Revised & Approved

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BIOCHEMISTRY TEAM
439

MED439
KING SAUD UNIVERSITY

Vitamin D, Rickets & Osteoporosis

Editing File

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Objectives

- Understand the functions, metabolism, regulation and deficiency 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

Overview

- Vitamin D distribution, metabolism, regulation and functions.
- Vitamin D in calcium homeostasis
- Vitamin D deficiency
- Nutritional and inherited rickets (types, diagnosis and treatment)
- Osteoporosis (Diagnosis, biomarkers, treatment and prevention

Vitamin D

1. Vitamin D is considered a steroid hormone
2. **Cholecalciferol (vitamin D3)** is synthesized in the skin by sunlight. Fish oil, fatty fish and egg yolk.
3. The biologically active form of cholecalciferol is **1,25-dihyroxycholecalciferol (calcitriol)**
4. **Ergocalciferol (vitamin D2)** is derived from ergosterol in plants
5. D3, D2 are also available as supplements
   Both can be converted to the active form (1,25-dihydroxycholecalciferol)

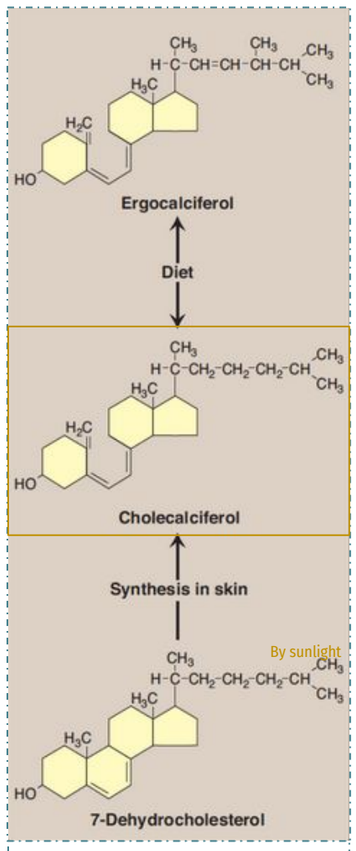
Vitamin D Distribution

Endogenous vitamin precursor

Vit D is the only vitamin that **cells can produce**

1. 7-Dehydrocholesterol is converted to vitamin D3 in the dermis and epidermis exposed to UV in sunlight

Cholesterol is a precursor of most steroid hormones and vitamin D, unlike the harmful is LDL.
- Vit D is of two types: endogenous and exogenous.
- Vit D is fat soluble, can be stored in the liver and cause toxicity so it is very important to take it in a controlled way



موجود بالبشرة و بعد التعرض للشمس يتحول ل cholecalciferol

Regardless of source (plant, endogenou), all forms get converted into cholecalciferol

Dietary sources

2.Ergocalciferol (vitamin D2) found in plants

3.Cholecalciferol (vitamin D3) found in animal tissue

كلهم متشابهين بس الفرق:
Methyl group and double bond in ergocalciferol.

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- a-hydroxylase enzyme converts 25-hydroxycholecalciferol to 1,25-dihydroxycholecalciferol (due to 2OH) (biologically active)

Transporter

Active vitamin D is transported in blood by **gc-globulin** protein
(vit D binding protein)
Because it is hydrophobic

Vitamin D Regulation and calcium homeostasis

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

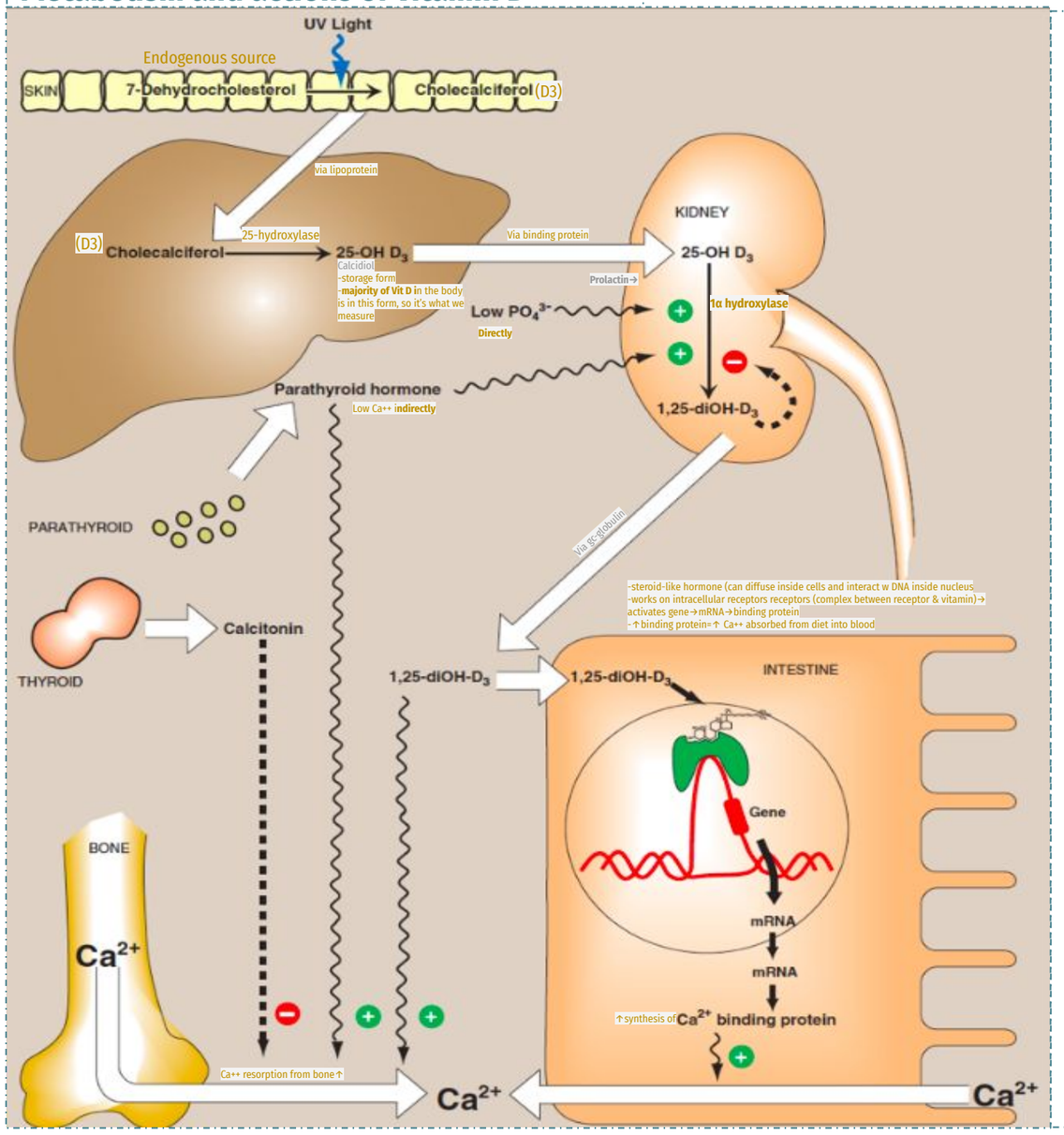
Vitamin D has essential role in calcium homeostasis

Activity of **1- a-hydroxylase** in kidneys is:

- 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

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

Metabolism and actions of vitamin D



- **In the skin**, after 7-dehydrocholesterol is converted to cholecalciferol, all cholecalciferol will transported by binding proteins (because it is hydrophobic) to the liver.

-**In the liver,** carbon number 25 on cholecalciferol is hydroxylated(by 25-hydroxylase), converting cholecalciferol to 25-hydroxycholecalciferol . 25-hydroxycholecalciferol will be transported by a binding proteins to the kidney.

-**In the kidney,** anther hydroxylation will happen(at carbon number 1) by 1-α-hydroxylase enzyme, converting 25-hydroxycholecalciferol to 1,25-dihydroxycholecalciferol.

-We call these two reactions **2 sequential hydroxylations**. The **2nd hydroxylation is very important** because it forms the most active form(1,25-dihydroxycholecalciferol), so it is **tightly regulated**:

A) Negative feedback to stop/slow down this reaction

B) Positively regulated by:

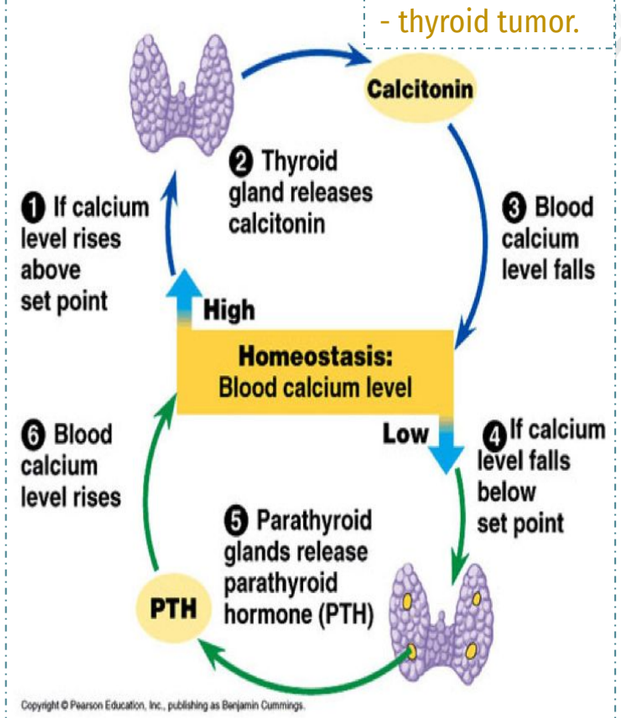
1. Directly will be stimulated by Low PDF
2. Indirectly by low Ca^{++} which lead to stimulation of PTH⇒stimulation of 1- a-hydroxylase enzyme.

1,25-dihydroxycholecalciferol is considered a steroid hormone. Because it has steroid-like activity, it can diffuse inside the intestinal cell. Then, the Vitamin D and receptor complex interacts with DNA inside the nucleus and stimulates the synthesis of Ca^{++}-like Protein. Ca^{++} like Protein is like a car (taxi) and will transport the Ca^{++} from intestine (Milk or food) to inside the body.

- 25-hydroxycholecalciferol: (عادة يتم تخزينه بالجسم و هو اللي يتم قياسه)

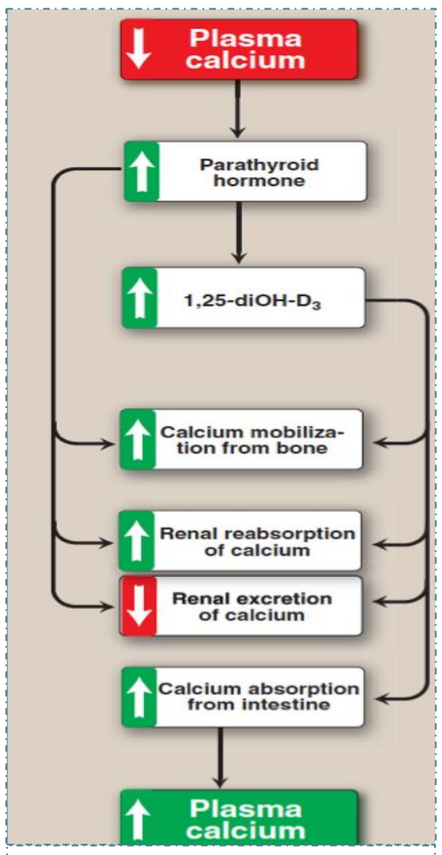
- 1,25-dihydroxycholecalciferol: the most powerful , and consider as steroid hormone because it has steroid like activity, it can diffuse inside the intestinal cell.

قياسه مهم :
- for bone.
- thyroid tumor.



Calcium homeostasis

Predominant action is for parathyroid



Vitamin D response to low plasma calcium

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 (when we need to have more Ca^{++}) or represses (when we have enough Ca^{++}) 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 (biggest reservoir of Ca^{++} in the body) to maintain plasma levels

Daily requirement (IU/day):

IU = International Unit

 Children:	Adults:	 Elderly:	 Boys slides ONLY Upper limit of intake:
400	600	800	4000

لانهم عادة ما يتعرضوا للشمس كثير، او اكلهم اقل، فنخاف عليهم من osteomalacia ونعطيهم كمية أكثر من ال adults

Vitamin D intake and toxicity

Boys slides ONLY

01 High doses (10,000 IU for weeks or months) can lead to toxicity

02 Hypercalcemia and deposition of calcium in arteries and kidneys

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

Rickets (Osteomalacia) الكساح

Rickets: normal or increase in collagen, with decrease in bone mineralization leading to disturbed ratio

Nutritional rickets

- A disease in children causing net **demineralization** of bone (ال minerals هي اللي تعطي الصلابة للعظام)
- With continued formation of collagen matrix of bone (normal collagen matrix but there is no minerals to make it more strong).
- Incomplete bone mineralization
- Bones become soft and pliable (تتقوس الأقدام لأنها ما تصير قوية، والوزن ينزل على الرجلين).
- Causes skeletal deformities including bowed legs
- Patients have low serum levels of **vitamin D**
- **Osteomalacia** ليونة العظام : demineralization of bones in adults due to nutritional deficiency of Vit. D

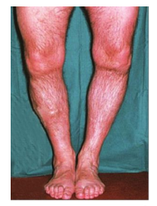


Figure 28.25
Bowed legs of middle-aged man with osteomalacia, a nutritional vitamin D deficiency that results in demineralization of the skeleton.

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)

Causes

Vitamin D deficiency because of:

- Poor nutrition
- Insufficient exposure to sunlight
- Renal osteodystrophy (causes decreased synthesis of active vitamin D in kidneys)
- Hypoparathyroidism (hypocalcemia) (due to loss of Ca^{++} Binding Protein, can't Absorb Ca^{++} ⇒ even with Good nutrition).

Diagnosis

Measuring serum levels of:

- 25-hydroxycholecalciferol (low)
- PTH (due to low Ca^{++} > will be high)
- Calcium (low)
- Phosphate (low)
- Alkaline phosphatase (high)

Treatment

Vitamin D and calcium supplementation

Osteoporosis هشاشة العظام

Osteoporosis: decrease in both collagen and mineralization, so the ratio is normal, but the whole bone mass is decreased

Definition	Secondary 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 الضربة البسيطة ممكن تسبب كسور مرضية • Increases susceptibility to fractures	**caused by:** -Drugs -Immobilization -Cushing syndrome -Smoking -Gonadal failure -Alcohol -Hyperthyroidism -GI disease

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

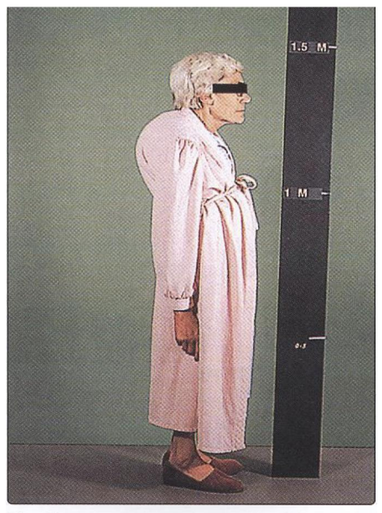
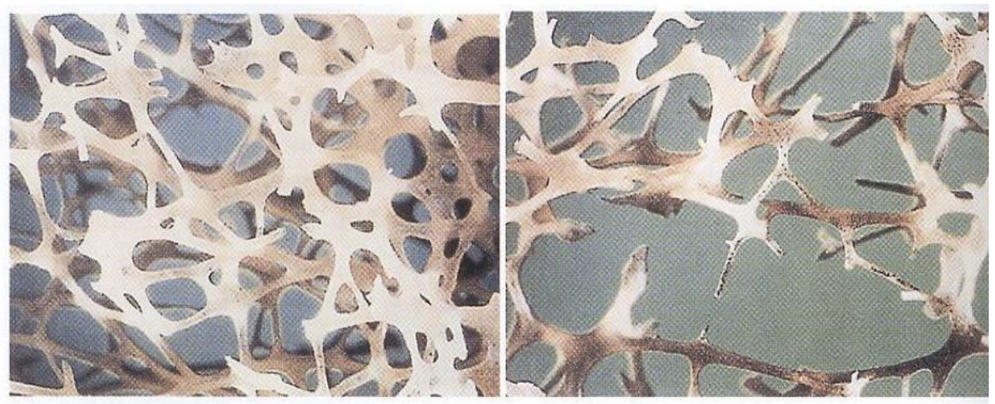


Fig. 3 **Elderly woman with so-called 'Dowager's hump' from collapsed vertebrae due to osteoporosis.**



(a) (b)

Fig. 1 **Bone showing (a) normal trabeculae and (b) bone loss in osteoporosis.**

Osteoporosis

Diagnosis of osteoporosis

To follow up the treatment of osteoporosis they used to do DEXA every 2 years to measure bone density, but now they can measure these markers every 2-3 months by these markers

- **WHO standard:** Serial measurement of bone mineral density
- Biochemical tests (calcium, phosphate, vitamin D) alone cannot diagnose or monitor primary osteoporosis. But Rule out Osteomalacia.
- The test results overlap in healthy subjects and patients with osteoporosis
- Secondary osteoporosis (due to other causes) can be diagnosed by biochemical tests e.g. Cushing syndrome = measure cortisol
  Gonadal failure = measure LH + FSH
  Hyperthyroidism = measure TSH + T_4

Biomarkers of Osteoporosis

1-Bone <u>formation</u> markers:

Osteocalcin aka Bone Gla Protein #GIT

- **Produced by osteoblasts during bone formation**
- Involved in bone remodeling process
- Released during bone formation and resorption (bone turnover) The higher the bone turnover, the higher the Osteocalcin
- Short half-life of few minutes so its useful only in research not day-to-day patients in hospital
- Blood levels are influenced by vitamin K status and renal function

Bone-specific Alkaline Phosphatase

- **Present in osteoblast plasma membranes**
- Helps osteoblasts in bone formation
- Non-specific marker since its also found in the liver(l#GIT) and placenta
- Its isoenzymes are widely distributed in other tissues
- The isoenzymes also interfere with the assay

P1NP (Procollagen type-1 amino-terminal propeptide) Listed under resorption markers in M slides

- **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 (best marker)** **P** for: **P**1NP and **P**rogression
- دائمًا يقيسونها To follow up on treatment

2- Bone <u>resorption</u> 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 <u>c</u>ircadian variation(a disadvantage)
- N-terminal telopeptide (NTx) Is also one of the resorption markers.

NTX

Treatment	Prevention
In confirmed cases of osteoporosis • Treatment options are unsatisfactory • Oral calcium, estrogens (for menopause lady), fluoride therapy may be beneficial • Bisphosphonates inhibit bone resorption that slow down bone loss أغلب الكبار يعطونهم أسبوعيًا	• 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

Extra **Summary**

Vitamin D	Distribution	**Endogenous precursor:** 7-Dehydrocholesterol **Dietary sources:** Ergocalciferol (D2) and Cholecalciferol (D3)
	metabolism	**Skin:** 7-dehydrocholesterol converted by the sunlight into Cholecalciferol (Vitamin D3) **Liver:** Cholecalciferol is converted to 25-hydroxycholecalciferol (calcidiol) by the enzyme 25-hydroxylase. **Kidneys:** 25-hydroxycholecalciferol is converted to 1,25-dihydroxycholecalciferol by the enzyme 1-a-hydroxylase (biologically active) **Transporter:** gc-globulin protein
	regulation	- Vitamin D synthesis is tightly regulated by plasma levels of **phosphate** and **calcium** - Activity of **1- a-hydroxylase** in kidneys is: ● 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
	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
	calcium homeostasis	● Vitamin D has essential role in calcium homeostasis ● Calcium homeostasis is maintained by **parathyroid hormone** (PTH) and **calcitonin**
	deficiency	● High prevalence in Saudi Arabia due to : (Low dietary intake, Insufficient exposure to sun, Lifestyle) ● Circulating level of >75 nmol/L is required for beneficial health effects
Rickets	Causes	**Vitamin D deficiency because of:** Poor nutrition ,Insufficient exposure to sunlight ,Renal osteodystrophy (causes decreased synthesis of active vitamin D in kidneys) ,Hypoparathyroidism (hypocalcemia)
	types	● **Nutritional rickets** ● **Inherited rickets**
	diagnosis	**Measuring serum levels of :** 25-hydroxycholecalciferol, PTH, Calcium, Phosphate, Alkaline phosphatase
	treatment	**Vitamin D and calcium supplementation**
Osteoporosis	Definition	● 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
	Diagnosis	● WHO standard: 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
	biomarkers	**Bone formation markers:**Osteocalcin ,Bone-specific alkaline phosphatase ,P1NP (Procollagen type-1 amino-terminal propeptide) ,**Bone resorption markers:** CTX-1 (Carboxy-terminal cross-linked telopeptides of type 1 collagen)
	treatment	● Oral calcium, estrogens , fluoride therapy may be beneficial ● Bisphosphonates inhibit bone resorption that slow down bone loss
	prevention	● Good diet and exercise prevent osteoporosis later ● Hormone replacement therapy in menopause may prevent osteoporosis

MCQs

1- Cholecalciferol is synthesized by			
A-liver	B-kidney	C-skin	D-bones
2- Vitamin D has essential role in . . . . . homeostasis			
A-calcium	B-sodium	C- Nitrogen	D-potassium
3-Activity of 1- a-hydroxylase in kidney indirectly increase via			
A-low plasma phosphate	B- PTH	C-iron	D-O2
4-nutritional rickets is?			
A-Bones become hard and pliable	B- demineralization of bones in adults	C-demineralization of bones in children	D-Patients have low serum levels of vitamin C
5- causes of secondary osteoporosis?			
A- Drugs	B-Immobilization	C-Cushing syndrome	D-all of them
6-bone resorption marker?			
A-P1NP	B- alkaline phosphatase	C-Osteocalcin	D-CTX-1

Answers key

1- C 2-A. 3- B 4- C 5- D 6- D

SAQs

1- mention 3 functions of vitamin D

- 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 kidney
- Mobilizes calcium and phosphate from bone to maintain plasma levels

2- What are the causes of rickets?

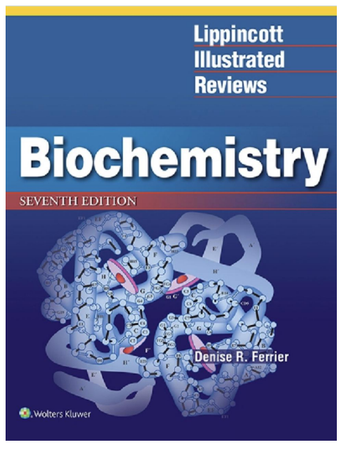
Vitamin **D deficiency because of:**
Poor nutrition ,Insufficient exposure to sunlight ,Renal osteodystrophy (causes decreased synthesis of active vitamin D in kidneys) ,Hypoparathyroidism (hypocalcemia)

3- Enumerate the Biomarkers of osteoporosis

Osteocalcin, CTX-1, Bone-specific alkaline phosphatase, P1NP

Resources

Click on the book to download the resource



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