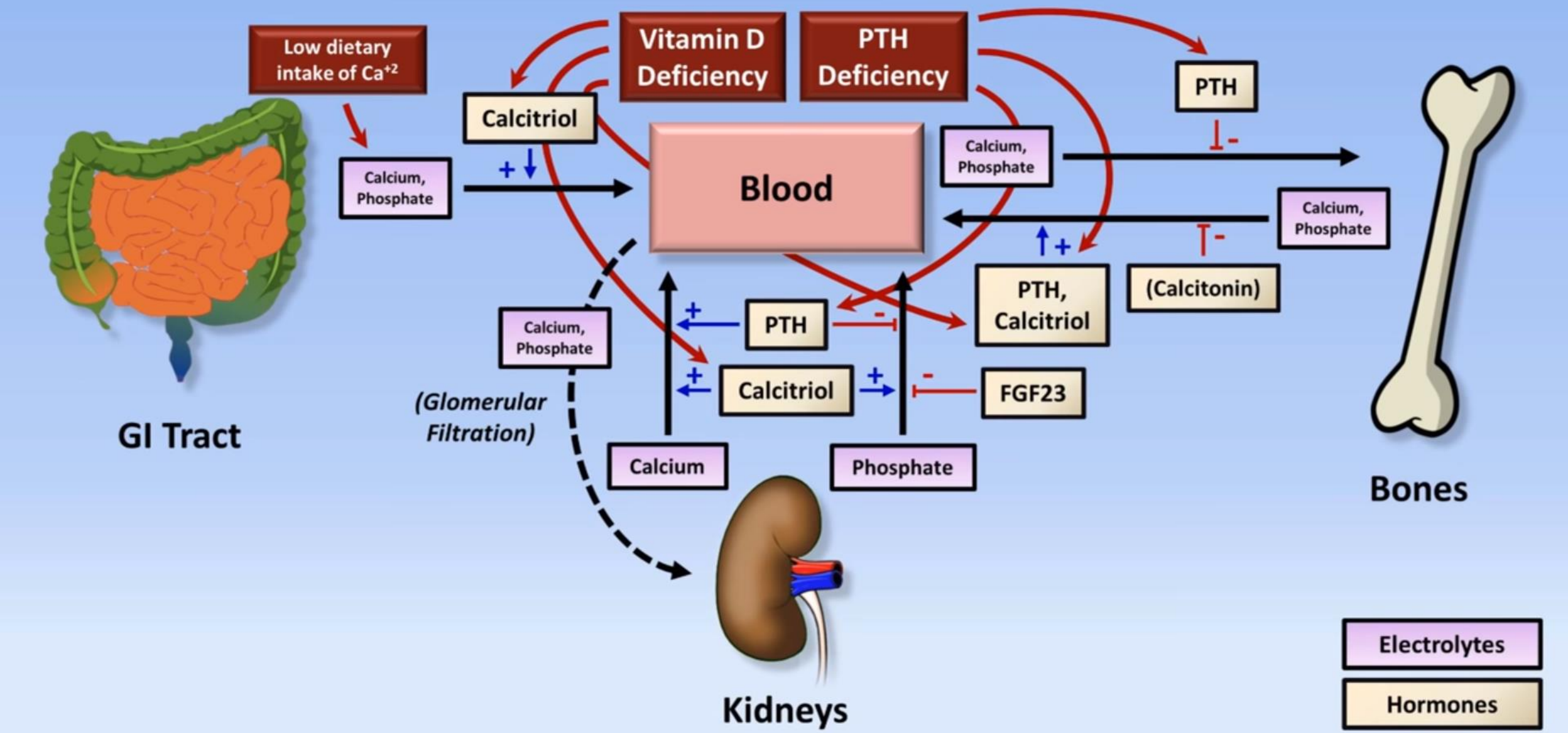
Chvostek sign

Clinical Manifestations



Trousseau's Sign

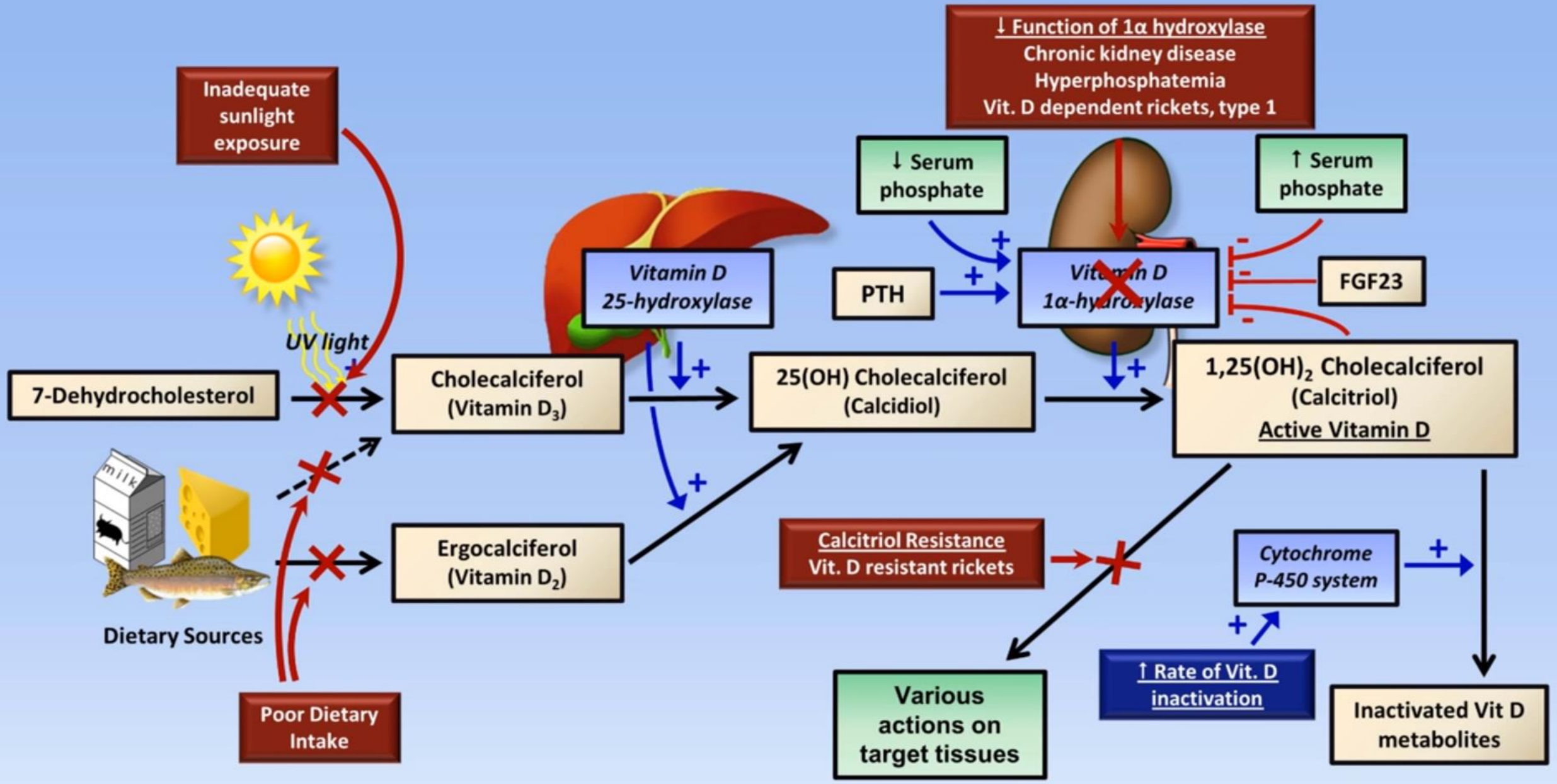
Actions of Hormones Involved in Calcium/Phosphate Homeostasis



Etiologies by Mechanism

Hypoparathyroidism	Vitamin D Deficiency	Low Dietary Intake of Ca^{+2}	Miscellaneous Mechanisms
s/p thyroidectomy or other neck surgery s/p I^{131} therapy for Graves disease or thyroid cancer Autoimmune hypoparathyroidism Infiltration of parathyroids Hypomagnesemia Genetic / Congenital PTH resistance	Low calcidiol ↓ intake of dietary vitamin D Inadequate sunlight exposure Malabsorption syndrome ↓ conversion of calcidol to calcitriol Advanced chronic kidney disease Hyperphosphatemia Vitamin D dependent rickets, type 1 Calcitriol resistance Vitamin D resistant rickets ↑ inactivation of vit D. Induction of cytochrome P-450 system (e.g. phenytoin, carbamazepine)	NA	Osteoblastic bone metastases Pancreatitis Hungry bones syndrome Multiple transfusions Acute respiratory alkalosis Hyperphosphatemia Bisphosphonates

Mechanisms of Vitamin D Deficiency



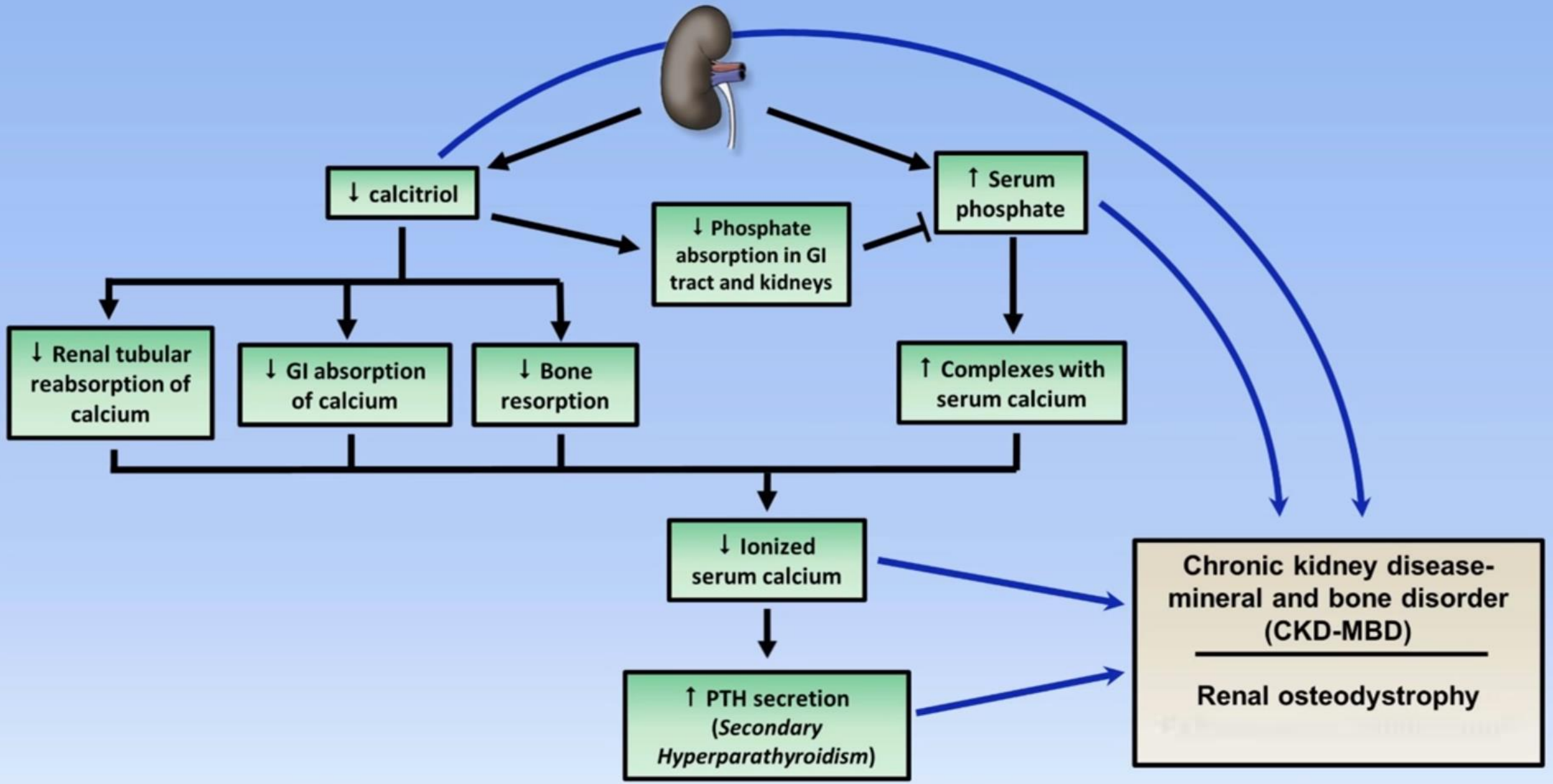
25(OH) vitamin D

People Who Should Be Tested

The following should be tested for vitamin D deficiency:

- Individuals who receive therapy to prevent or treat osteoporosis
- Elderly people, especially those with minimal exposure to sunlight
- Patients with signs and symptoms of hypocalcemia or hypercalcemia
- Children and adults with suspected rickets and osteomalacia, respectively
- Patients receiving vitamin D therapy who do not demonstrate clinical improvement

Chronic Kidney Disease and Secondary Hyperparathyroidism



Diagnostic Evaluation

Step 1: Correct calcium for low albumin

$$[\text{Calcium}]_{\text{corrected}} = [\text{Calcium}]_{\text{measured}} + \left(0.8\,(\,4 - [\text{albumin}])\right)$$

Correcting total serum calcium for hypoalbuminemia

Step 2: Measure PTH, creatinine, phosphate, magnesium, calcidiol, and calcitriol

Diagnostic Evaluation

Typical Lab Values

	PTH	Phosphate	Magnesium	Calcidiol 25 (OH) vit D	Calcitriol 1,25 $(OH)_2$ vit D	Creatinine
Hypoparathyroidism	↓	↑	Normal	Normal	Normal / ↓	Normal
Hypomagnesemia	Normal / ↓	Normal	↓	Normal	Normal	Normal
PTH resistance	↑	↑	Normal	Normal	Normal / ↓	Normal
Vitamin D deficiency (i.e. low calcidiol)	↑	Normal / ↓	Normal	↓	Variable	Normal
Chronic kidney disease	↑	↑	Normal / ↑	Normal	↓	↑

Calcium and Phosphate Disorders

Hyperphosphatemia

Etiologies by Mechanism

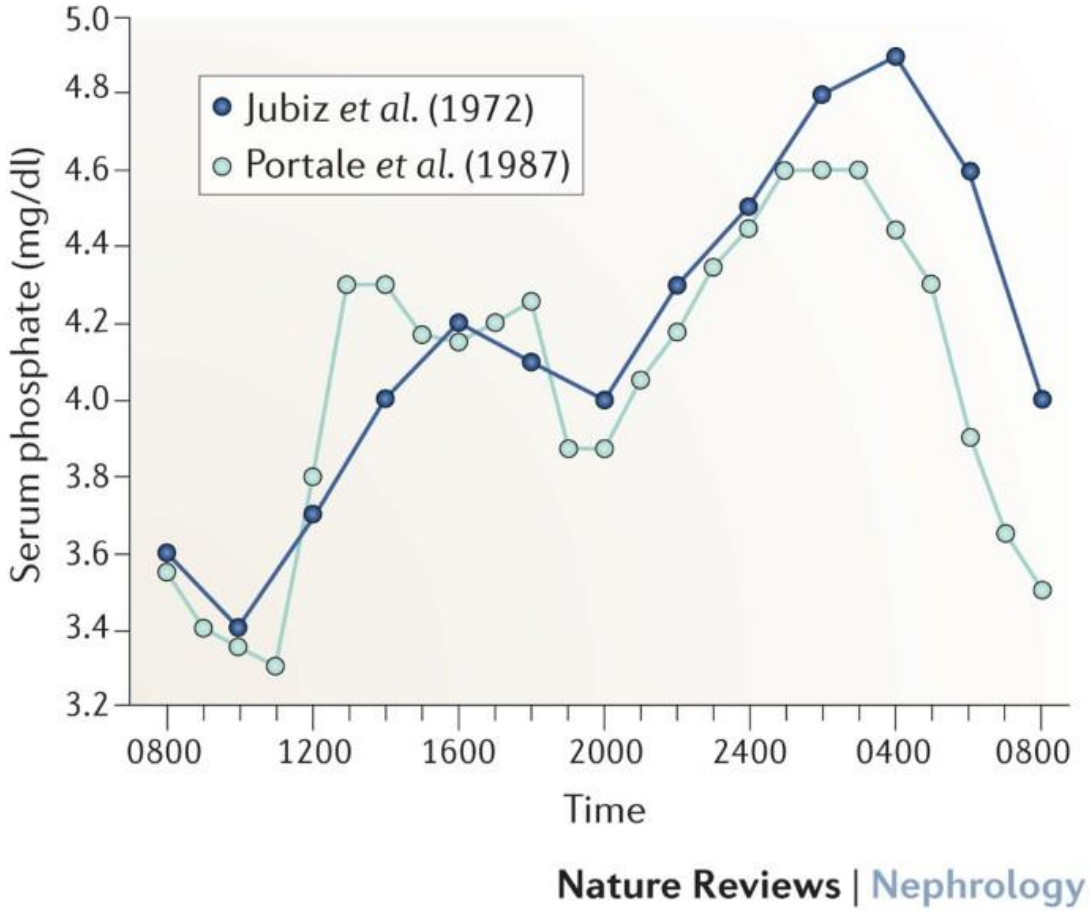
Increased GI Intake	Decreased Urinary Excretion	Internal Redistribution
Phosphate-containing laxatives (e.g. Fleet Phospho-Soda)	Renal failure (occurs when GFR < 20-25mL/min) Familial tumoral calcinosis Hypoparathyroidism Vitamin D excess Acromegaly	Tumor lysis syndrome Rhabdomyolysis Lactic acidosis

Calcium and Phosphate Disorders

Hypophosphatemia

Phosphate

- Adult: 0,80 – 1,45 mmol/L



- Circadian rhythm!
- Diet!

Clinical Manifestations

Mechanism	Early Manifestations (Serum phosphate 1-2 mg/dL)	Late Manifestations (Serum phosphate < 1 mg/dL)
Dysfunctional bone metabolism	↓ Bone mineralization Bone pain	Rickets (children) Osteomalacia (adults)
↓ Intracellular ATP	↓ Myocardial contractility	Heart failure
	Proximal muscle weakness	Rhabdomyolysis
	↑ RBC rigidity	Hemolysis
	Encephalopathy	Seizures, coma
↓ RBC 2,3 DPG	↑ affinity of Hb for O_2	Systemic ischemia

Etiologies by Mechanism

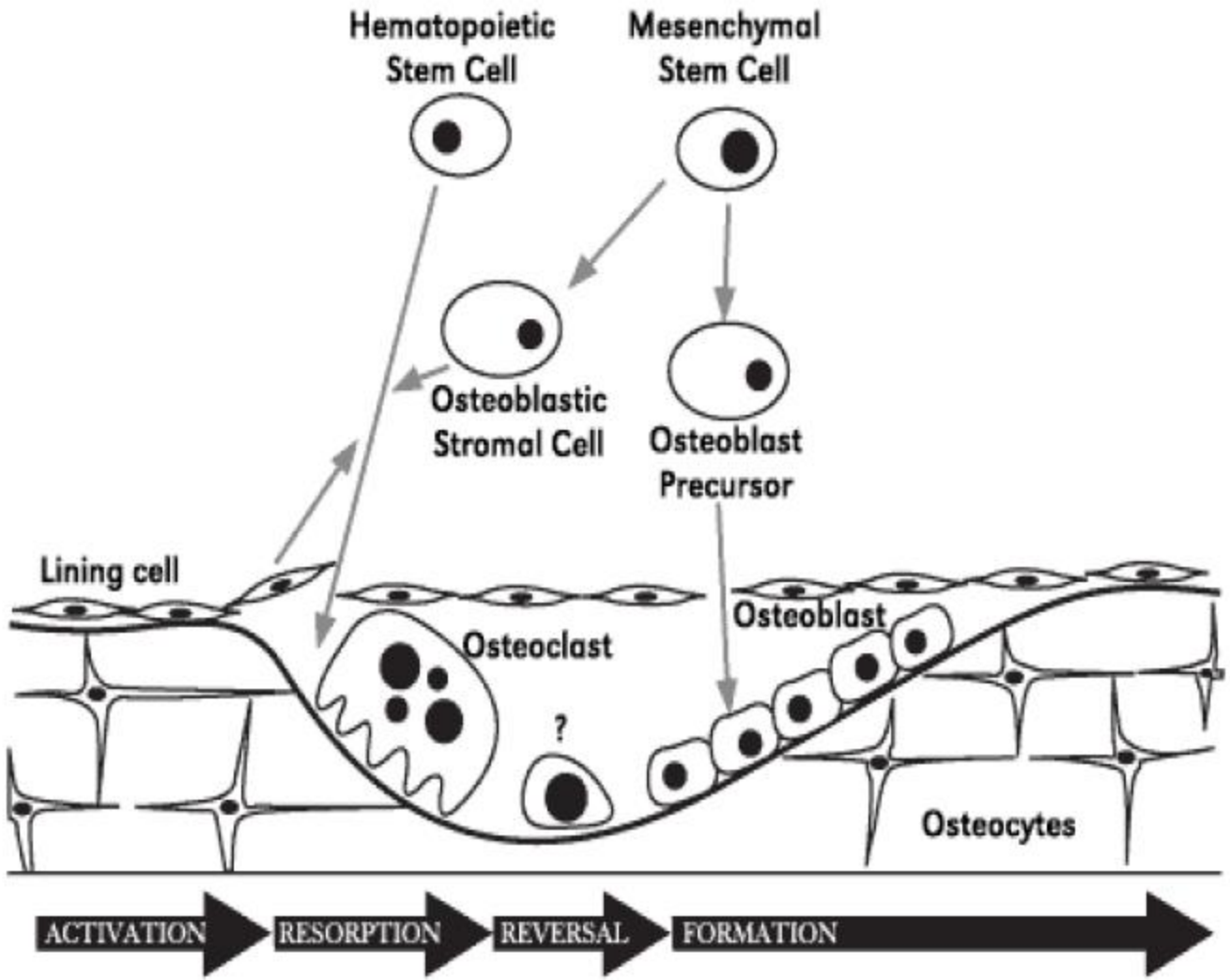
Decreased GI Absorption	Increased Urinary Excretion	Internal Redistribution
↓ Intake of dietary phosphate (only seen in malnourished alcoholics)	Vitamin D deficiency	Refeeding syndrome
Malabsorption	Hyperparathyroidism	Hungry bones syndrome
Phosphate binders (e.g. calcium acetate, Al^{3+} and Mg^{2+} containing antacids)	Variety of rare genetic diseases	During treatment of DKA or HHS
	Fanconi syndrome (general proximal tubule dysfunction) Wilson's disease (in children) Cystinosis (in children) Multiple myeloma (in adults)	Acute respiratory alkalosis
	Tumor-induced osteomalacia	

FGF-23

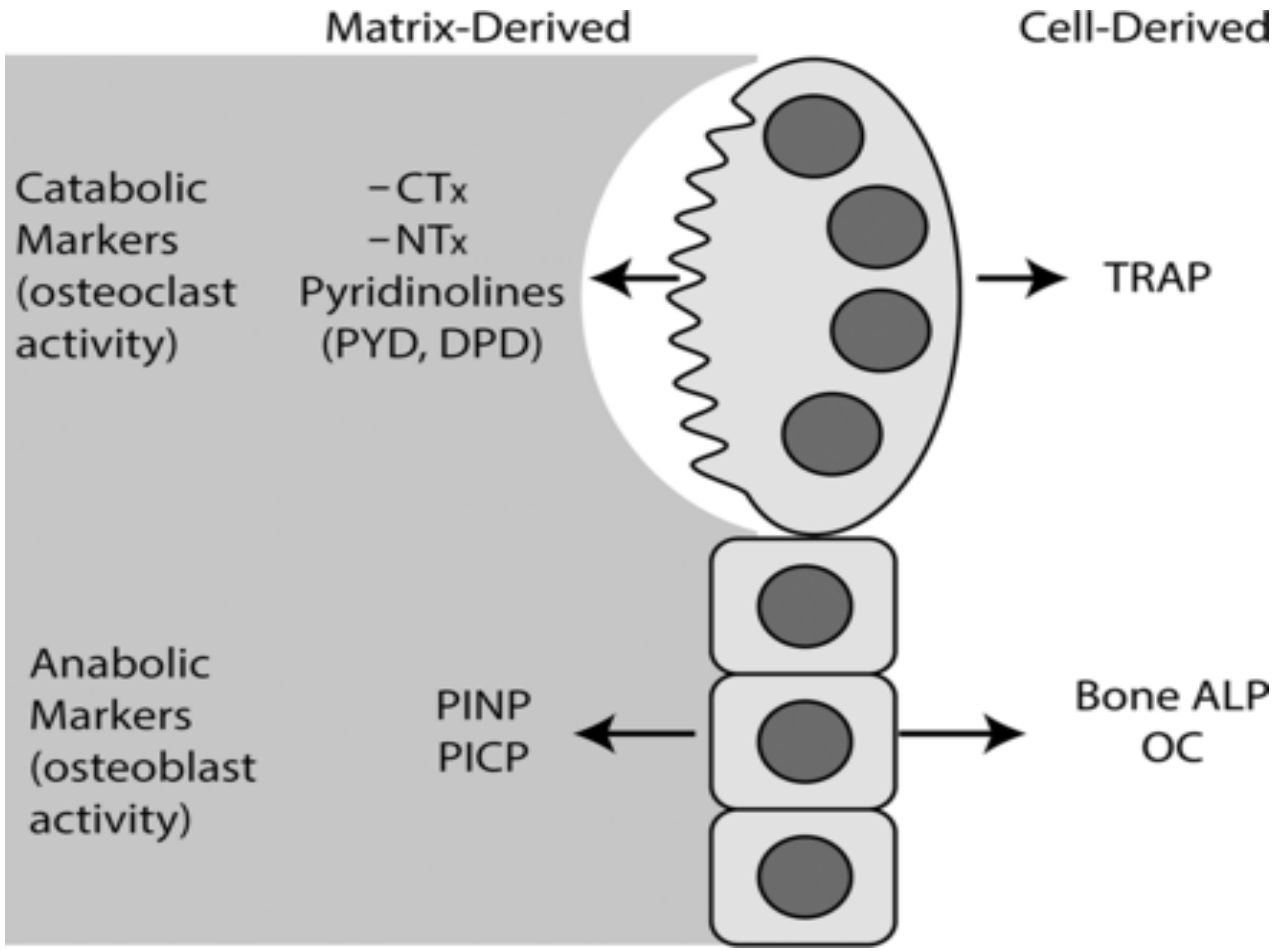
- Diagnosing and monitoring **oncogenic osteomalacia**
- **X-linked hypophosphatemia** and **AD hypophosphatemic rickets**

- (predicting treatment response to calcitriol or vitamin D analogs in patients with renal failure)

Bone Remodeling Mechanism

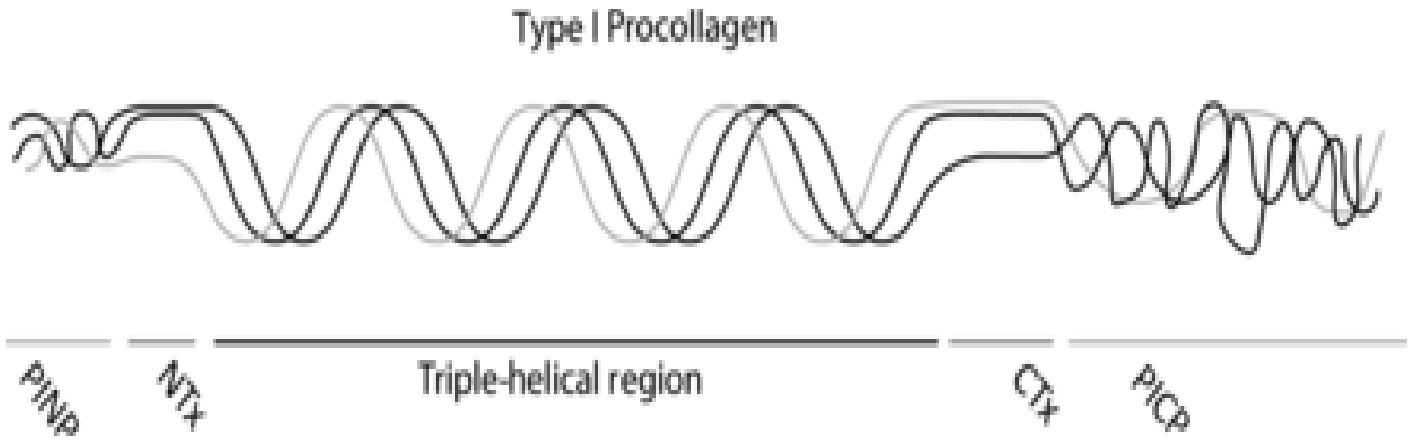


Assessing bone resorption and bone formation



Clin Chem, Volume 63, Issue 2, 1 February 2017, Pages 464–474, https://doi.org/10.1373/clinchem.2016.259085

(pro)collagen: structure



Clin Chem, Volume 63, Issue 2, 1 February 2017, Pages 464–474, https://doi.org/10.1373/clinchem.2016.259085

Pre-analytical considerations (1)

- Urine: 24h or urine specimen (creatine correction)
- Serum markers: more popular

- Children have higher values due to growth
- Elderly have lower values, especially postmenopausal women

- Elevated during pregnancy and lactation
- Elevated during immobilisation
- Elevated after bone fractures (6 months)
- Unreliable if impaired kidney function

Pre-analytical considerations (2)

- Circadian rithm: highest levels during the morning
- Phase in menstrual cycle
  - Formation markers are elevated in luteal phase
  - Resorption markers are elevated in follicular phase
- Seasonal variation: markers are higher in winter (when vitamin D is low)
- Meat etc. increase markers → SOBER
- Smoking or low BMI: higher values
- Other organs then bone contain collagen: heart, connective tissue…

Analytical problems

- No standardization
- No good EQC available

- IFCC working group on standardization:

  - CTx and P1NP
  - In serum
  - Work in progress

Table 2

Changes in bone biomarker levels during different anti-osteoporosis therapeutic regimes

Bone marker	Type	Therapy	Target levels	Follow-up period
ß Crosslaps	Resorption marker	Anti-resorptive	min. 35% ↓	Baseline and every 6 months
Total P1NP	Formation marker	Anti-resorptive	min. 40% ↓	Baseline and every 6 months
		Anabolic	min. 40% ↓	Baseline and every 6 months
Osteocalcin	Turnover marker	Anti-resorptive	min. 20% ↓	Baseline and every 6 months

Monitoring of anti-osteoporosis therapy: USEFUL

- Prediction of evolution of BMD
- Anti-resorptieve therapy: destruction of osteoclasts → lower markers
- Every 3-6 months = **faster** then DXA (reacts after 1 year)
- Different types of therapy:
  - **Bisfosfonates (IV, SC, oral)**
  - Estrogen receptor modulators
  - Denosumab
  - others

Predict fracture risk: LIMITED

- Elevated markers indicate:
  - Bone loss
  - Elevated risk of pathological fractures

- BUT:
  - Efficacity and cost-effectiveness not proven on an individual basis
  - Screening is absolutely contra-indicated

Use in nephrology: LIMITED

- End stage renal disease = renal osteodystrophy
  - Low serum calcium
  - Elevated PTH (secundary hyperparathyroidism)
  - High bone turnover (and high marker levels)
- TRAP en BSAP are the only markers NOT cleared by the kidney
- Monomer P1NP, osteocalcine and CTx are not good in renal patients
- PTH en BSAP have a good correlation with bone turnover in renal patients

Use in oncology: LIMITED

- Prostate, lung, breast… → bone metastasis
- Multiple myeloma

- Osteolytic metastasis: elevated resorption markers
- Osteoblastic metastasis: elevated formation markers

- Can be tumor marker if secreted by the primary tumor:
  - Osteocalcin in osteoid osteoma
  - BSAP in osteosarcoma

Use in rheumatology: LIMITED

- Rheumatic diseaseare inflammatory diseases:
  - Elevated resorption markers
  - Lowered formation markers

- Might be useful in:
  - Rheumatoid arthritis
  - Psoriatic arthritis
  - Ankylosing spondylitis
  - Reactive arthritis

Paget disease: USEFUL

- Extremely high bone turnover
- Malformations of the affected bones
- All bone markers are elevated
- P1NP correlates with the activity of the disease
- P1NP is usuful for monitoring therapy

Conclusion on bone turnover markers

- Bone markers reflect bone homeostasis
- Numerous pre-analytical interferences
- Good for monitoring anti-resorptive therapy
- Good for monitoring compliance
- Might be useful to assess bone loss or fracture risk, but always in combination with DEXA
- Useful in monitoring and follow-up of some specific bone diseases

Up to date (october 2020)

The use of BTMs in clinical trials has been helpful in understanding the mechanism of action of therapeutic agents.

However, their role in the care of individual patients is not well established.

Biologic (circadian variation, dietary influence) and laboratory (standardization, automation) variability in BTM values has confounded their widespread use in clinical practice

Guidelines

The use of biochemical markers of bone turnover (BTMs) for managing osteoporosis is not a central component of most osteoporosis guidelines.

When BTMs are addressed, guideline committees typically recommend against their routine use, due to the limitations of measuring and interpreting BTMs in individual patients.

Exception: postmenopausal osteoporosis