Professional Practice in Clinical Chemistry

Calcium Homeostasis and Bone Metabolism

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Presented by AACC and NACB

Learning objectives

- Discuss calcium homeostasis
- Describe hormonal control of calcium concentration, specifically vitamin D and parathyroid hormone
- Describe bone remodeling
- Assess markers of bone turnover
- Describe laboratory testing of Calcium, PTH and Vitamin D

Case study

- 11 year old female presented to ED with “hand spasms” and abdominal pain
- Initial Labs

	US units		International units	
Calcium	**5.6**	8 – 11 mg/dL	**1.4**	2 – 2.75 mmol/L
iCa	**0.72**	1.12 – 1.32 mmol/L	**0.72**	1.12 – 1.32 mmol/L
Mg^{++}	**1.5**	1.7 – 2.4 mg/dL	**0.62**	0.7 – 0.99 mmol/L
Phos	**8.3**	3.4 – 5.4 mg/dL	**2.68**	1.10 – 1.74 mmol/L

Calcium

Calcium:

- Fifth most common element in the body (O2, C, H2, N2)
- Nearly all extracellular
- ~99% in hard tissues as hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$
- Serum concentrations well controlled - involved in important processes:
  - Muscle contraction, coagulation, neural transmission, bone metabolism

Ca^{2+}

Calcium in blood:

- ~ 50% in the form of ionized calcium (iCA) – active form
- ~ 40% is protein bound (albumin 80%)
- ~10% complexed to small diffusible ligands (lactate, phosphate, citrate, bicarbonate)
- Acidosis increases iCA form, alkalosis decreases iCA

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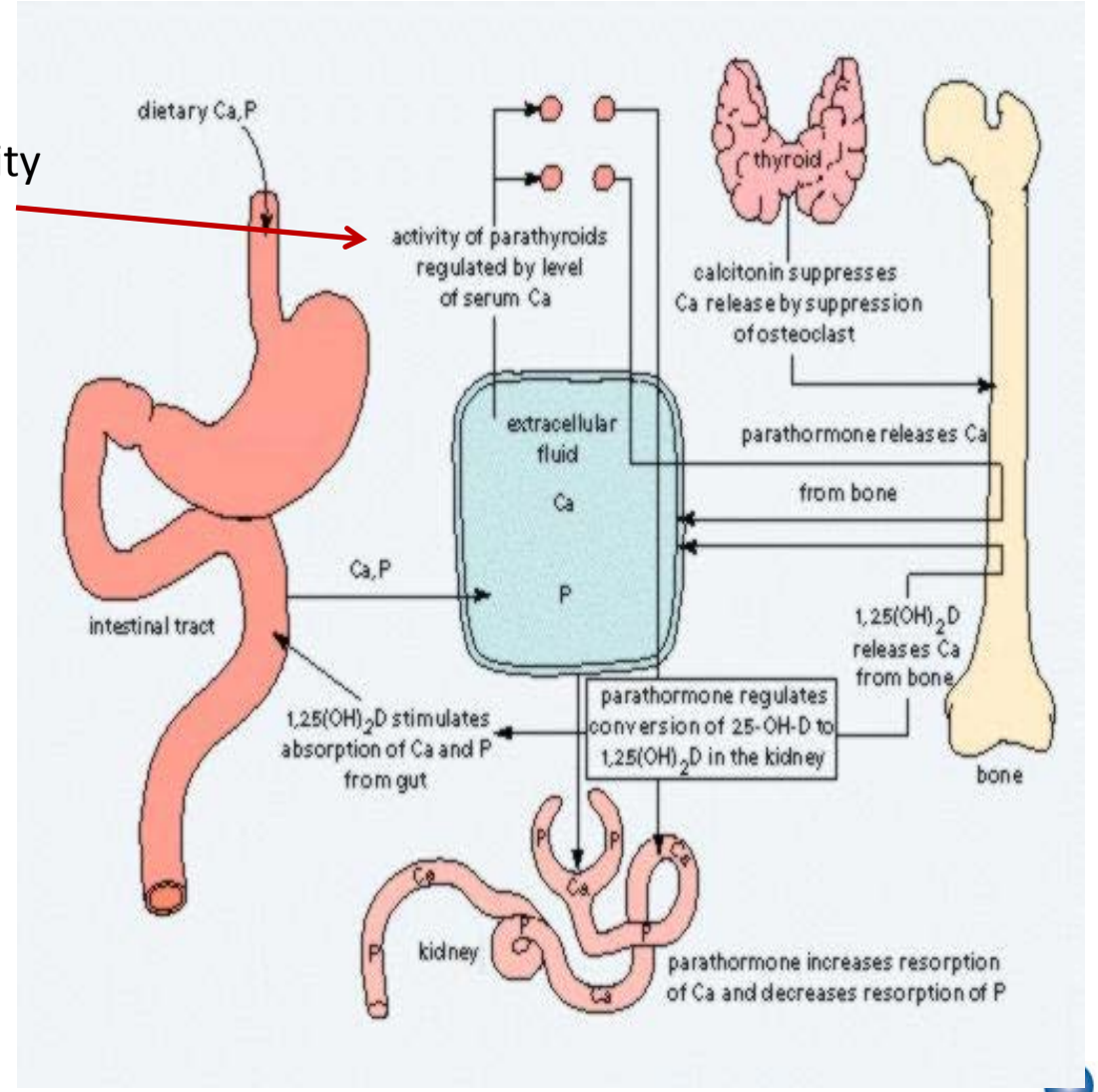
Systemic control of calcium balance

- Two hormones primarily responsible for calcium homeostasis
  - Parathyroid Hormone - PTH
  - 1,25-dihydroxy-vitamin D
  - Calcitonin – lowers serum calcium by stimulating bone accretion (suppressing osteoclast activity) – minor physiological role – thyroidectomy has no adverse affect on bone strength or density

Ca^{2+} Balance

Serum Calcium regulates activity of parathyroid glands



www.orthoteers.com/…/images2/metab3.jpg

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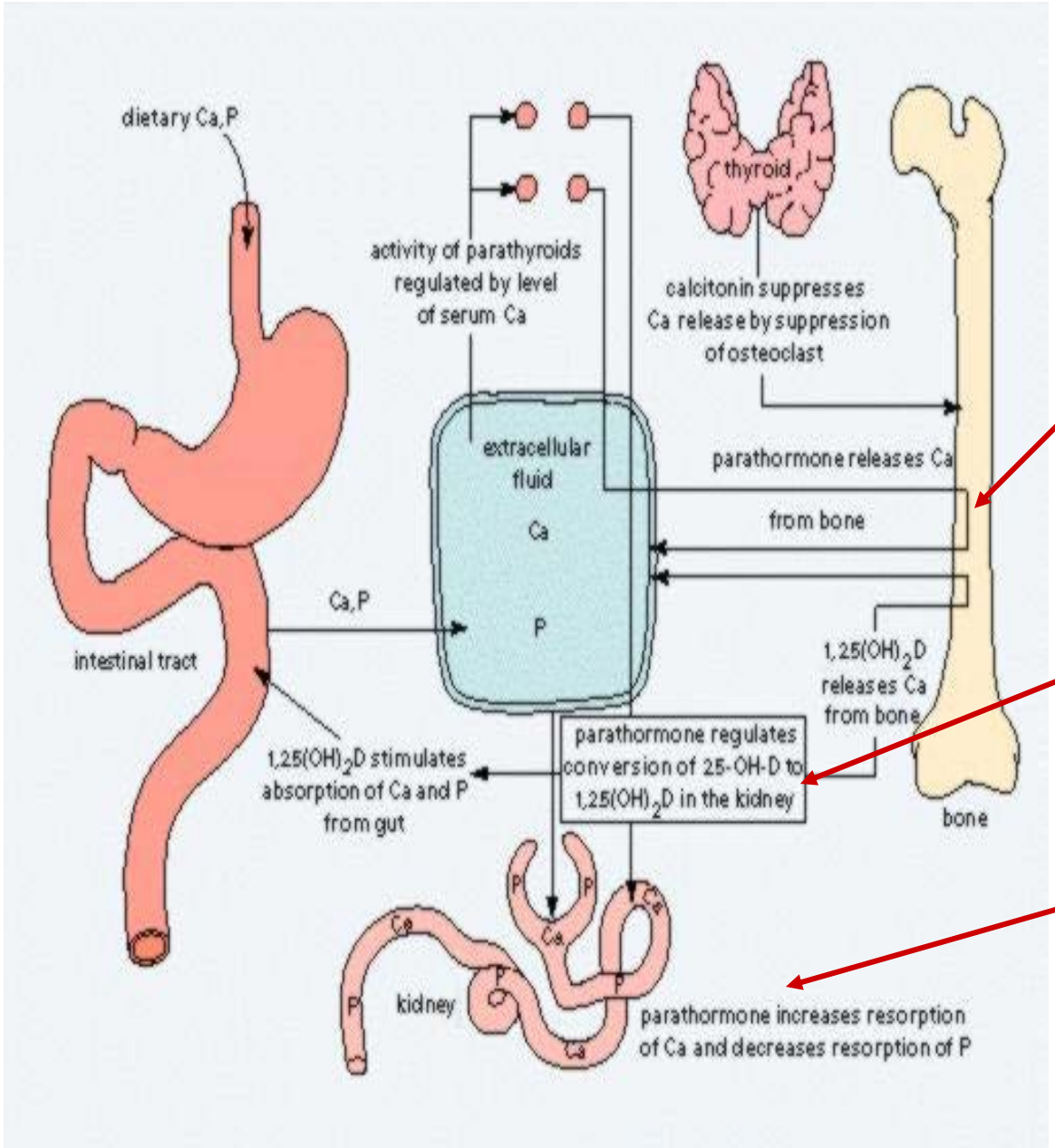

PTH:

-up-regulates Ca mobilization from bone

-up-regulates Vit D conversion from 25-OH to 1,25-diOH in kidney

-increases Ca reabsorption in kidney

-decreases Phos reabsorption (more Phos loss)

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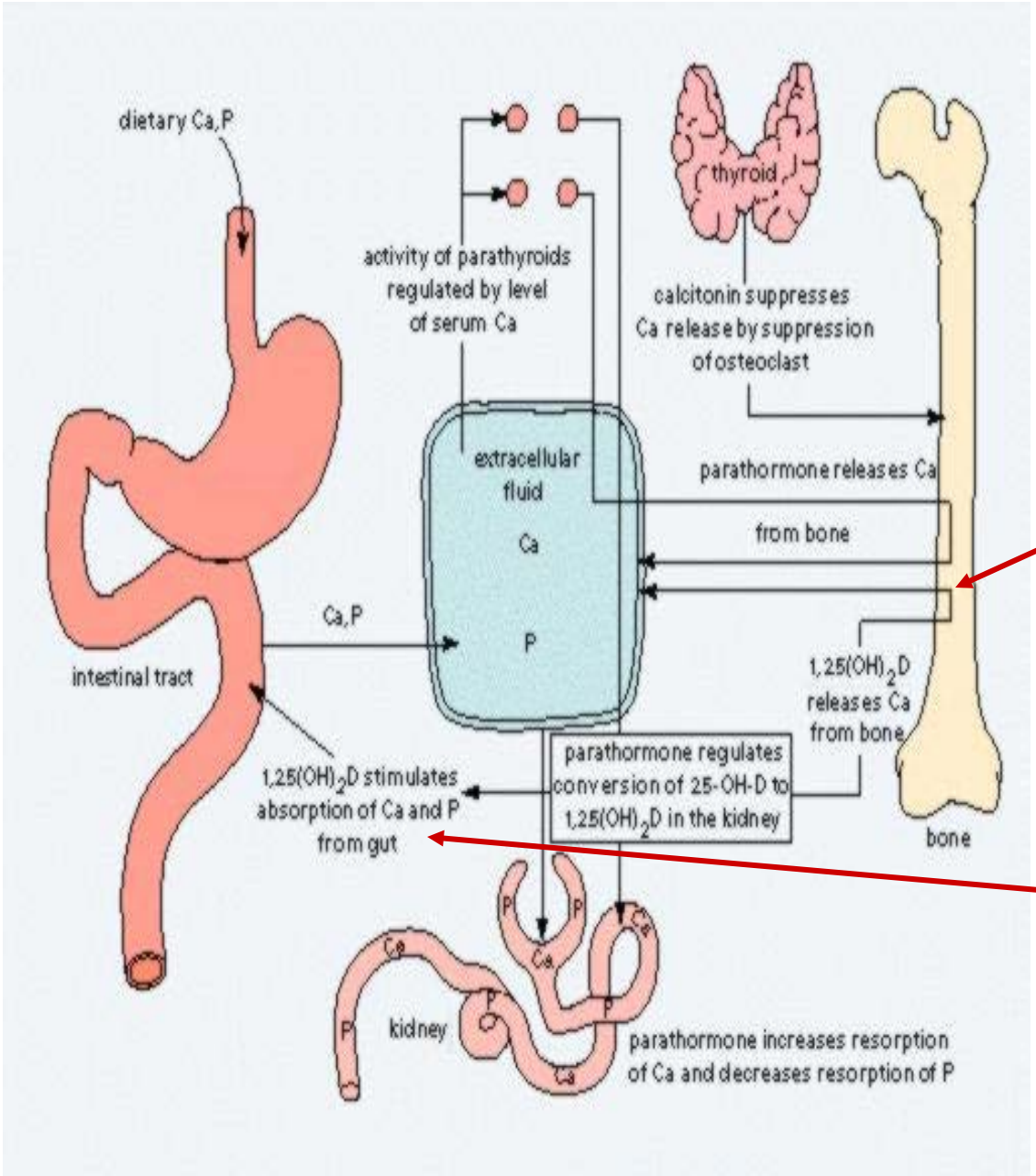

1,25-diOH-D_3:

-up-regulates Ca mobilization from bone

-increases Ca & Phos absorption from intestine

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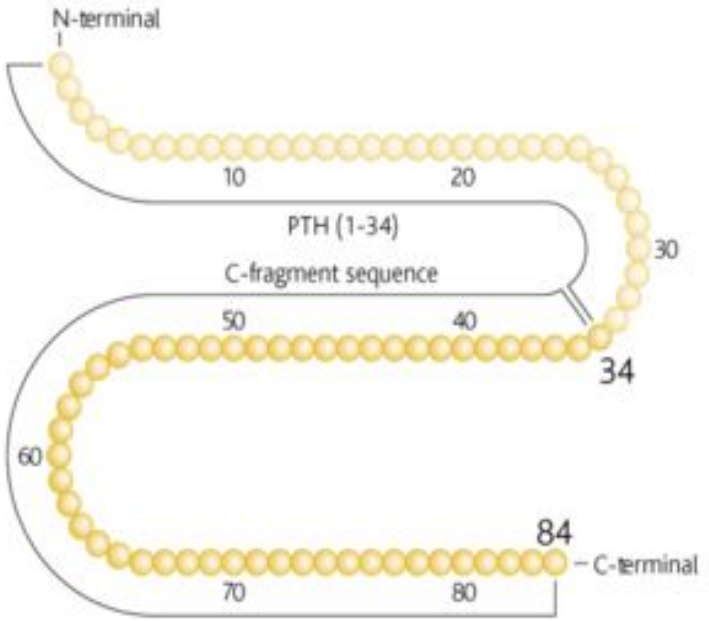
Hormonal control of calcium balance

- PTH: produced in response to low serum calcium; is suppressed when serum calcium is elevated
  - Increased mobilization of Ca from bone
  - Increased kidney reabsorption of Ca, decreased reabsorption of Phos
  - Increased kidney conversion of 25-OH to 1,25 diOH- Vitamin D
- 1,25-diOH D: formation regulated by PTH, indirectly by serum calcium
  - Increased Ca and Phos absorption from gut
  - Increased Ca mobilization from bone

Parathyroid hormone



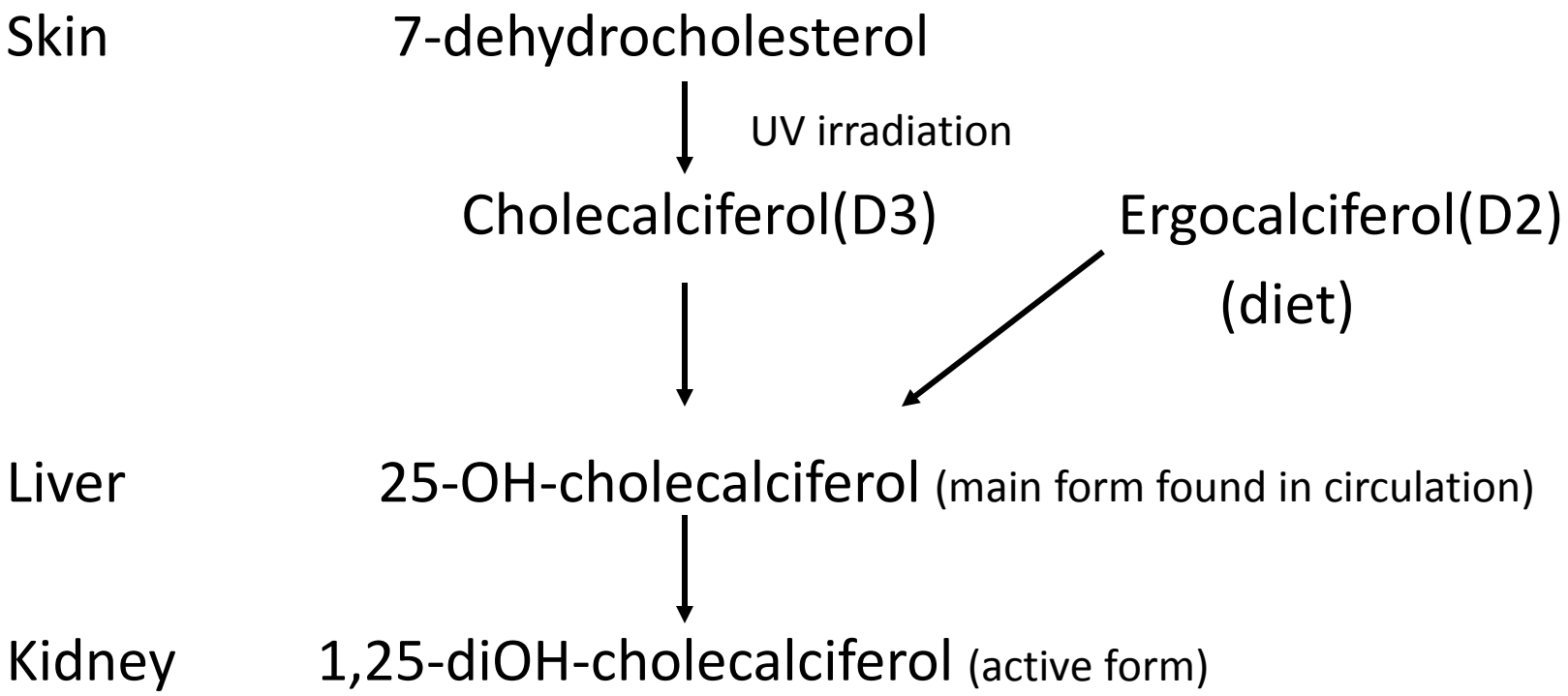
-Parathyroids secrete intact, 1-84; 7-84 molecule; 1-34 molecule produced from 1-84 molecule

-All thought to have biological activity, (7-84 may lower serum calcium)

- Original assays against C-terminal

- Most of the “intact” assays cross-react to some extent with molecules besides the 1-84

Vitamin D metabolism



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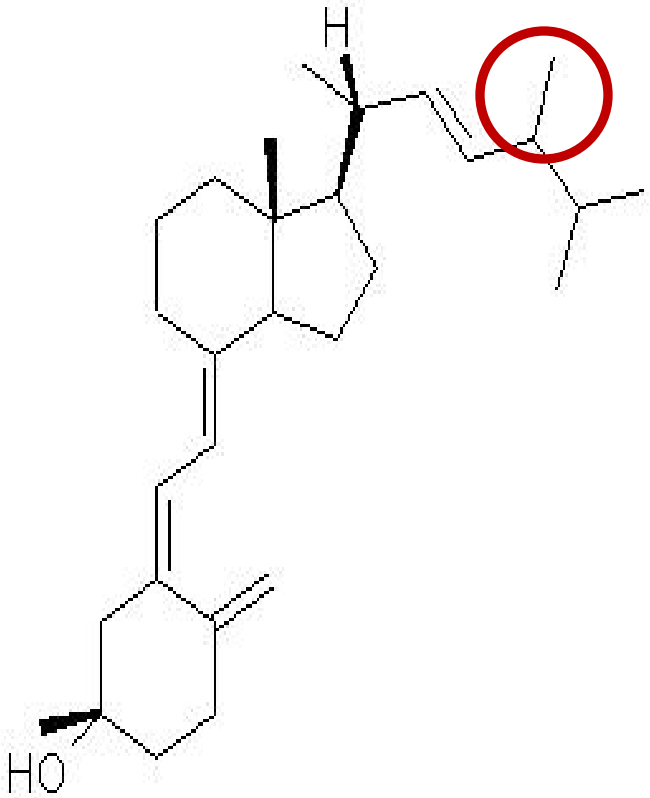
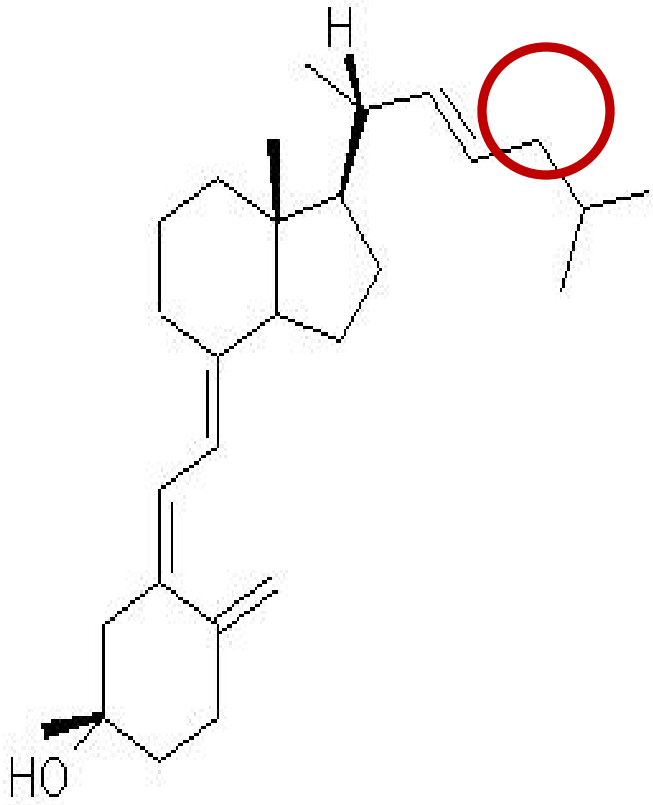
Vitamin D_2 and D_3

Vitamin D_2 (Ergocalciferol)



Vitamin D_3 (Cholecalciferol)



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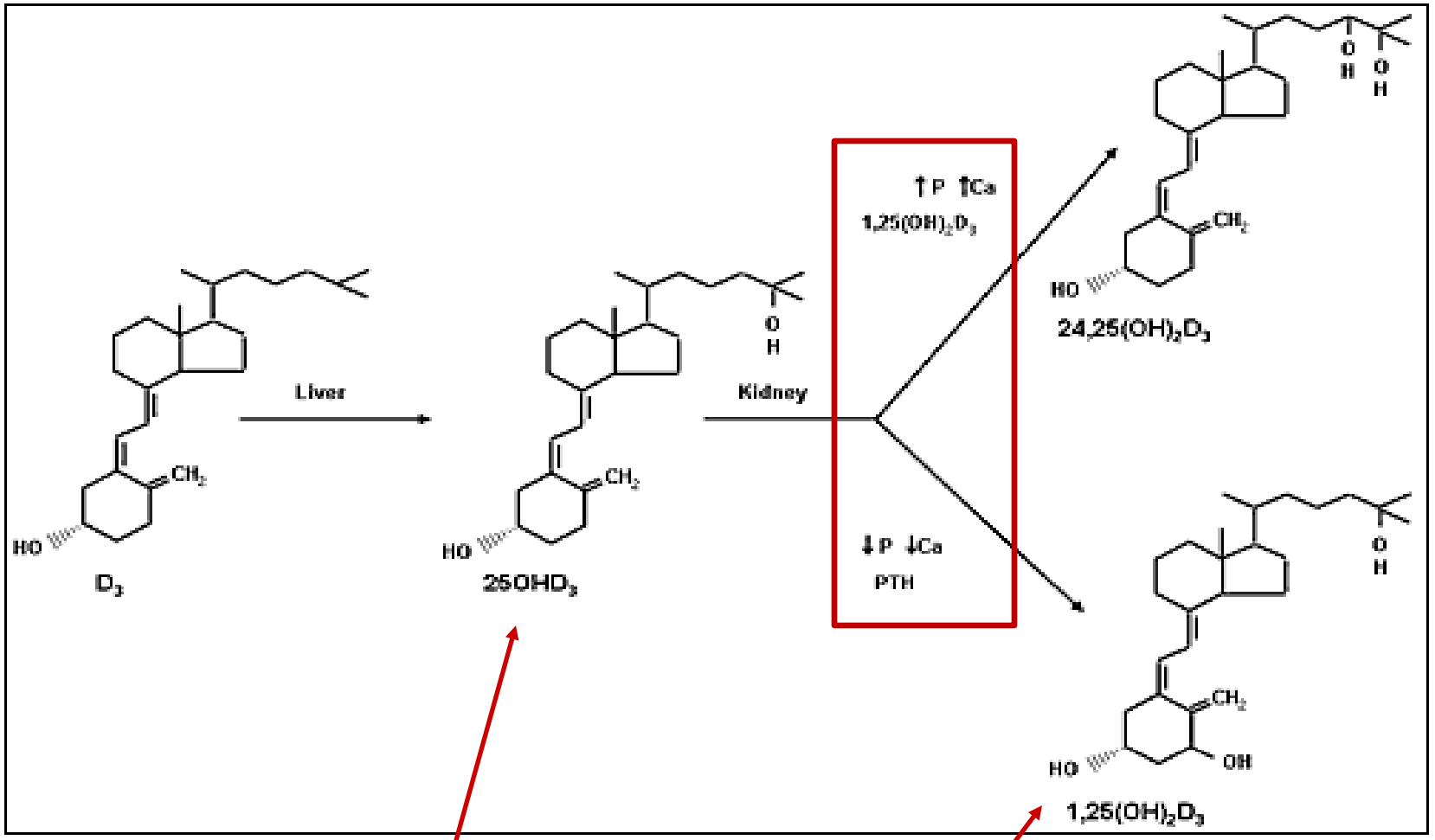
Vitamin D metabolism



2 – 3 week half-life

4 – 6 hour half-life

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Case study

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Hypocalcemia

- Hypoparathyroidism
  - Idiopathic, post surgery, hypomagnesemia,
  - low PTH
- PTH resistance (pseudohypoparathyroidism)
  - Increased PTH, hypocalcemia, hyperphosphatemia
- Non-parathyroid
  - Vitamin D deficiency
  - Malabsorption
  - Liver disease
  - Renal disease

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Phos	**8.3**	3.4 – 5.4 mg/dL	**2.68**	1.10 – 1.74 mmol/L
PTH	**57.5**	1.3 – 6.8 pmol/L		
25-OH-Vit D	**14**	30 – 80 ng/mL		
1,25-diOH- D	**42**	15 – 75 pg/mL		

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Case study - pseudohypoPTH

- 6 days in hospital receiving calcium carbonate prn and calcium gluconate IV, calcitriol 1 mcg po daily
- Labs
  - Calcium 7.5 – 8.1 for 24 hrs (8 – 11 mg/dL)
  - iCa trending up (1.07) (1.12 – 1.32 mmol/L)
  - Phos 5.0 – 6.0 (3.3 – 5.4 mg/dL)
  - PTH 50 - 80 (1.3 – 6.8 pmol/L)
  - Vit D 4 - 14 (30 – 80 ng/mL)
  - 1,25 – Vit D 22 - 45 (15 – 75 pg/mL)

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Hypercalcemia

- Primary hyperparathyroidism (HPT)
  - Most common in outpatients

- Hypercalcemia of Malignancy (HCM)
  - Most common in inpatients

Hypercalcemia

- Primary hyperparathyroidism (HPT)
  - Parathyroid gland adenoma
  - High PTH, high Calcium, low phos, renal stones

- Secondary HPT
  - Response to hypocalcemia
  - Renal failure
    - Losing calcium into urine
    - High phosphate - suppresses 1α-hydroxylase (less Ca absorption from gut), Ca complexes to phos
    - High PTH, normal to low serum calcium, high urine calcium

Hypercalcemia

– Hypercalcemia of Malignancy –

- Skeletal involvement
  - Bone resorption – metastasis
- No skeletal involvement
  - PTHrP – PTH-related peptide
    - » protein produced in fetal development and by tumors (squamous cell, breast, lymphoma)
    - » mimics PTH action, binds to PTH receptors
- Hematological malignancy (multiple myeloma)
  - Increased cytokines (IL, TNF)

Case – 2° hyperPTH due to renal failure

- 13 year old female with ESRD presents for dialysis
- Labs:

	US units		International units	
Creatinine	**13.5**	0.3 – 1.1 mg/dL	**1193**	27 – 97 mmol/L
Calcium	**7.4**	8 – 11 mg/dL	**1.85**	2 – 2.75 mmol/L
Phos	**6.3**	3.4 – 5.4 mg/dL	**2.03**	1.10 – 1.74 mmol/L
PTH	**128.3**	1.3 – 6.8 pmol/L		
25-OH-Vit D	**36**	30 – 80 ng/mL		

- Ordered: bone density scans, bone age determination
- Cases like this lead to renal osteodystrophy

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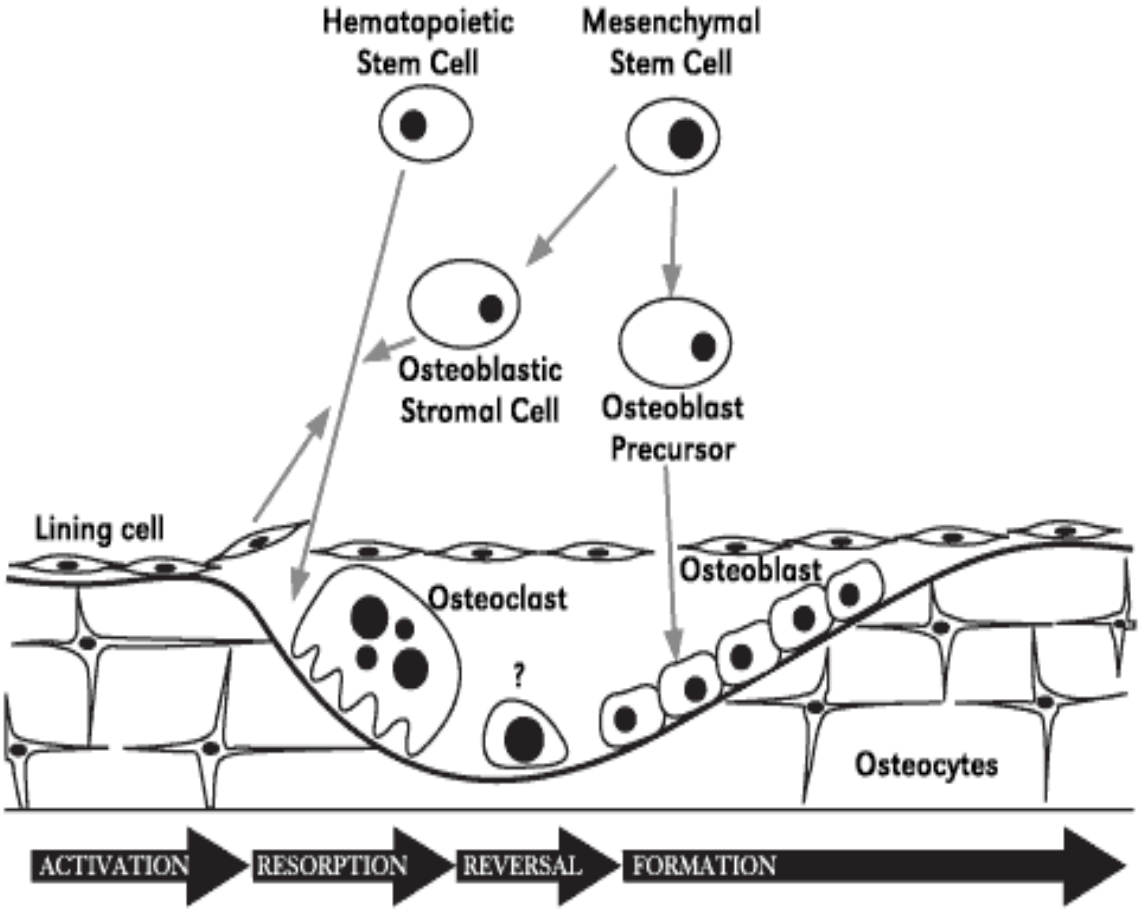
Bone Metabolism

- Bone acts as a reservoir for calcium and phosphate
- Bone remodeling allows for release and uptake of calcium – thus one control of bone remodeling is **calcium** level
- Bone remodeling is a constant, not random process – always going on but rate determined at multiple levels
  - Hormone – PTH, Vitamin D
  - Serum calcium levels
- Most of the adult skeleton is replaced ~ every 10 years (10-30% replaced per year)

Bone Remodeling Mechanism



www.surgeongeneral.gov/library/bonehealth/chapter_2.html

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Bone Remodeling Regulation

- Regulated **systemically** by:

Factor	Effect on osteoblast	Effect on osteoclast	Effect on bone
PTH	⇧	⇧	Variable
1,25 di-OH-D	⇧	⇧	Variable
IL-1/TNF	⇩	⇧	Bone loss
T3/T4	⇔	⇧	Bone loss
Cortisol	⇩	⇧	Bone loss
Calcitonin	⇔	⇩	Bone gain
Estrogen/ testosterone	⇧	⇩	Bone gain
Mechanical load	⇧	⇩	Bone gain
Growth hormone /IGF-1	⇧	⇔	Bone gain

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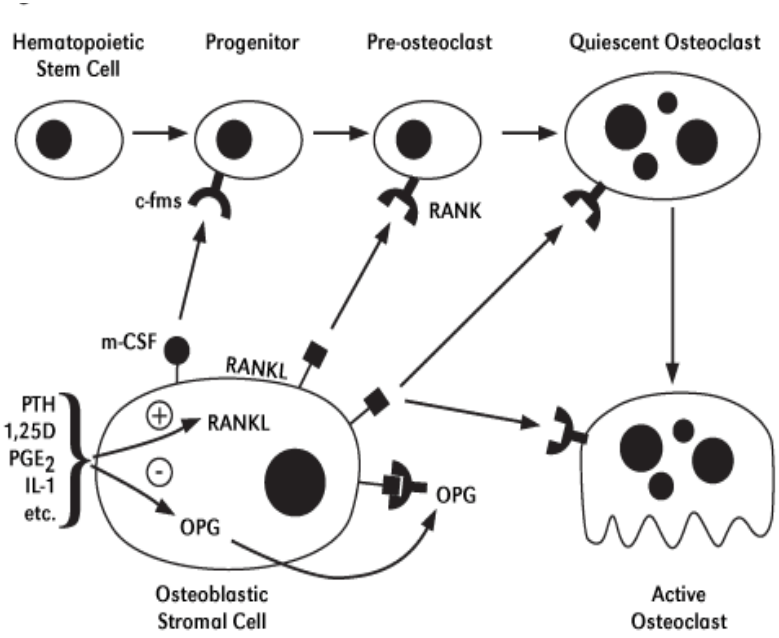
Bone Remodeling Regulation

- Regulated **locally** (at level of osteoclast / osteoblast) by:
  - Macrophage colony stimulating factor (m-CSF)
  - Receptor activator of nuclear factor kappa B ligand (RANKL)
  - Osteoprotegrin (OPG)



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Assessing bone remodeling

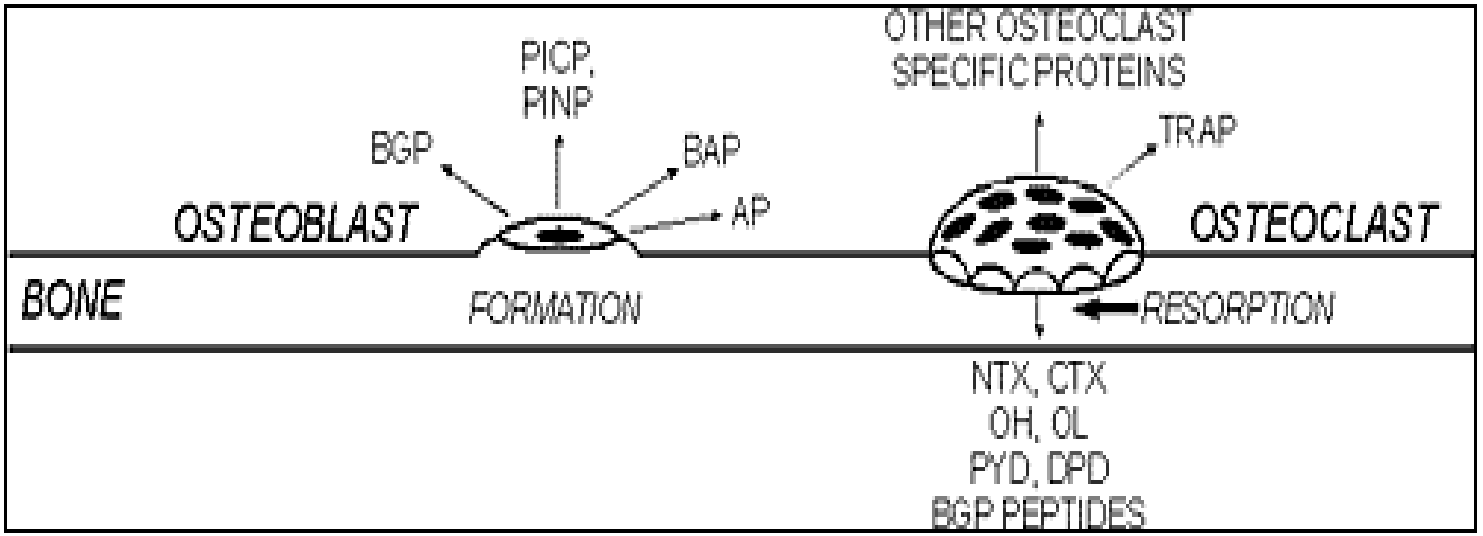

Figure 5 - Schematic Representation of the Cellular and Skeletal Sources of Serum and/or Urinary Markers of Bone Formation and Bone Resorption
(www.endotext.com Chapter 2, LJ Deftos MD,JD,LLM

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Assessing Bone Formation

- Proposed tests for bone formation
  - BGP - Bone gamma carboxyglutamic acid protein (osteocalcin, bone gla protein)
    - produced by osteoblasts, most incorporated into the new bone matrix
  - PICP - C-terminal propeptide of type I procollagen
  - PINP - N-terminal propeptide of type I procollagen
    - cleaved ends of newly synthesized procollagen molecules
  - BAP - bone-specific alkaline phosphatase
    - activity increases at deposition of osteoid, as osteoblasts begin making new bone

Tests for bone formation

Analyte	Utility	-vantages
BAP and total Alk Phos	⇑ - osteoporosis, osteomalacia, rickets, HyperPT, thryotoxicosis, Paget's, acromegaly, etc -Highest diagnostic sens & spec for Paget's	+ stable molecule, easily measured -BAP needs Chromatography Electrophoresis
Osteocalcin	⇑ - as above, ⇓ -hypoPT, GH deficiency, estrogen replacement therapy	-5 minute half life, non-stable - increased in impaired renal function (cleared by glomerulus)
PICP **PINP**	±; type 1 collagen not only found in bone	-PINP at reference labs (RIA)

Assessing Bone Resorption

Proposed tests for bone resorption

- TRAP - tartrate-resistant acid phosphatase
- BSP – bone sialoprotein

2 proteins, ⇑ in serum during bone resorption

- NTX - N-terminal telopeptide cross-links of type I collagen
- CTX - C-terminal telopeptide cross-links of type I collagen
- PYD – pyridinoline
- DPD – deoxypyridinoline
- ICTP – C-terminal pyrodinoline cross-links

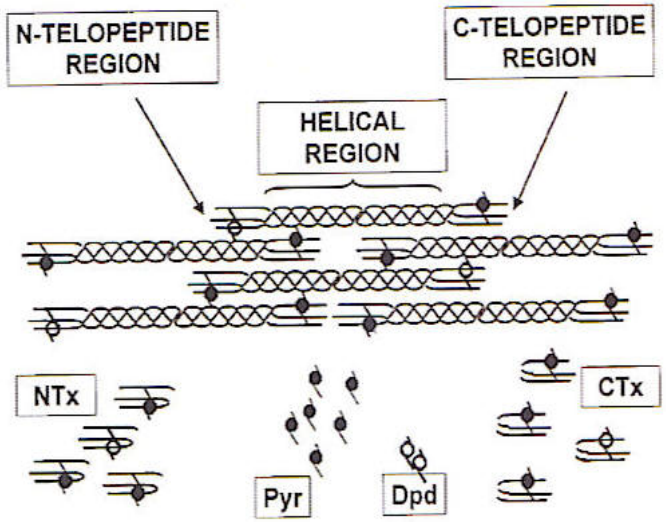

Fig. 2. Structure of type I collagen and cross-link degradation products. ○, Dpd; ●, Pyr.

Watts NB, Clinical Utility of biochemical markers of bone remodeling. Clin Chem 45(8):1359-58. 1999

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Tests for bone resorption

Analyte	Utility	-vantages
TRAP	Not used much	-failure to distinguish osteoclastic TRAP from other TRAPs
BSP	Not proven	
NTX **CTX**	⇑ In increased bone remodeling; measure response to therapy	+NTx – commercially available assay, can use serum
DPD **PYD**	DPD – most useful, appears to be from bone only	+DPD – commercially available

Utility of tests for bone remodeling

- No consistency between assays
  - No reliable synthetic standard
  - Follow treatment or disease progression – must get all samples run at same lab
- Not readily available assays
  - Essentially all reference lab assays
  - Few analyzers have these assays
- Samples:
  - Except for alk phos, most markers have significant diurnal variation
  - Degradation products best measured in either early morning urine or 24 hr urine sample

Utility of tests for bone remodeling

- Primarily useful for monitoring response to therapy, especially for metabolic bone diseases
  - Osteoporosis
    - Uncoupling of bone turnover
    - Increased resorption and/or decreased formation
    - Especially in women after estrogen loss
  - Paget's Disease
    - Increased osteoclast activity and bone turnover
    - ⇑ alk phos, and collagen degradation products
  - Osteomalacia
    - Defective mineralization of osteoid in bone
    - Often related to defects in Vitamin D metabolism
- Baseline level at start of therapy - monitor

Laboratory testing of Calcium

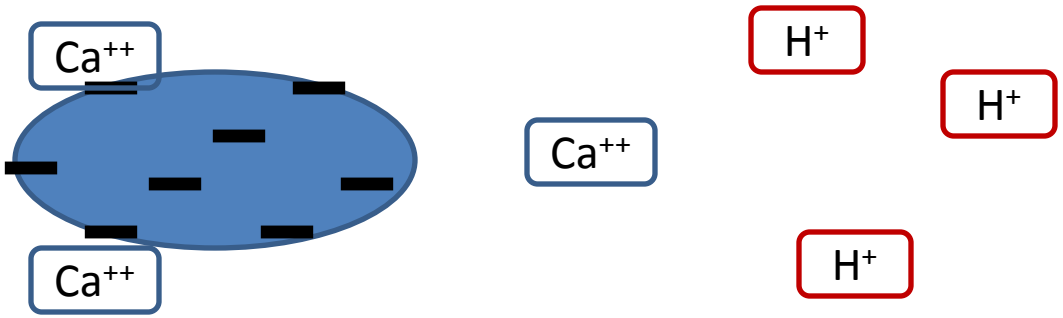
- Total Calcium
  - Measurement on most chemistry analyzers – spectrophotometric
  - Measured in heparinized plasma or serum
  - Affected by serum protein concentration
  - "Adjusted" Calcium for albumin concentration –
    - Adj Ca = TCa (mg/dL) + 0.8(4 – albumin[g/dL])
    - Below 4 g/dL: for every 1 g/dL albumin decrease, Ca decreases 0.8 mg/dL
    - Above 4 g/dL: for every 1 g/dL albumin increase, Ca increases 0.8 mg/dL

Laboratory testing of Calcium

- Free Calcium (ionized Calcium)
  - Better reflects Ca metabolism and status than Total
  - Biologically active and tightly regulated
  - Measured by ISE, generally whole blood sample, blood gas

Laboratory testing of Calcium

- Free Calcium (ionized Calcium)
  - Free calcium concentrations affected by pH
    - Acidic – more iCa available
    - Basic – less iCa available



  - Some analyzers “correct” iCa to normal pH
    - Should NOT report

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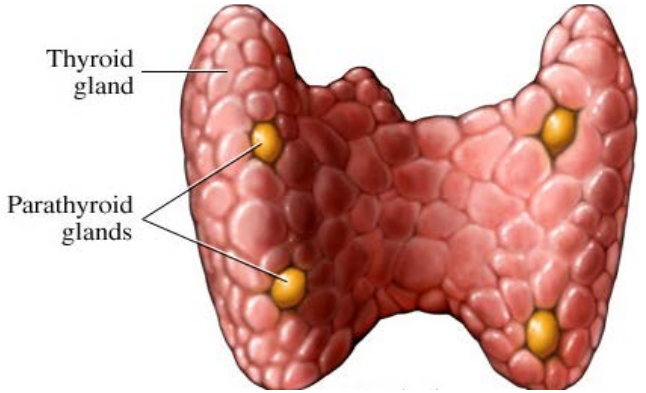
Laboratory testing of PTH

- PTH
  - Immunoassay, usually sandwich type, for intact PTH
  - ALWAYS report with Ca level
  - PTH stable at room temperature in EDTA
  - Can’t perform calcium on EDTA tube
  - Useful for differential diagnosis of hypercalcemia and hypocalcemia

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

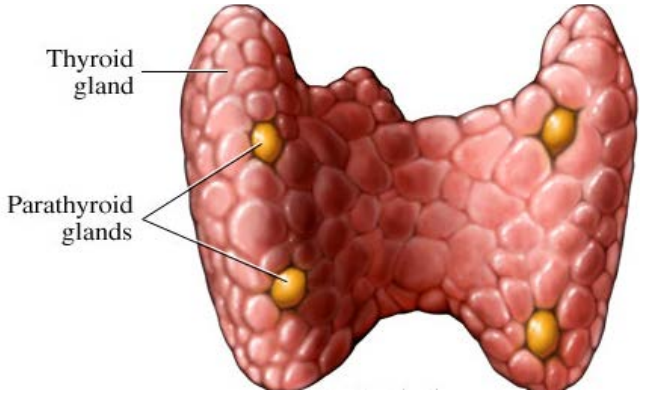


Laboratory testing of PTH

- PTH
  - Intra-operative PTH

    on fluid (saline)
    - Thyroidectomy, leaving

      parathyroid glands intact
    - Flush tissue with saline and send saline to lab for PTH
    - LDT!!!



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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

  - Used to have population based reference intervals - Different intervals for summer and winter (or north and south!)

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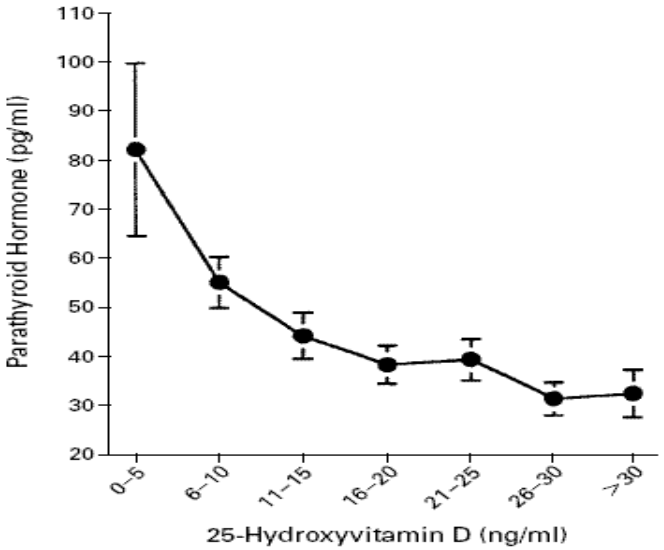
Vitamin D Reference Intervals

- If Vitamin D levels are low, PTH should rise to activate more to the bioactive form
  - Measured Vitamin D and PTH in samples
  - Determined concentration of Vitamin D at which PTH concentration goes up



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Laboratory testing of Vitamin D

- Vitamin D
  - Changed to health based reference intervals
    - < 20 ng/mL – deficient
    - 20 – 29 ng/mL – insufficient
    - 30 – 80 ng/mL – sufficient
    - > 80 ng/mL – toxic
  - 2011 IOM report
    - Serum 25-OHD range – **20 – 50** ng/mL
  - Problem? – Not all D assays created equal
    - Same sample, 8 methods, results = **23** to **85** ng/mL

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Summary

- Hormonal control of calcium homeostasis is via PTH and Vitamin D
- Bone formation and resorption processes both result in biochemical markers which are most useful for monitoring therapy for metabolic bone disorders
- Measurement of free calcium provides the most information on calcium status but has not replaced total calcium measurement
- In order to allow for more correct interpretation of PTH results, a calcium result should be provided with a PTH determination
- Vitamin D measurement is currently not standardized between assays

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Self Assessment Questions

1. Which of the following sets of lab results is consistent with pseudohypoparathyroidism?

	PTH	Serum Calcium	Serum phosphate	Urine calcium
A	↑	↑	N to ↓	↑
B	↑	Normal	Normal	↓
C	↑	↓	↑	↓
D	↓	↓	↑	↓

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Self Assessment Questions

2. Serum calcium concentration:
   a. Directly effects activation of 25-OH-Vitamin D to 1,25 diOH-D
   b. Directly causes suppression or induction of PTH production
   c. Is independent of albumin concentration
   d. Provides more useful information if only total calcium is measured rather than total and ionized

Self Assessment Questions

3. Markers of bone resorption include:
   a. Osteocalcin, osteoprotegrin and N-telopeptide crosslinks
   b. N- telopeptide crosslinks, tartrate –resistant acid phosphatase, and deoxypyridinoline
   c. Osteocalcin, C-terminal propeptide of type 1 collagen and bone alkaline phosphatase
   d. C-terminal telopeptide crosslinks, bone sialoprotein and bone alkaline phosphatase

Self Assessment Questions

4. 25-OH-Vitamin D:
   a. Has the hydroxyl group added to the 25 position in the liver
   b. Is usually measured by immunoassays that differentiate between D2 and D3 forms
   c. Gives comparable results with all methods and thus can use one reference interval
   d. Is the biologically active form

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Answers

1. C
2. B
3. B
4. A

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