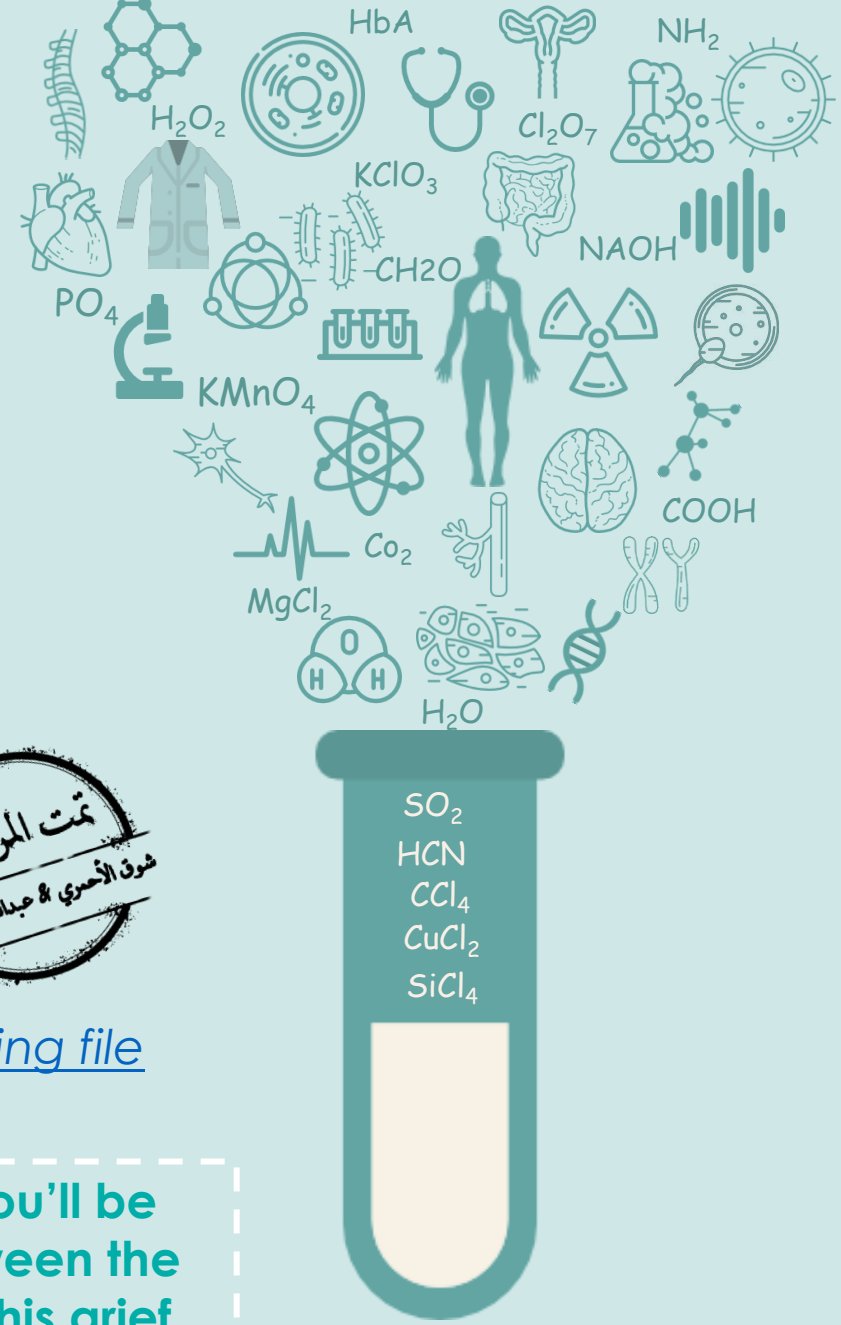


Important Doctors slides

Extra Information Doctors notes

تمت المراجعة

شوق الأحمري & عبدالعزيز العنقري

[Editing file]

Biochemistry

Vitamin D, Rickets and Osteoporosis

One day you'll be person between the patient and his grief. Please study well ..

OBJECTIVES

By the end of this lecture, the students should be able to:

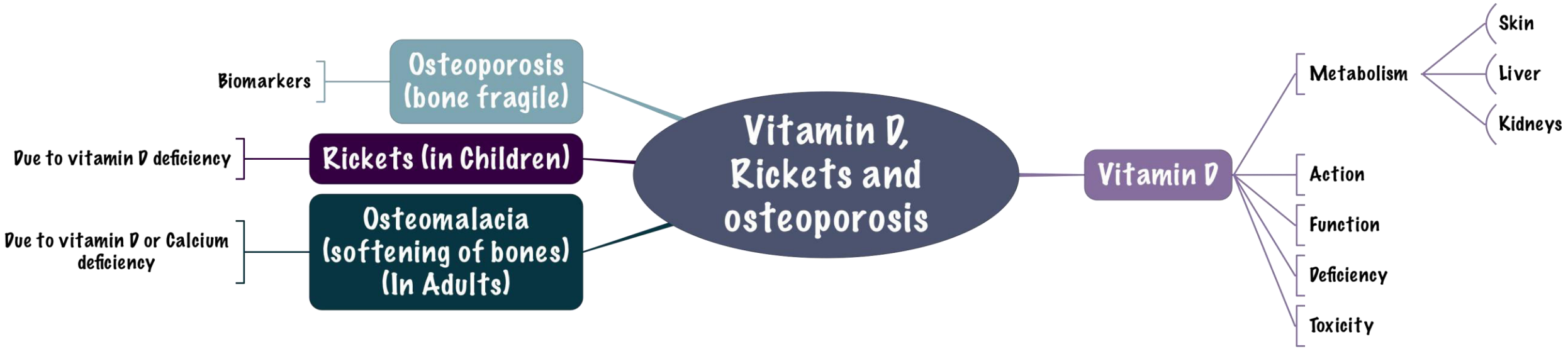
- Understand the functions, metabolism and regulation of vitamin D
- Discuss the role of vitamin D in calcium homeostasis
- Identify the types and causes of rickets
- Correlate vitamin D and calcium deficiency in osteoporosis
- Identify biomarkers used for the diagnosis and follow up of osteoporosis

Lippincott's Biochemistry 6th Edition

Clinical Biochemistry: An illustrated colour text 4th Edition by Allan Gaw (Churchill Livingstone)

Wheater, G. et al. The clinical utility of bone marker measurements in osteoporosis. J. Trans. Med. 2013, 11: 201-214.

Overview


Vitamin D, Rickets and osteoporosis
Osteoporosis (bone fragile)
Biomarkers
Rickets (in Children)
Due to vitamin D deficiency
Osteomalacia (softening of bones) (In Adults)
Due to vitamin D or Calcium deficiency
Vitamin D
Metabolism
Skin
Liver
Kidneys
Action
Function
Deficiency
Toxicity

Vitamin D

- It considered a steroid hormone

1-Cholecalciferol (vitamin D3):

is synthesized in the skin by the sunlight (UV) (UV type B- rays)

The biologically active form is

1,25-dihydroxycholecalciferol (calcitriol)

2-Ergocalciferol (vitamin D2) is derived from

Ergosterol in plants

D3, D2 are also available

as supplement

2 vitamins that we consider as hormones: (Steroid in type)
1- Vitamin D. 2- Vitamin A.
Why?
Recall: hormones are chemical messengers released from endocrine cells and circulating in the blood and work to accomplish intercellular interactions.
And since they are steroid hormones they diffuse into the cellular membrane and they bind to their receptors inside the cell and then go to the nucleus to binds with hormone-response element (HRE) (which is a specific sequence in the DNA) to affect the transcription of multiple types of genes نفس ما درسنا بالميد

Vitamin D distribution

Dietary sources:

- Ergocalciferol (vitamin D2) found in plants.
- Cholecalciferol (vitamin D3) found in animal tissue.

So it is either from the diet or exposure

Endogenous vitamin precursor:

- 7-Dehydrocholesterol is converted to vitamin D3 in the dermis and epidermis exposed to UV in sunlight.

يعني هو الريدي يبكون موجود داخل الجسم ولما يتعرض للشمس يتحول لـD3

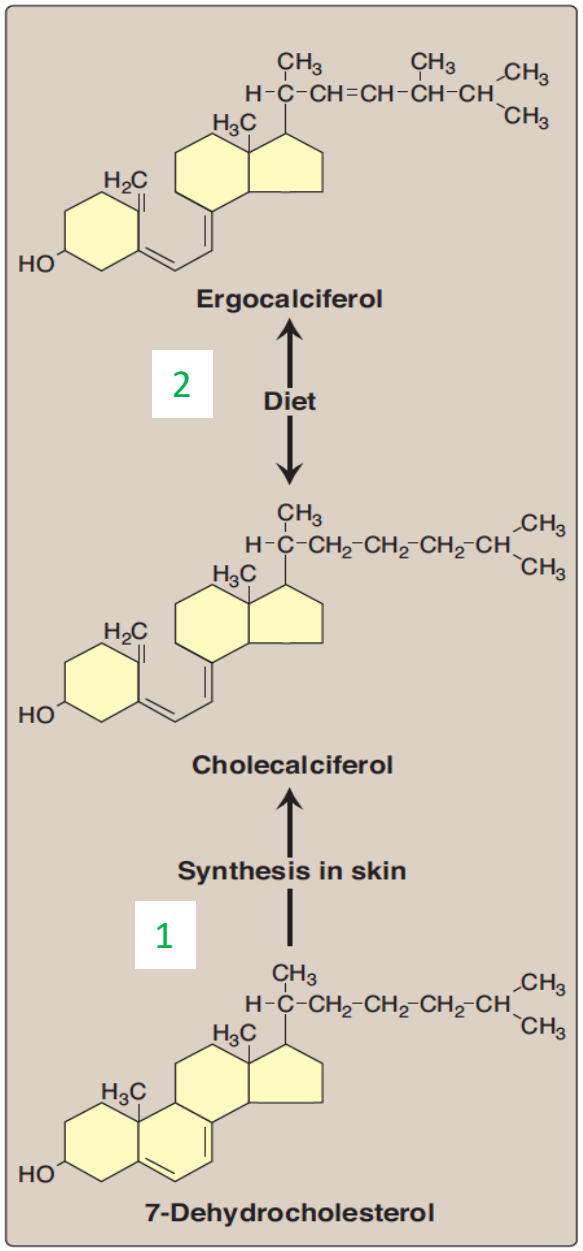
D2 and D3 both can be converted into its biologically active form which is 1,25-dihydroxycalciferol.

Daily requirement (IU/day):

- Adults: 600
- Children: 400
- Elderly: 800
- Upper limit of intake: 4000

- High doses (10,000 IU for weeks or months) can lead to toxicity.
- Hypercalcemia and deposition of calcium in arteries and kidneys.

Sources of Vitamin D



1. After your skin exposed to sun UV light it convert 7-Dehydrocholestrol (which is already synthesized in your body) into Cholecalciferol (D3).

2. Another form is from the plants (Ergocalciferol) also the body can convert it into Cholecalciferol.

- Cholecalciferol still need to be modified by the addition of 2 hydroxyl groups, the first one in the first carbon and the second one at 25th carbon and, then you can get the biologically active form which is Calcitriol.
- Cholesterol is the precursor of cholecalciferol

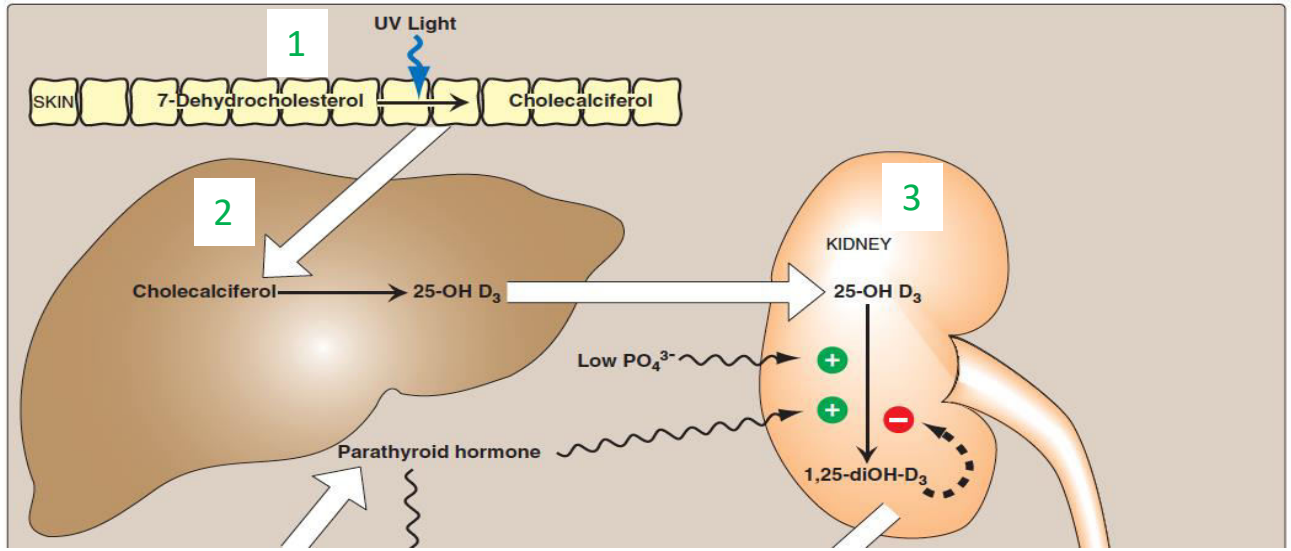
Metabolism of vitamin D

Follow the numbers pleases :

1. In the skin you synthesize Cholecalciferol from 7-Dehydrocholesterol (which is already in your body) when it exposed to sun UV light.

2. then it will go to the liver, because the liver has the enzyme (25- hydroxylase) to add the hydroxyl group in the 25th Carbon, so it becomes 25-hydroxycolecalciferol, this form is the most abundant form in the plasma and this is the storage form and the form we check in vitamin D blood test.

3. After that it will go to the kidney which have the enzyme (1alpha-hydroxylase) which will add a hydroxyl group to the first Carbon to get the final form which is 1,25-dihydroxycholecalceferol which is the final functional form of vitamin D and the form which will be released from the kidney to the circulation.



Summery of Vitamin D metabolism

- storage form.
- most abundant form.
- form we check in vitamin D blood test.

Ergocalciferol (Vit.D2) (from plants)

Converted By body into:

7-Dehydrocholesterol

Sun UV light

Cholecalciferol (Vit. D3)

25- Hydroxylase (+OH in 25th C)

25-hydroxyColecalciferol

Liver

Skin

(1-α-hydroxylase) (+OH in 1st C)

25-hydroxyColecalciferol

1,25-dihydroxyColecalciferol (Calcitriol) (Active form of Vit.D)

Kidney

Actions of vitamin D

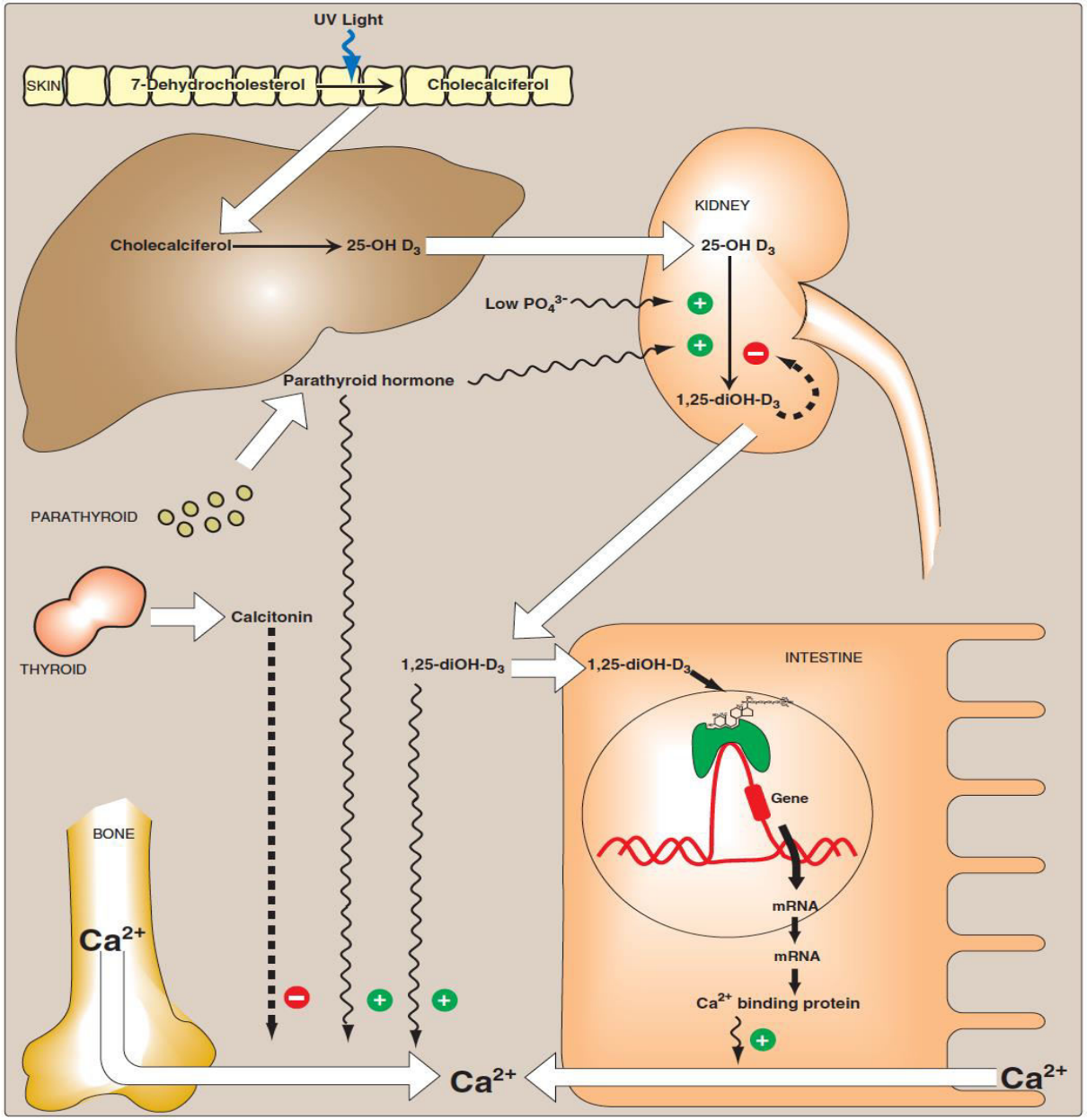
1,25 diOH- D3 has a lot of effects, one of the them are intestinal cells it helps in the absorption of Ca, it does it by going to intestinal cells and affects gene expressions of some of the proteins which includes Ca+2 or cab binding protein and this helps in the absorption of ca2 from the intestine to the bloodstream (which will increase the Ca+2 ions in the plasma)

Decrease in Ca causes the release of parathyroid hormone, which activates 1-alpha hydroxylase which will increase the functional form of vitamin D (1,25-diOH-D3) that can increase Ca concentration, So 1,25 diOH-D3 and PTH cause increase in Ca level.

Calcitonin: cause decrease in Ca level

Q: What will stimulate 1,25-diOH-D3 synthesis?
A: PTH and low PO4

Q: What will inhibit 1,25-diOH-D3 synthesis?
A: 1,25-diOH-D3 itself by –ve feedback



Vitamin D metabolism

In skin: Cholecalciferol (Vitamin D3) is derived from 7-dehydrocholesterol by the sunlight

In liver: Cholecalciferol is converted to 25-hydroxycholecalciferol (calcidiol) by the enzyme 25-hydroxylase

In kidneys:

- The 1-α-hydroxylase enzyme converts 25-hydroxycholecalciferol to 1,25-dihydroxycholecalciferol (biologically active).
- Active vitamin D is transported in blood by gc-globulin protein

- We always measure 25-hydroxycholecalciferol BUT if the patient has Renal problem then we measure 1,25-dihydroxycholecalciferol, Why? Because in this case the 25-hydroxycholecalciferol will not reflect your functional or active Vit.D amount, due to the problem in the kidney.
- Why we don't' measure both from the beginning? because 1,25-dihydroxycholecalciferol measurement is expensive and complicated so we don't do it for all patients.

Gc-protein or vitamin D binding protein(other name).

Vitamin D regulation and calcium homeostasis

Vitamin D synthesis is tightly regulated by plasma levels of phosphate and calcium.

Activity of 1-α-hydroxylase in kidneys is: (Point of regulation)

- Directly increased due to low plasma phosphate.
- Indirectly increased via parathyroid hormone (PTH) due to low plasma calcium.
- PTH increases vitamin D synthesis in kidneys.

Vitamin D has essential role in calcium homeostasis.

Calcium homeostasis is maintained by parathyroid hormone (PTH) and calcitonin.

Function of calcitonin: When Ca plasma levels is increased it stimulates calcitonin to be released and decreases the activity of osteoclasts (cells responsible for bone resorption), so it inhibits bone resorption then it doesn't lead to furthermore production of calcium.

Recall:
PTH: comes from para thyroid gland
Calcitonin: comes from thyroid gland

Vitamin D action

Vitamin D action is typical of steroid hormones.

It binds to intracellular receptor proteins.

The receptor complex interacts with target DNA in cell nucleus.

This stimulates or represses gene expression.

Vitamin D functions

- Regulates plasma levels of calcium and phosphate.
- Promotes intestinal absorption of calcium and phosphate.
- Stimulates synthesis of calcium-binding protein for intestinal calcium uptake.
- Minimizes loss of calcium by the kidneys.
- Mobilizes calcium and phosphate from bone to maintain plasma levels.

It can increase bone mineralization and formation by acting upon osteoblasts

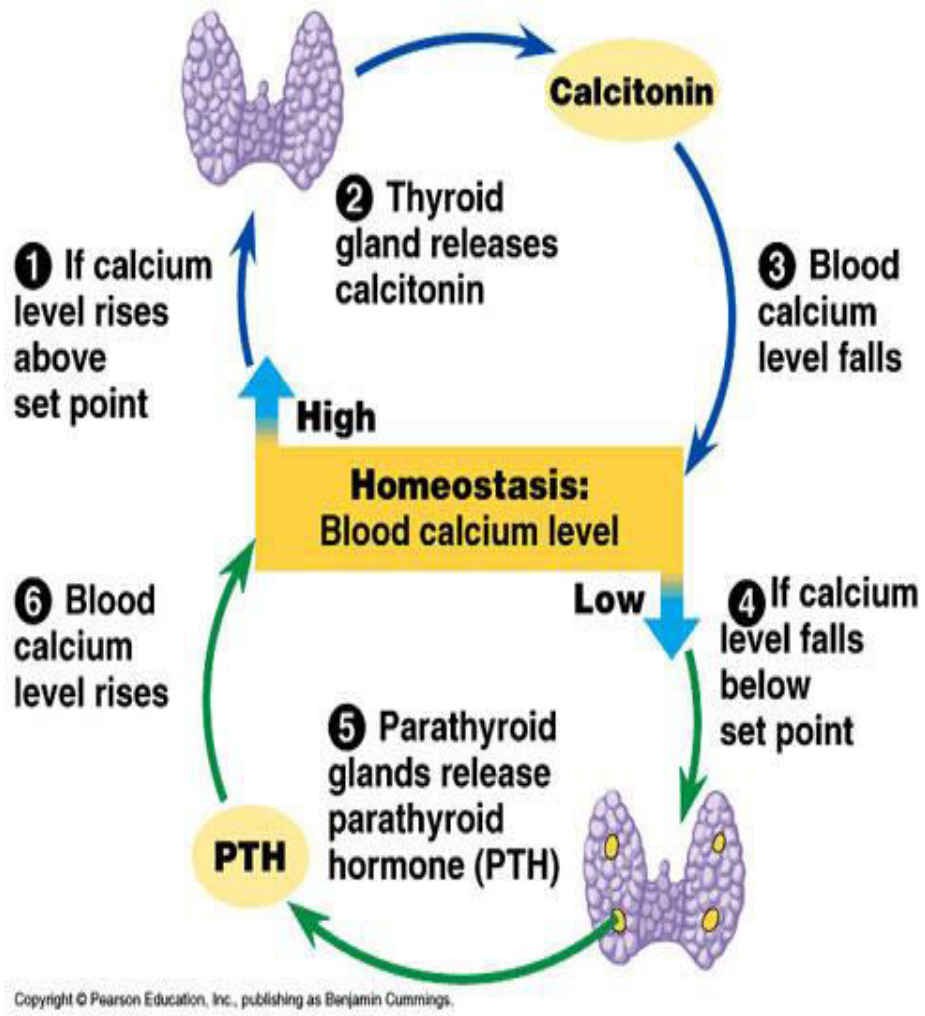
By increasing calcium reabsorption from the kidney.

By acting on the osteoclasts to mobilize the Ca and PO4 from the bone to maintain plasma levels.

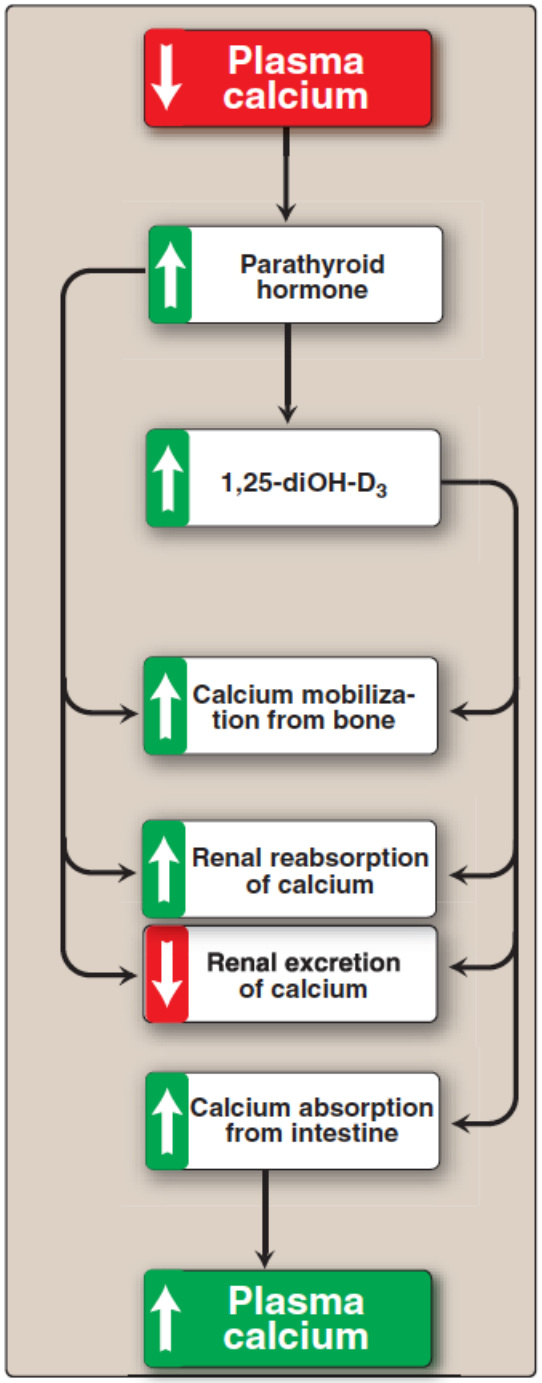
Vitamin D response to low plasma calcium

So vitamin D helps the intestinal cells to absorb of calcium via increasing the synthesis of Ca-binding protein (by stimulation of gene expression in that responsible for this protein synthesis), this protein will enhance Ca absorption from intestine (see image in slide 9).

So the enzyme 1-alpha-hydroxylase is actually regulated by calcium. How? When you have low plasma levels of calcium It causes the release of parathyroid hormone (so low Ca is a stimulus for PTH) and what PTH is released it goes and activates the enzyme 1alpha-hydroxylase and therefore 1alpha-hydroxylase increase the synthesis of the functional form of vitamin D that can go again and increase calcium level concentration by PTH.



Intestine are only affected by vitamin D and not by PTH.



Vitamin D deficiency

Deficiency most common worldwide.

High prevalence in Saudi Arabia due to:

- Low dietary intake.
- Insufficient exposure to Sun.
- Lifestyle (eg. clothing esp. in women)

Circulating level of >75 nmol/L is required for beneficial health effects.

Actually we have 3 categories for measurements:
-Below 50= Vitamin D deficiency.
-Between 50 – 75 =. Vitamin D insufficiency.
-Above 75 = Vitamin D sufficiency.

Nutritional Rickets

A disease in children causing net demineralization of bone.

With continued formation of collagen matrix of bone.

Incomplete bone mineralization.

Bones become soft and pliable.

Causes skeletal deformities including bowed legs.

Patients have low serum levels of vitamin D.

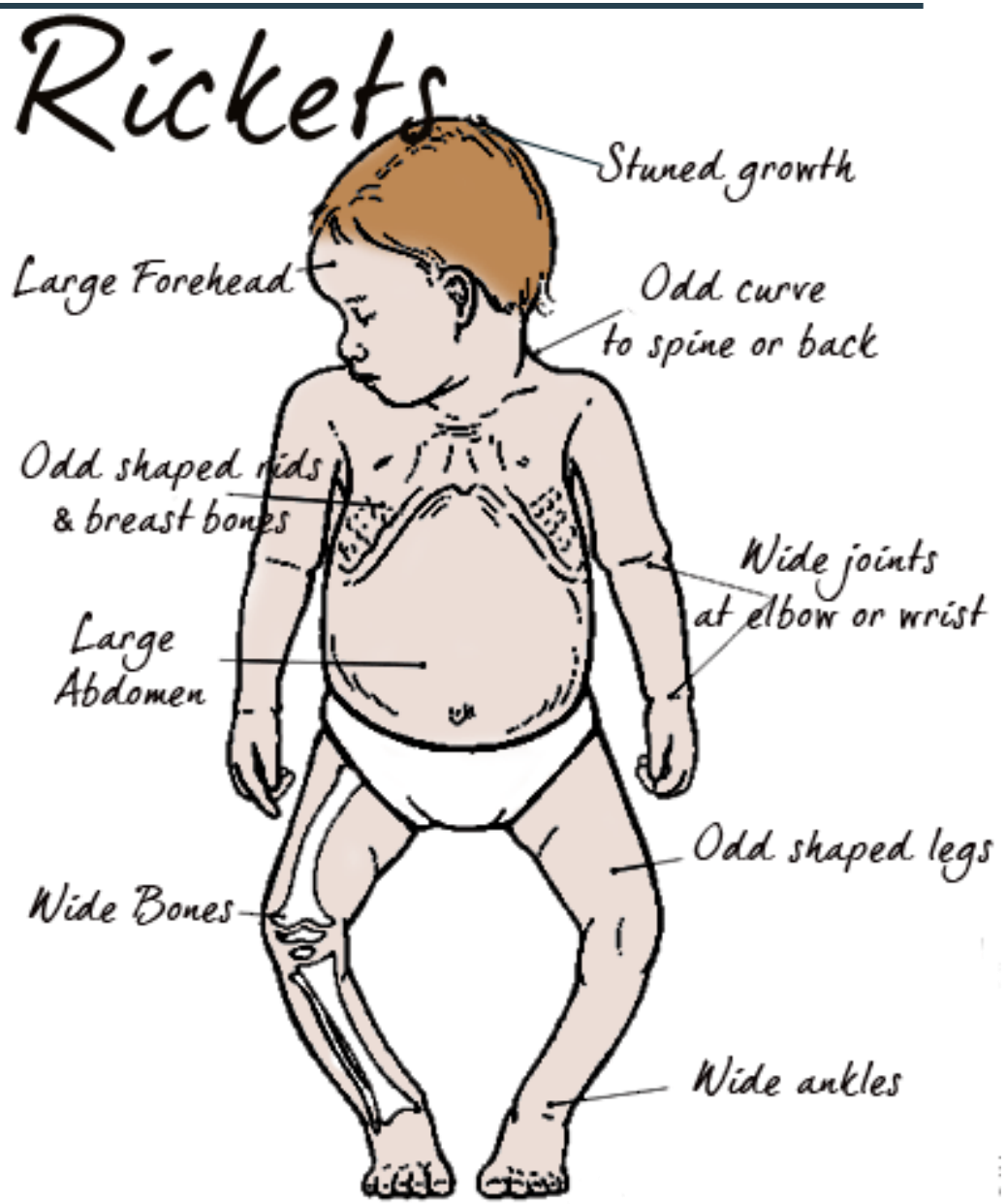
(Osteomalacia: demineralization of bones in adults, due to nutritional deficiency of vitamin D)

Here we are talking about bone development.
If the bone is not getting mineralized properly it becomes weak.

Mainly in Rickets not in osteomalacia.

The Bone starts from a healthy bone.
But when the bone is being remodeled it doesn't have proper mineralization.

Rickets


Rickets
Stuned growth
Large Forehead
Odd curve
to spine or back
Odd shaped ribs
& breast bones
Wide joints
at elbow or wrist
Large
Abdomen
Odd shaped legs
Wide Bones
Wide ankles

Rickets

Rickets has 2 causes:
1- Vitamin D deficiency.
2- Genetic defect leading to vitamin D deficiency.

❖ Causes

1. Vitamin D deficiency because of:

- Poor nutrition
- Insufficient exposure to sunlight
- Renal osteodystrophy (causes decreased synthesis of active vitamin D in kidneys).
- Hypoparathyroidism (hypocalcemia)

Leading to insufficient hydroxylation of the storage form of vitamin D into the functional form.

2. Inherited rickets

Vitamin D-dependent rickets (types 1 and 2)

Rare types of rickets due to genetic disorders

Causing vitamin D deficiency mainly because of genetic defects in:

- Vitamin D synthesis
- Vitamin D receptor (no hormone action), This type is much more prevalent.

Diagnosis and treatment of Rickets

Measuring serum levels of:

- 25-hydroxycholecalciferol Low
- PTH
- Calcium
- Phosphate
- Alkaline phosphatase

We studied it in GIT block as one of the tests done in liver function tests and was not specific in the case of the liver, but it has an isotype specific for bones (high levels indicates problem in the bones) because it is produced by the osteoblast.

Treatment:

- Vitamin D and calcium supplementation

Osteoporosis

- Reduction in bone mass per unit volume
- Bone matrix composition is normal but it is reduced
- Post-menopausal women lose more bone mass than men (primary osteoporosis)
- Increases fragility of bones
- Increases susceptibility to fractures

So rickets the quality of bone is defected, while in osteoporosis the quality is good but the problem is in bone density and amount of matrix (no problem in mineralization but there is loss or reduction in bone mass).

Osteoporosis

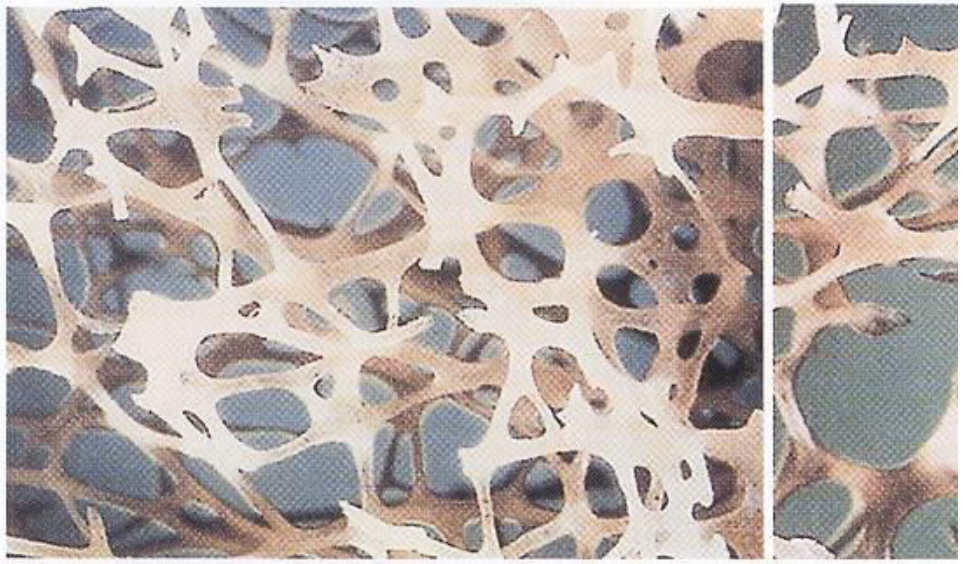
Secondary osteoporosis may be caused by:

- Drugs e.g. asthma drugs
- Immobilization
- Smoking
- Alcohol
- Cushing's syndrome
- Gonadal failure
- Hyperthyroidism, Thyroid hormones affect the synthesis of collagen & osteoblast activity.
- GI disease

Most of these causes are somehow related to vitamin D deficiency (or improper absorption)

Primary osteoporosis: is that happen in Post-menopausal women and we don't know the exact mechanism.

Osteoporosis



(a) (b)

Fig. 1 **Bone showing (a) normal trabeculae and (b) bon**

Fig. 2 **Crush fractures of vertebral bodies in a patient with osteoporosis.**

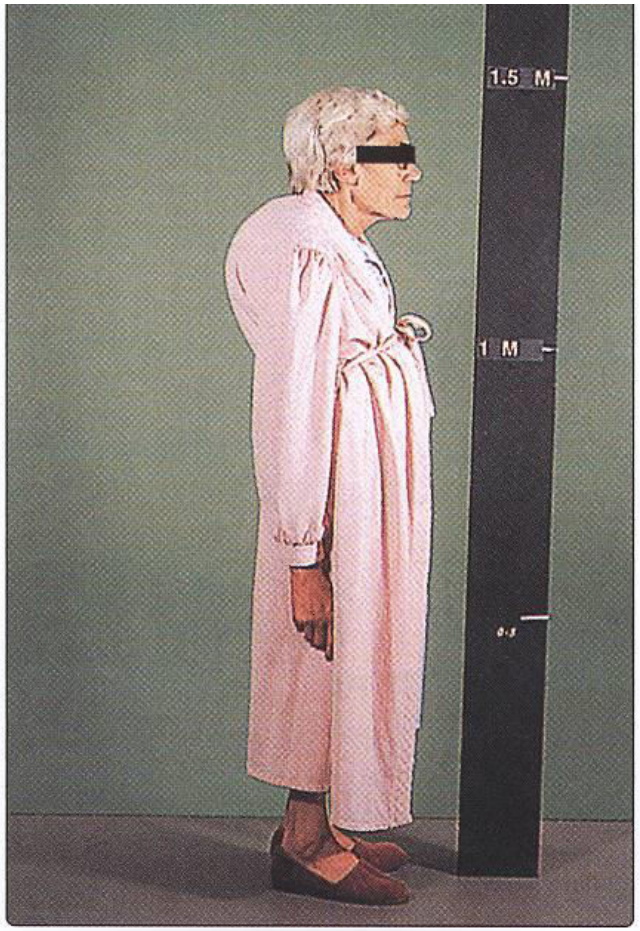


Fig. 3 **Elderly woman with so-called 'Dowager's hump' from collapsed vertebrae due to osteoporosis.**

Diagnosis of osteoporosis

- **<u>WHO standard:</u>** Serial measurement of bone mineral density.
- Biochemical tests (calcium, phosphate, vitamin D) alone cannot diagnose or monitor primary osteoporosis
- The test results overlap in healthy subjects and patients with osteoporosis
- Secondary osteoporosis (due to other causes) can be diagnosed by biochemical tests

DEXA scan: is a technique where the bone density is measure.
It measures the amount of ca per square cm of the bone comparing it to what should it there for 30 year individual

Biomarkers of osteoporosis

❖Bone formation markers

Osteocalcin

- Produced by osteoblasts during bone formation
- Involved in bone remodeling process
- Released during bone formation and resorption (bone turnover)
- Short half-life of few minutes
- Blood levels are influenced by Vitamin K status and renal function.

Recall from GI: It is related to vitamin K when they carboxylate blood coagulation factors to maturate them.

Bone-specific alkaline phosphatase

- Present in osteoblast plasma membranes
- Helps osteoblasts in bone formation
- A Non-specific marker
- The isoenzymes also interfere with the assay
- Its isoenzymes are widely distributed in other tissues

P1NP (Procollagen type-1 amino-terminal propeptide)

- Produced by osteoblasts
- Involved in the process of type 1 collagen formation
- Shows good assay precision
- Stable at room temperature
- Blood levels are highly responsive to osteoporosis progression and treatment

Involved in type 1 collagen synthesis.
So when there is bone synthesis the P1NP levels will be elevated.

Biomarkers of osteoporosis

❖Bone resorption markers

CTX-1 (Carboxy-terminal cross-linked telopeptides of type 1 collagen)

- A component of type-1 collagen
- Released from type-1 collagen during bone resorption
- Blood and urine levels are highly responsive to post-resorptive treatment
- Levels vary largely by circadian variation

In the osteoclasts.
So when there is osteoclastic activity its levels will be elevated (during resorption)

- So the blood samples has to be taken at the same time (morning or afternoon)

Treatment and prevention of osteoporosis

❖Treatment

- In confirmed cases of osteoporosis
  - Treatment options are unsatisfactory
- Oral calcium, estrogens, fluoride therapy may be beneficial
- Bisphosphonates inhibit bone resorption that slows down bone loss

❖Prevention

- Prevention from childhood is important.
- Good diet and exercise prevent osteoporosis later.
- Hormone replacement therapy in menopause may prevent osteoporosis.

Take home messages

- Overview of vitamin D metabolism and regulation.
- Importance of vitamin D functions.
- Vitamin D deficiency is common in populations.
- Rickets and osteomalacia are due to vitamin D deficiency.
- Various biochemical markers clinically important for assessment of osteoporosis.

QUIZ

Q1 : Which one of the following is correct in Vit.D deficiency?

A. Associated with sufficient exposure to sun
B. Has nothing to do with a human's lifestyle
C. It's related to vaccinations during childhood
D. Associated with loss of calcium from the kidneys

Q2 : 22 year old smoker, came to the E.R. with a bone fracture. His brother mentioned that he has been inactive for the past few years because of med school and that he also had a fracture 2 months ago when playing football with his high school friends.
Which one of the following is most probably the cause of his fracture?

A. Osteomalacia
B. Hypothyroidism
C. Osteoporosis
D. Crohn's disease

Q3 : Which one of the following is the most common cause of rickets?

A. Nutritional
B. Inherited

Q4 : Which one of the following is the active form of Vit.D?

A. 25-Hydroxycholecalciferol
B. Calcitriol
C. 7-dehydrocholestrerol
D. Cholecalciferol

Q5 : Which one of the following is a protein responsible for transportation of the active form of Vit.D in the blood?

A. Albumin
B. GC-Globulin
C. Membrane transport protein
D. Rhodopsin

Q6 : 12 year old boy, his monthly intake of Vit.D is 9000 IU. Which one of the following is correct about him?

A. His daily intake is deficient by 100 IU
B. His daily intake is excessive by 100 IU
C. His daily intake is deficient by 150 IU
D. His daily intake is excessive by 150 IU

QUIZ

Q7 : 6 year old boy came to your clinic with his older sister who is a medical student.
She says that she is worried about him because his legs have been bent slightly.
But because she wants to make sure you're a good doctor, she asked you a few questions.

A) Can you tell me what activates Vit.D?

Low Phosphate, Low Calcium & High Parathyroid hormone

B) Mention 3 clinical features that are associated with rickets.

1. Soft pliable bones
2. Bowed legs
3. Low serum levels of vitamin D

C) How is rickets diagnosed?

By measuring levels of: 25-Hydroxycholecalciferol, PTH, Calcium, Phosphate and Alkaline phosphatase.

D) What is the possible treatment ?

Vit.D and calcium supplementation.

Suggestions and recommendations

1) D 2) C 3) A 4) B 5) B 6) A

Biochemistry Team 436

THANK YOU

FOR CHECKING OUR WORK

PLEASE CONTACT US IF YOU HAVE ANY ISSUE

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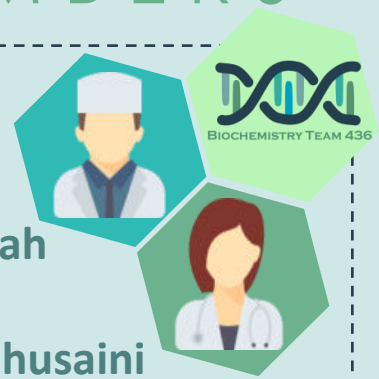

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