CHAPTER 1 : LET US START WITH THE BASICS !!

A journey of thousand miles must begin with a single step.
–LAO-TZU, Tao Te Ching

1.1 INTRODUCTION

1.2 DEVELOPMENT OF BONE

1.3 ORGANIZATION OF THE BONES

1.4 PHYSIOLOGICAL ROLE OF CALCIUM IN SKELETAL SYSTEM

1.5 EFFECTS OF VITAMINS, MINERALS AND OTHER NUTRIENT ON BONE HEALTH

1.6 A SUMMARY OF BONE EFFECT OF VARIOUS MICRONUTRIENTS

1.1 INTRODUCTION:

Bone is a specialized connective tissue, which gives shape to the human body by forming a rigid skeleton.

Functions of bone:

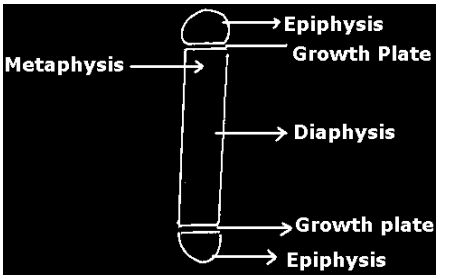
a. Locomotion
b. Haemopoiesis
c. Protective function to the underlying organs
d. Mechanical shape & support to body.
e. Storage of calcium

Types of bones: Depending on shape:

a. Long e.g. femur, tibia
b. Short e.g. phalanges
c. Flat e.g. scapula, skull
d. Irregular e.g. pelvic bones
e. Sesamoid e.g. developing within substance of tendon or fascia (like sesame seeds) e.g. patella

Structure of a long bone: The part of long bone comprises of:

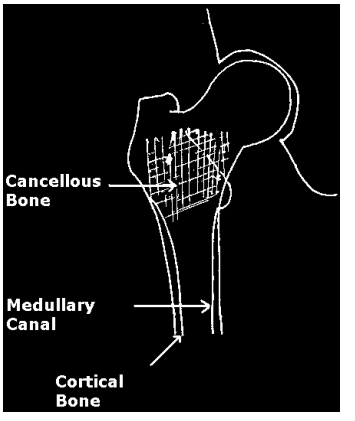
a. Epiphysis
b. Physis (Growth plate)
c. Metaphysis
d. Diaphysis



Epiphysis: It is the expanded portion at the end of the bone, which supports the joint surface.

Diaphysis: The intermediate portion between the 2 epiphysis which forms the shaft of the bone is known as diaphysis. It is made up of compact cortical bone & hence healing is slow as compared to cancellous metaphysis.

Metaphysis: It is the part of the shaft, which separates diaphysis from epiphysis. Made up of cancellous bone, it takes part in remodeling of bone & provides attachment to ligaments & tendons.



Growth plate (physis): It is a thin plate of growth cartilage between epiphysis & metaphysis on each end, helping in longitudinal growth.

Macroscopic features: The shaft of the long bone has a central cavity containing bone marrow. A connective tissue sheath, the periosteum, covers the outer part of the shaft. The articular surface at the ends of the bones is covered by hyaline cartilage.

Microscopic features: Microscopically, bone exists in two forms compact & cancellous; compact bone appearing as a solid mass, cancellous bone consisting of a branching network of trabeculae.

Microscopically bone can be classified into woven or lamellar bone:

Woven bone: It is characterized by random arrangement of cells & connective tissue, seen in early callus formation.

Lamellar bone: It has an orderly distribution of cells & proper orientation of collagen fibers. It forms the organized bone, both cortical & cancellous.

Structural unit of bone: The basic structural unit of the bone is known as osteon. Osteon comprises of a central canal ' harvesian canal' running longitudinally surrounded by a series of concentric lamellae. The harvesian canal connects freely with each other & with volkman's canals. Volkman's canal is a horizontally arranged canal connecting the endosteal to periosteal surfaces.

Bone Marrow: It occupies the marrow cavity of long & short bones & interstices of cancellous bone in flat & irregular bone. At birth the marrow is red & haematopoietic. From 7 years onwards, gradually it is replaced by yellow marrow so that by adulthood red marrow is restricted to vertebral column, bones of skull, gridle bones, thoracic cage, the head of humerus and femur.

Periosteum: This layer of connective tissue covers all bony surfaces except articulating surfaces. It has an abundant vascular supply & is firmly united to bone at the site of enthesis (sites of attachment of muscles, tendous & ligaments). The cells on the deeper surface of periosteum are osteogenic. The periosteum receives a rich nerve supply & is adhered to underlying bone by bundles of collagen fibers known as sharpey's fibers running transversely.

Bone Cells: Three types are seen.

a. *Osteophyte*: Mature bone cells with varied activity, may assume the form of an osteoclast or reticulocyte.
b. *Osteoclasts*: These multinucleated mesenchymal cells play an important role in bone resorption.
c. *Osteoblasts*: These play an important role in ossification.

Extracellular Matrix:

2 forms

- Organic matrix (30 – 35% of dry wt)
- Inorganic matrix (65 – 70 %)
  - Calcium
  - Phosphorus salts

  Especially hydroxyapetite

1.2 DEVELOPMENT OF BONE:

Bone grows by 2 methods

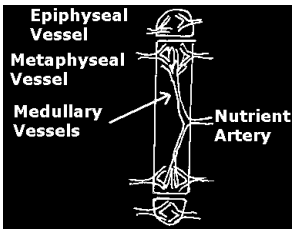
- Membranous
  Bone develops directly from a Connective tissue membrane e.g. skull
- Endochondral
  Cartilaginous model is initially laid down & is later replaced by bone e.g. all long bones (except clavicle)

Endochondral ossiffication: The ossification commences in the middle of shaft before birth (primary center of ossification). The secondary center of ossification, which appears at the end of the bone, mostly after birth (except at distal end of femur which is present at birth) is known as epiphysis.

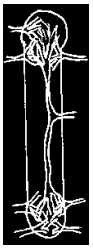
The longitudinal growth of the bone occurs at growth plate whereas growth in the girth is contributed by the subperiosteal new bone deposition.

Blood supply of bones: The blood supply comprises of:

a. *Nutrient Artery:* It enters the bone near its middle & divides into 2 medullary branches running towards the either end of the bone. It divides into multiple parallel branches at metaphysis.



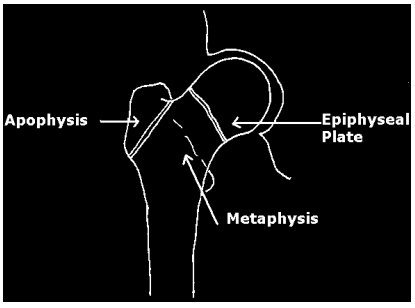
BLOOD SUPPLY BEFORE FUSION OF EPIPHYSIS WITH DIAPHYSIS



ANASTOMOSIS AFTER FUSION OF EPIPHYSIS WITH DIAPHYSIS

b. *Metaphyseal Vessels:* There are multiple small vessels which enter the metaphysis along the line of attachment of joint capsule.
c. *Epiphyseal Vessels:* Entering directly into epiphysis.
d. *Periosteal vessels:* The blood supply to the periosteum supplies roughly the outer third of cortex in adult bones.

Apophysis: The secondary center of ossification which doesnot contribute to longitudinal growth of the bone is known as apophysis e.g. apophysis of tibial tuberosity, greater trochanter.



Cartilage: It is a specialized form of connective tissue containing a gel like matrix (which is responsible for its firmness & resilience) in which cells & fibers are embedded.
Hyaline cartilage: It covers the articular surfaces of all synovial joints & has a great resistance to wear.
Fibrocartilage: It has abundant collagen fibers embedded in a small amount of matrix e.g. discs within T-M joint, sternoclavicular joint, knee joint etc or articular surfaces of clavicle & mandible.
Elastic cartilage: It is highly flexible due to a large number of elastic fibers & is found in the ear & epiglottis.

1.3 ORGANIZATION OF THE BONES:

The bones are 206 in number and are grouped into 2 subdivisions:

Axial Skeleton		**Appendicular Skeleton**	

Bone	Number	Bones	Number
SKULL		SHOULDER GRIDLE	
Cranium	08	Clavicle	02
Auditory ossicles	06	Scapula	02
Face	14	UPPER EXTREMITIES	
Vertebrae	26	Humerus	02
Sternum	01	Radius	02
Ribs	24	Ulna	02
Hyoid	01	Carpals	16
Total	80	Metacarpals	10
		Phalanges	28
		Pelvic Gridle	02
		LOWER EXTREMITIES	
		Femur	02
		Tibia	02
		Fibula	02
		Patella	02
		Tarsals	14
		Metatarsals	10
		Phalanges	28
		TOTAL	126

1.4 PHYSIOLOGICAL ROLE OF CALCIUM IN SKELETAL SYSTEM :

Calcium is the most abundant mineral in the human body. It is an essential nutrient for bone health and is also required for normal functioning of the heart, muscles, nerves and coagulation system.

Percentage of calcium stores :	
Skeletal System	99%
Soft tissue	0.9%
Blood stream and extra cellular fluid	0.1%

Calcium balance: Calcium balance is depended on the absorption rate of calcium ingested as well as the rate of calcium excretion. The body excretes calcium through urine, faeces, sweat and shed skin, hairs and nails.

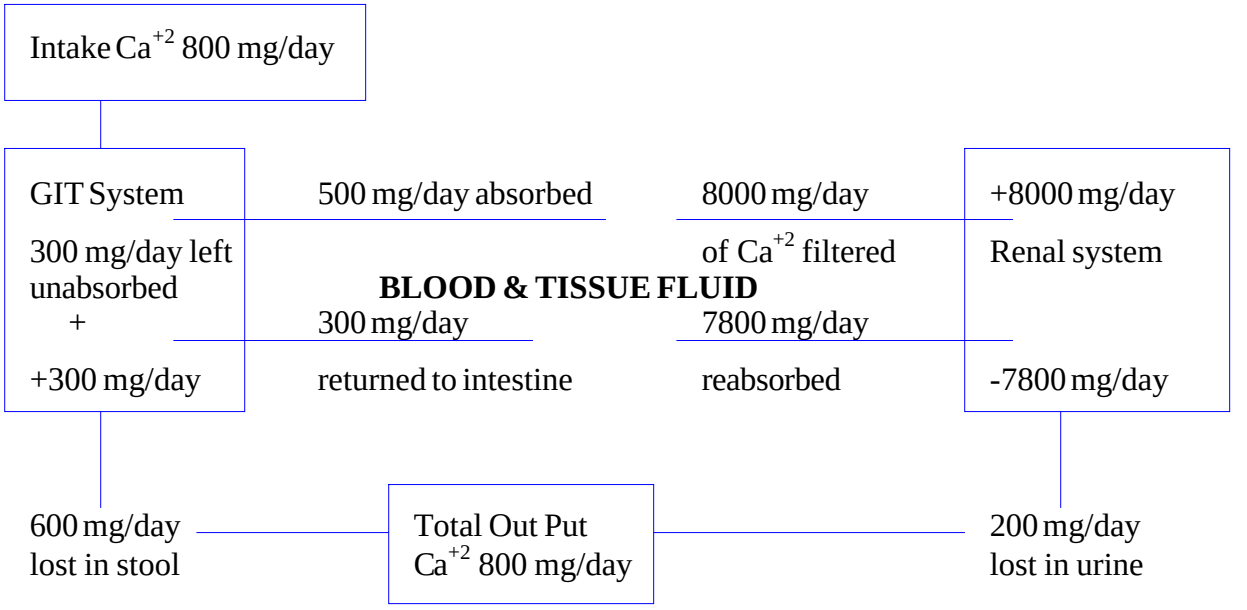
Absorption of calcium :

About 30% to 80% of the ingested calcium is absorbed. The absorption is adjusted to body needs, it is increased in the presence of Ca^{+2} deficiency and decreased in the presence of Ca^{+2} excess.

Active transport primarily occurs in the upper small intestinal lumen. There is also some absorption by passive diffusion.

Active transport is facilitated by 1,25-dihydroxy cholecalciferol, which induces the synthesis of a Ca^{+2} binding protein in the mucosal cell. Ca^{+2} absorption is also facilitated by protein. It is inhibited by phosphates and oxalates because they form insoluble salts with Ca^{+2} in the intestine.

Dynamics of calcium homeostasis:



INPUT = OUTPUT

Calcium requirements for skeletal maintenance:

The requirements fluctuate throughout a woman's life. It is more in a rapidly growing skeleton of teen-age and low intake during that time may impede reaching high peak bone mass. It has been suggested that calcium deficiency in youth can cause 5-10% deficiency in peak bone mass and also increase risk of hip fracture in later life. In third decade, the bone turnover stabilizes and peak adult bone mass is achieved thus calcium requirement remains stable until menopause. In postmenopausal state due to decreased ovarian estrogen secretion, there is an increase in bone resorption and also reduced utilization of dietary calcium which results in increased calcium needs.

Recommended Calcium Intakes (mg/day)

National Institute of health		**National academy of Science**	
Birth – 6 months	400	Birth-6 months	210
6 months-1year	600	6 months to 1 year	270
1-10 years	800-1200	1-3 years	500
11-24 years	1200-1500	4-8 years	800
25-50 years (women and men)	1000	9-13 years	1300
51-64 years (women on ERT and men)	1000	14-18 years	1300
51+ (women not on ERT)	1500	19-30 years	1000
65 or older (women and men)	1500	31-50 years	1000
Pregnant/lactating	1200-1500	51-70 years	1200
		70 years or older	1200
		Pregnant or Lactating	
		14-18 years	1300
		19-50 years	1000

Standing committee on the scientific evaluation of dietary reference intakes food and nutrition board, institute of medicine guidelines for daily dietary calcium requirements for women :

Premenopausal women	1000 mg/d
Postmenopausal woman on HRT	1000 mg/d
Postmenopausal woman not on HRT	1500 mg/d
Adolescent girls, aged 9-17 years old	1300 mg/d
Pregnancy, </=18 years of age	1300 mg/d
Pregnancy, 19-50 years of age	1000 mg/d
Lactation, </=18 years of age	1300 mg/d
Lactation, 19-50 years of age	1000 mg/d

Factors influencing calcium absorption: These include:

1. ***Dietary factors :*** Factors affecting calcium absorption include :

- *Protein :* It is required for tissue building and repair/replacement throughout the life cycle. It plays an important role in immunological functioning and for fracture healing.
  Role on calcium balance: Sulfate, the product of protein metabolism increases calcium excretion through the kidney. Recommended intake for protein is around 44 grams in women and 56 grams for men.
- *Oxalate :* Oxalate interferes with absorption of calcium in the same food as it binds with calcium. It does not interfere with calcium absorption from other food taken along with oxalate rich food. For example, if sweet potatoes are consumed with cheese, the oxalate contents of sweet potatoes would not interfere with calcium absorption from cheese.

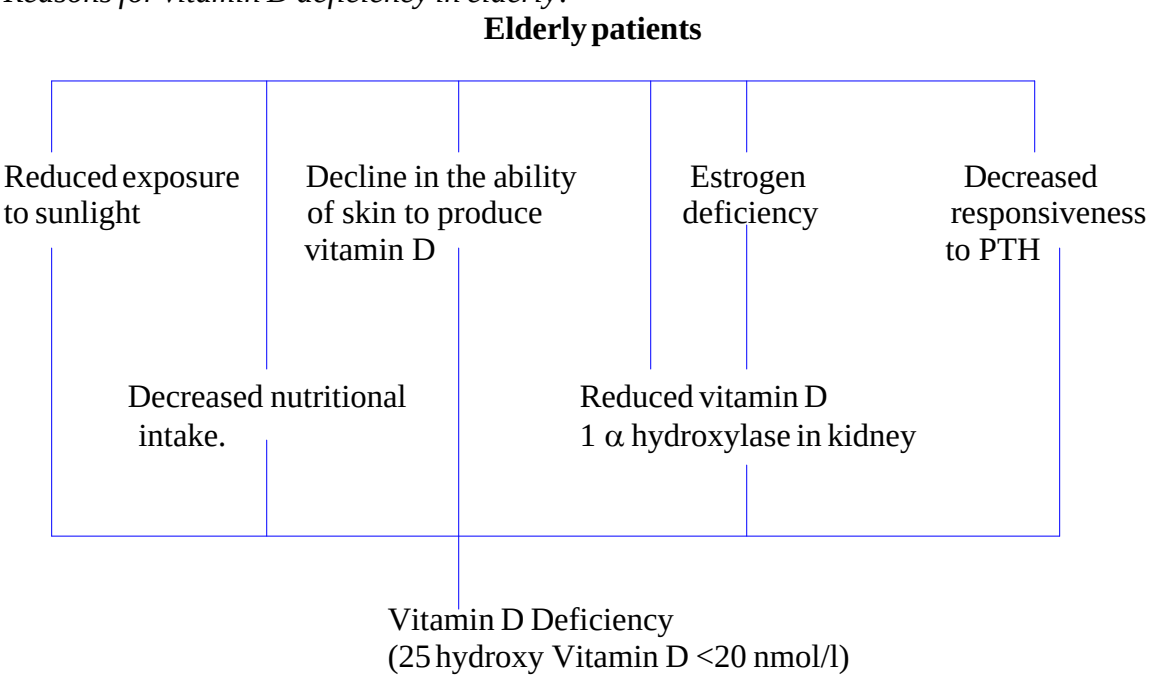
Foods rich in oxalates :
- Spinach
- Rhubarb
- Sweet potatoes

- *Vitamin D :* The role of vitamin D is discussed later.
- *Phosphorus :* It may interfere with calcium absorption.
- *Fiber :* Excess amount of fiber may interfere with calcium absorption albeit not to a clinically significant level.
- *Tannius* (found in tea) may also interfere with calcium absorption.

2. Non Dietary factors :

- *Age :* The calcium absorption increases during adolescent growth spurt. The absorption decreases with age and at around age of 65, calcium absorption efficiency is reduced to half. One of the major factors for the same is vitamin D deficiency in elderly.

Reasons for vitamin D deficiency in elderly:



Beneficial effects of calcium:

1. ***Role in osteoporosis:***

a) Calcium monotherapy (or in combination of vitamin D) has been shown to reduce or halt bone loss in healthy postmenopausal women as well as in postmenopausal women with substantial bone loss or previous fracture.

Reference : Nordin BE. Calcium and osteoporosis nutrition 1997; 13:664-86

b) Calcium may potentiate the effect of exercise on BMD in postmenopausal women.
c) Supplemental calcium improves the efficacy of ERT/HRT.

2. ***Colorectal cancer*** *:* Low calcium intake is one of the risk factors associated with an increased incidence of colorectal cancer. Calcium phosphate (source in high milk diet) is supposed to be less irritating to mucosal cells of colon than calcium carbonate. It has been postulated that high calcium intake decrease proliferation of colorectal epithelial cells.
3. ***Hypertension :*** Calcium has beneficial effect on hypertension. It has been suggested that supplemental calcium intake lowers systolic pressure by 0.15 mm Hg/ 100mg per day of calcium, diastolic pressure by 0.051 mm Hg/100 mg per day of calcium. In a study of the DASH diet (Dietary approaches to stop hypertension) using fruits, vegetables and low fat dairy products as the primary source of calcium, diastolic and systolic blood pressure declines of 3 mm and 5 mm respectively were observed.
4. ***Obesity*** *:* It has been suggested that children and adolescents with high milk intake weighed less and has less body fat than those with low milk intakes. The theory put forward is that low calcium intake increases PTH and 1.25 dihydroxy vitamin D levels, stimulating the adipose cell metabolism to switch from lipolysis to lipogenesis, which in turn increases fat deposition.
5. ***Nephrolithiasis*** *:* Calcium intake from natural sources of upto 1500 mg/day do not increase the risk of nephrolithiasis and may reduce the risk. Same could not be said about supplementation; if at all supplementation is needed, it should be taken with food with a large glass of water.

Side Effects of Calcium : The calcium intake upto the recommended levels donot seem to have any serious side effect profile. Intakes greater than 2500 mg/day may cause hypercalcemia and in extreme cases renal failure.

Precautions : Patient with renal calculus should have their urine calcium excretion measured. In women with no previous history, urinary excretion measurement is not indicated.

Contraindications : Renal Calculus, hypersensitivity to the contents of drugs.

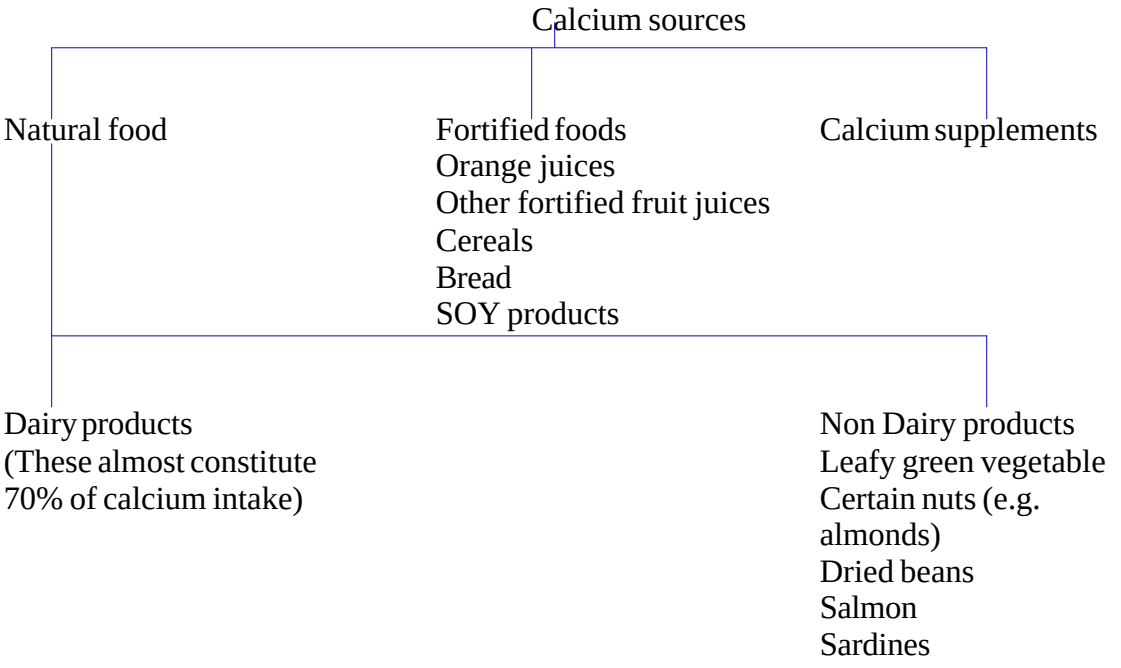
Combination with other nutrients :

1. *Vitamin D :* Vitamin D is essential for intestinal absorption of calcium
   Serum Levels : Normal range is in between 28-32 ng/ml
2. *Magnesium :* Magnesium is a necessary nutrient for the metabolic activity of all cells. In frail elderly women and women with gastrointestinal disease, magnesium supplements may be needed.

Assessment of calcium deficiency:

- *Serum calcium:* It may remain in normal ranges even in dietary deficiency, hence its reliability is limited.
- *BMD :* Bone Mineral Density is affected by many factors other than diet.
- *Urine calcium :* It has a large range of normal value and hence it is of limited utility.

Sources of calcium:



Food A	Amount B	Mg. Of Calcium Content C
Milk and milk products		
Buffalo's milk	1 cup 250ml	300
Cow's milk	1 cup 250ml	244
Ice cream	1 cup 250ml	200
Curd from cows milk	100 gms	120
Milk powder (skimmed)	100 gms	1370
Milk powder (whole)	100 gms	910
Paneer	1 pcs	27
Cheese	1 pcs	203
Khoa	50 gms	478
Vegetables		
Dark green leafy vegetables	1 cup	200
Potato	100 gms	5
Methi	50 gms	235
Onion (raw)	100 gms	180
Cereals/Pulses		
Wheat – chappati	4 no thin	28
Idly-rice	2 nos	10
Dosa-rice	1 no.	10
Rice cooked	100 gms	10
White bread	1 slice	32
Soyabean	100 gms	240
Dal	100 gms	160
Bengal gram	100 gms	202
Dry fruits		
Cashewnuts	25 gms	12
Almonds	25 gms	63
Pista	25 gms	140
Egg/Meat/Fish		
Chicken	100 gms	30
Mutton (muscle)	100 gms	150
Prawn (without shell)	100 gms	145
Eggs (hen)	2 nos	40
Sardines (fish)	1 average size	409

Calcium supplements : These include :

Forms	Contents
1. Tablets	1. Calcium Carbonate
2. Chewable tablets	2. Calcium Citrate
3. Dissolvable tablet	3. Calcium phosphate
4. Liquid	4. Calcium related to an amino acid e.g. bisglycinocalcium
	5. Bone meal, dolomite etc.

Recommendations for calcium supplementations:

1 Total calcium dose in a supplement is based on the amount of elemental calcium it contains e.g. 1,250 mg of calcium carbonate (which contains 40% elemental calcium) provides 500 mg of elemental calcium.

Generic name	Calcium content (per tablet) (%)	Strength of each tablet	No. of tablets needed for 500mg dose of calcium.
Calcium carbonate	40	1500	1
		1250	1
		835	3
		650	2
Calcium citrate	21	950	2.5
Calcium gluconate	9	1000	5.5
		650	9
Tricalcium phosphate	39	800	2
Calcium lactate*	13	650	6
		325	12
Dicalcium phosphate	23	500	4.5

2 Recommended calcium levels refer to elemental calcium.

3 True milk intolerance or allergy is rare, calcium supplements may be required only if dairy foods intake is severely restricted. These people may use yogurt and lactase treated milk. Another way is to increase milk intake gradually thereby allowing the intestinal flora to condition itself to produce lactase.

4 Calcium carbonate contains the highest percentage of elemental calcium (40%) and is the cheapest and most widely used calcium supplement.

5 Calcium supplements are better taken with meals for maximum absorption.

6 Diary products have high calcium content, high calcium bioavailability and relatively low cost and hence are the best source of natural calcium.

7 Calcium in some food (e.g. spinach) is not well absorbed.

8 Calcium has been shown to lower the rate of iron absorption and it is generally advisable that iron supplements should not be taken with calcium.

9 It is better to avoid calcium from unrefined oyster shell, bone meal, or dolomite as these historically have contained higher lead levels than other calcium supplements and may contain other toxic metals.

10 Calcium is best absorbed if taken in amounts of 500 mg or less several times a day, than given in large single doses.

11 Calcium carbonate preparations should be taken at or near mealtime, while calcium citrate preparations can be taken either with a meal or on an empty stomach.
12 When calcium is taken with the antibiotic tetracycline, the absorption of tetracycline may be reduced.
13 Patients should be advised to drink the recommended 6-8 glasses of fluid every day.
14 A high intake of calcium, i.e. more than 2,000 mg per day is not recommended. It may increase the likelihood of developing kidney stones in people prone to them. Individuals with a personal or family history of kidney stones should consult their doctor before increasing their calcium intake. Calcium rarely causes kidney stones in people with normal kidney function.
15 Antacids made up of calcium carbonate can be used as a calcium supplement, but be sure that they do not contain aluminium. Large doses of aluminium can be harmful to the bone.

1.5 EFFECTS OF VITAMINS, MINERALS AND OTHER NUTRIENT ON BONE HEALTH:

A. Vitamin D:

Vitamin D is a collective name for several structurally similar chemicals and their metabolites.

- Vitamin D is required to maintain normal blood levels of calcium and phosphates, that are in turn needed for the normal mineralisation of bone, muscle concentration, nerve conduction, and general cellular function in all cells of the body.
- Vitamin D also modulates the transcription of cell cycle proteins, that decrease cell proliferation, and increase cell differentiation of a number of specialized cells of the body (e.g. Ostoclastic precursors, enterocytes, keratinocytes, etc.).
- Vitamin D also possesses immuno-modulatory properties that may alter responses to infection in vivo.

Vitamin D and its metabolites may be categorized into two families of steroids, the chloecalciferols and ergocalciferols.

1) *Cholecalciferol :* (Vitamin D_3, cholecalciferol, calciol) is produced in the skin on exposure to sunlight. The UV_B portion of sunlight converts 7-dehydrocholesterol to provitamin D_3, which undergoes thermal isomerization to form vitamin D_3. Latitude, season, aging, sunscreen use, clothing and skin pigmentation influence production of vitamin D_3 by the skin.
2) *Ergocalciferol (vitamin D_2, ercalciol):* Is manufactured by irradiation of ergosterol (provitamin D_2) produced by yeasts.
   Both vitamins are translocated from the skin to the blood, where they are bound to a Gc globulin, which is called vitamin D – binding protein (VDBP).

25-OH Vitamin D_3:

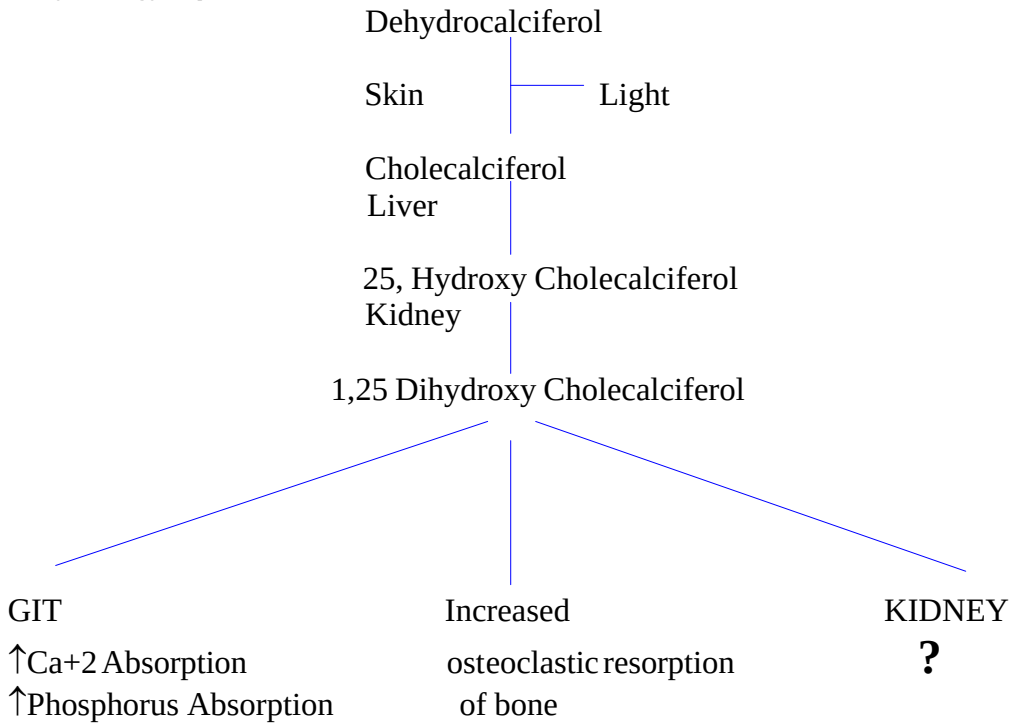
25-hydroxy vitamin D, also known as 25-hydroxy chlolecalciferol, calcidiol 25-OH Vit D, is the main vitamin D metabolite circulating in plasma. It is synthesized in the skin from endogenous or dietary cholesterol on exposure to ultraviolet light (sunlight). This is sometimes called synthetic vitamin D, a misnomer because it is obtained by ultraviolet irradiation of ergosterol, a steroid found in plants and some fungi. 25-OH Vit D is the storage form of vitamin D in the human body.

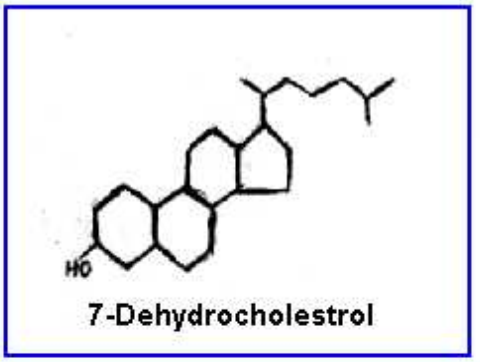
Physiology of 25-OH Vit D_3:

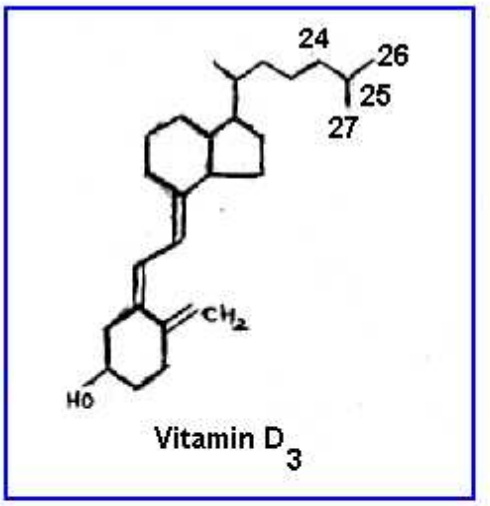
1) Normally, epidermal cells that are exposed to sunlight will produce cholecalciferol, which is then bound to a serum vitamin D binding protein and transported to the liver.
2) In the liver, cholecalciferol is metabolized to 25-hydroxy cholecalciferol which is the major form of vitamin D in the circulation.

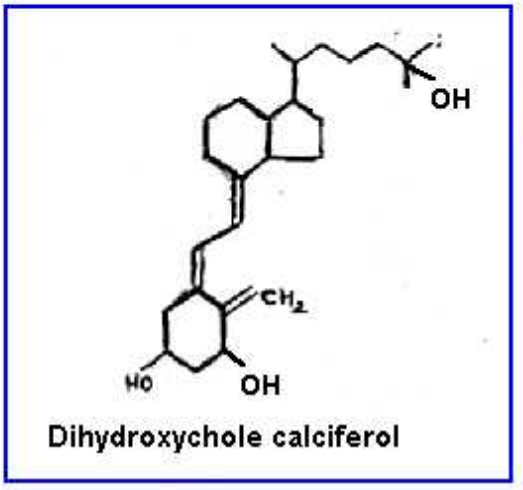
3) The vitamin D metabolite is transported to the kidney where 25-hydroxy cholecalciferol is converted to 1,25 dihydroxy cholecalciferol, which is the most potent metabolite.
4) This metabolite is transported to multiple organs and binds to nuclear receptors and causes a biologic response (calcium absorption or resorption, increased bone resorption, etc.).
5) The vitamin D binding protein increases the serum half life of 25-OH cholecalciferol and serves to provide a stable serum concentration of vitamin D.

Normal Physiology Of Vitamin D Metabolism:



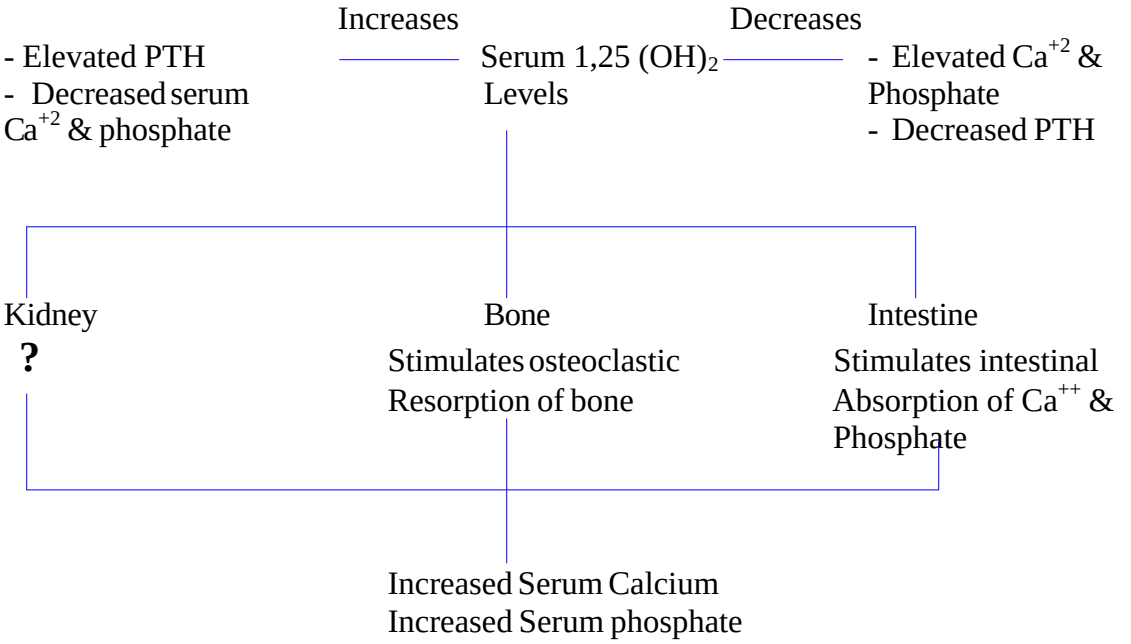






Effect on calcium and phosphorus:

Role of 1.25 $(OH)_2$ D in calcium and phosphate metabolism can be best summarized as below :



Sources of vitamin D :

Sunlight : Sunlight exposure provides most people with entire vitamin D requirement. Children and young adults who spend a short time outside two or three times a week will generally synthesize all the vitamin D they need.

If sun exposure is not sufficient, it is necessary to obtain performed vitamin D from the diet. A good food source of vitamin D contains a substantial amount of vitamin D in relation to its caloric content and contributes at least 10 percent of the recommended daily allowance (RDA) for vitamin D in a selected serving size.

The RDA for vitamin D is 400 IU for adults (except pregnant or lactating women) and children over 4 years of age.

Foods: Milk and milk products are the major source of preformed vitamin D because milk is routinely supplemented with vitamin D.

Eggs are also a good source, and since vitamin D is stored in the liver; animal livers are rich in this vitamin. Because ultra violet light is filtered out by water, fish synthesize their own vitamin D and are also a rich source of the vitamin.

Vitamin D is found naturally in very few foods. Foods containing vitamin D include some fatty fish (herring, salmon, sardines), fish liver oils, and eggs from hens that have been fed vitamin D.

The vitamin D content of some vitamin D rich foods are listed in the table below in both international units (IU) and micrograms (mcg)

Food	**Serving**	**Vitamin D (IU)**	**Vitamin D (mcg)**
Cod liver oil	1 tablespoon	1360	34
Salmon	3 ounces	425	10.6
Herring	3 ounces	765	19.1
Shrimp, canned	3 ounces	90	2.3
Sardines, canned	3 ounces	255	6.4
Cereal, fortified	1 serving (usually 1 cup)	40 to 50	1 to 1.3
Egg yolk	1	25	0.63
Cow's milk, fortified	8 ounces	100	2.5

Vitamin D is included in many, if not most, multivitamins. It can be found alone in over-the-counter preparations in strengths from 50 IU to 1,000 IU as soft gel capsules, tablets and liquid. Higher-dose preparations are also available by prescription. For those who have trouble digesting fat, vitamin D injections are also available by prescription.

***Vitamin D intake guideline* : Institute of medicine (1997)**

19-50 years	200 IU/day
51-70 years	400 IU/day
Over 70 years	600 IU/day
Maximum limit	2000 IU/day

The guidelines assumes no vitamin D is synthesized from sunlight exposure.

***Risk factors for vitamin deficiency*:**

1) *Infants:* Infants who have little or no sun exposure, and do not consume vitamin D-fortified formula are at increased risk, especially those born just before winter in northern and southern latitudes.
2) *Elderly individuals with minimal sun exposure:* The elderly have reduced capacity to synthesize vitamin D in response to sunlight exposure, and are more likely to stay indoors or use sunscreen for the prevention of skin cancer.

3) *Dark skin individuals:* Individuals with darkly pigmented skin, e.g., those of African or Indian descent living in northern or southern latitudes synthesize less vitamin D on exposure to sunlight than those with light skin.
4) *Those covering all exposed skin when outside:* Osteomalacia has been documented in Arab women who cover all of their skin at all times when going outside, for religious or cultural reasons.
5) *Malabsorption syndromes:* Cystic fibrosis and cholestatic liver disease impair the absorption of dietary vitamin D.
6) *Kidney failure:* Severe kidney disease can impair the conversion of calcidiol to the biologically active form of vitamin D, calcitriol.
7) *Genetic disease:* A rare disease affects the activity of the 1-hydroxylase enzymes in the kidney that convert calcidiol to its active form, calcitriol.
8) *Seizure disorders (epilepsy):* Long-term treatment with anticonvulsant medications, such as phenytoin, can stimulate liver enzymes that break down and inactivate calcitriol.

Signs and symptoms of Vitamin D_3 deficiency:

Maternal osteomalacia can lead to metaphyseal lesions and tetany in the newborn. Young infants are restless and sleep poorly. They have reduced mineralization of the skull (carniotabes) away from the sutures. In older infants, sitting and crawling are delayed as is fontanelle closure, and there is bossing of the skull and costochondral beading (rachitic rosary). In children aged 1 to 4 years, walking is delayed. In older children and adolescents, walking is painful, and in extreme cases, deformities such as bowlegs and knock-knees develop.

Rachitic tetany: It is caused by hypocalcemia and may accompany infantile or adult vitamin D deficiency.

Bone changes: Visible on x-rays, these precede clinical signs, becoming evident in the 3rd or 4th month of life-even at birth if the mother is vitamin D deficient.

In adults: In adults demineralization (osteomalacia) occurs, particularly in the spine, pelvis and lower extremities.

Rickets and osteomalacia are discussed later in a separate topic.

Signs of vitamin D_3 toxicity:

The main signs are sudden hypercalcemia (excessive levels of calcium in the blood) and/or hypercalciuria (excessive levels of calcium in the urine). Toxicity symptoms are headache, nausea, dizziness, vomiting, loss of appetite and dry mouth.

Hypercalciuria is discussed later in a separate topic.

***Clinical significance of 25-OH vitamin D_3 estimation**:*

1) To allow precise diagnosis and better management of clinical or suspected cases of rickets or osteomalacia and rickets.
2) To ensure adequate control of populations at risk (old age pensioners, neonates, pregnant women and populations with a mismatch between skin pigmentation and sun exposure).
3) To confirm suspicion of subclinical deficiency state.
4) To complement the diagnostic workup of some hepatic, renal or gastrointestinal diseases.
5) To monitor vitamin D status during long term therapy with anticonvulsant drugs.
6) To ensure adequate control of the clinical efficacy of vitamin D preparations and food products fortified with vitamin D.
7) To diagnose vitamin D intoxication, as a part of differential diagnosis of hypercalcemia.

Estimation of 25-OH vitamin D_3 is considered a better indicator of vitamin D deficiency due to the following reasons:

1) Vitamin D has a much shorter half-life than 25-OH Vit D_3, 1-2 days versus 2-3 weeks.
2) Vitamin D levels fluctuate more widely with exposure to sunlight and dietary intake.
3) 25-OH Vit D_3 is present in much greater concentrations than vitamin D.
4) 25-OH Vit D_3 is an early indicator (even before bone densitometry) for osteoporosis.
5) Very effective for evaluating a patient in Vitamin D_3 (alpha calciferol) therapy and follow up.

Diagnosis:

A variety of methods have been published and the literature is full of detailed description of amendments and improvements of the methodology.

1) *Bioassay procedures:* These procedures have been used in the past and depend upon evidence of alleviation of the rachitic state. This set up does not lend itself to routine application.

2) *Mass spectrometry:* This could be used for analytical purposes but is not a common tool in routine clinical laboratories.

3) *UV detection after HPLC:* UV absorption is a sensitive method for 25-OH vitamin D_3 detection. Before any measurements can be made, the blood sample have to be submitted to exhaustive extraction and purification steps to exclude interfering UV absorbing materials. Hence this method remains a good reference method only for research purpose.

4) *Competitive protein binding assay:* This assay requires a sensitive vitamin D binding protein. It's affinity for 25-OH vitamin D_3 is high but is equally high for 1,25-OH vitamin D_3 and several dihydroxy related metabolities. Hence these metabolities will interfere in the essay, if the sample is used without prior purification.

5) *RIA:* Radio-immunoassays are highly sensitive and specific assays. Hence, these assays are preferred by many laboratories.

Interpretation of 25-OH vitamin D_3 levels :

The serum 25-OH vitamin D_3 level is the best initial test for vitamin D deficiency. If there is a high level of clinical concern for vitamin D deficiency and a low-normal 25-OH vitamin D_3 level is found, serum parathyroid hormone (PTH) concentration and a 24-hour urine calcium should be checked.

Although experts differ, the following definitions have been proposed :

1) *Vitamin D sufficiency :* 25-OH vitamin D_3 is 20 to 80 ng/mL (50 to 200 nmol/L) and there is normal calcium homeostasis/bone metabolism.

2) *Vitamin D insufficiency :* 25-OH vitamin D_3 is 4 to 20 ng/mL (10 to 50 nmol/L) with mild hyperparathyroidism, sub-optimal calcium absorption and decreased bone density.

3) *Vitamin D deficiency :* 25-OH vitamin D_3 is 0 to 4 ng/mL (0 to 10 nmol/L) with secondary hyperparathyroidism and malabsorption of calcium causing osteomalacia.

Decreased levels of 25-OH vit D appear in patients with:

- Nutritional rickets/osteomalacia.
- Senile/postmenopausal osteoporosis.
- Celiac disease.
- Inflammatory bowel disease (e.g. Crohn's disease).
- Insufficiency of the exocrine pancreas.
- Short bowel syndrome.
- Biliary cirrhosis, liver dysfunction.
- Renal osteodystrophy.
- Nephritic syndrome.
- Neonatal hypocalcemia.

- Hypocalcemia.
- Treatment with anticonvulsant drugs (enhanced metabolism)

Elevated levels of 25-OH vit D appear in patients with:

- Hypercalcemia.
- Vitamin D intoxication.

Precautions:

Because of the potential of side effects and interactions with medications, dietary supplements should be taken only under the supervision of a knowledgeable healthcare provider.

Taking too much vitamin D (more than 1,000 IU daily) can cause a number of adverse effects including excessive thirst, metal taste, poor appetite, weight loss, bone pain, tiredness, sore eyes, itching skin, vomiting, diarrhoea or constipation, a need to urinate and muscle problems.

Getting too much sunlight, however, will not provide excess of vitamin D, nor is one likely to get too much vitamin D from food sources alone. Generally, excess of vitamin D is a result of taking supplements in too high a dose.

People with the following conditions should exercise caution when considering taking vitamin D supplements :

- High blood calcium or phosphorus levels.
- Heart problems.
- Kidney problems.

B. Magnesium :

Role:

- Many enzymatic reactions.
- Synthesis of proteins.
- Synthesis of nuclear acids
- Used in management of premature labour.
- Used for prophylaxis and treatment of seizures in toxaemia of pregnancy.
- Active ingredient in antacids.

Impact on bone health :

- It affects bone remodeling, strength and preservation.
- It is needed for secretion and action of PTH, an important regulator of calcium status.
- Useful in muscular contraction and nerve transmission.

Recommended daily intake :

For men it is 420mg and for women 320mg.

Sources of magnesium :

- Whole grains.
- Dark green vegetables.
- Milk.
- Bananas.
- Meat
- Nuts

Magnesium deficiency : It has been found associated with :

- Necrotizing enterocolitis.
- Development of atherosclerosis.
- Migrain headaches.

C. Zinc :

Role : It is an essential trace mineral.

- Forms a part of enzymes, hormones, proteins.
- Wound healing.
- Sperm protection
- Fetal development.

Impact on bone health : It is a cofactor for enzymes required for healthy bone metabolism.

Recommended daily intake : It is 11mg/day for men and 8mg/day for women with upper limit of 40mg/day (in a person with normal kidney).

Sources of zinc :

- Fortified cereals.
- Beef
- Poultry
- Red and white meat
- Eggs
- Dairy products
- Nuts
- Peas
- Shell fish

Deficiency of zinc : It is associated with :

- Decreased appetite
- Dwarfism
- Delayed development of sex organs
- Reduced immune function
- Impaired wound healing

D. Selenium :

Role : Essential trace element.

- ? Anticancer effect.
- ? Component of enzyme involved in antioxidant protection.
- ? Effects on thyroid hormone metabolism.

Impact on bone health : Not known

Sources :

- Grains
- Meats
- Fish
- Brazil nuts

Deficiency : It may be associated with :

- Juveline cardiomyopathy.
- Chondrodystrophy
- ? decreased immune efficiency

E. Chromium :

Role :

- Normal sugar and fat metabolism
- It potentiates the action of insulin

- Helps in energy release

Role in bone health :

Not known.

Sources :

- Fruits
- Vegetables
- Seeds
- Grains

Chromium deficiency :

- Impaired glucose metabolism
- Altered lipid profile

F. Boron:

Role: Positive impact on:

- Calcium metabolism
- ? increased bone mass

Recommended daily intake : No such levels have been established. Tolerable upper limit is 20 mg/day

Sources :

- Egg
- Potatoes
- Fluids
- Milk
- Vegetables
- Legumes
- Pulses

G. Fluoride:

Role: It is required for the growth of teeth and bone.

Impact on bone health : High doses of fluoride increases bone mass significantly. But as the new bone formed is brittle, there is no significant reduction in risk of fracture.

Recommended daily intake : Adequate intakes are 4 mg/day for adult men and 3 mg/day for adult women.

Tolerable upper limit : 10 mg/day.

H. Strontium*:*

Role: Research suggest that strontium may increase bone strength.

Recommended daily intake : Average daily intakes is around 1 to 3mg. Very high intake may increase bone fragility, impaired vitamin D metabolism and bone mineralization.

Sources :

- Whole milk.
- Wheat bran
- Poultry
- Root vegetables

***I.* Vitamin A (retinol):**

Role:

- Vision
- Tissue repair

- Reproduction
- Growth
- Anti cancer effects
- Antioxidant
- Hormone synthesis

Impact on bone health : High levels can lead to reduced bone mass and increased fracture rates.

Recommended dietary allowance (RDA) for vitamin A in international units (IU)

Age years	Children	Men	Women	Pregnancy	Lactation
1-3	1000 IU				
4-8	1333 IU				
9-13	2000 IU				
14-18		3000 IU	1330 IU	2500 IU	4000 IU
19+		3000 IU	2330 IU	2565 IU	4335 IU

Source : Institute of medicine.

Common food sources of vitamin A

Food sources	International units (IU) of vitamin A
Liver, beef, cooked 3 oz	30325
Liver, chicken, cooked 3 oz	13920
Egg substitive, fortified, ¼ cup	1355
Fat free milk, fortified with vitamin A, 1 cup	500
Cheese pizza 1/8 of A 12" diameter pie	380
Milk whole 3.25% fat, 1 cup	305
Cheddar cheese 1 ounce	300
Whole egg, 1 medium	280

Source : NIH office of dietary supplements.

Dietary deficiency :

- Rough skin
- Night blindness
- Impaired bone growth
- Susceptibility to infection

J. Vitamin K *:*

Role :

- Blood clotting
- Blood calcium regulation
- Protein synthesis

Impact on bone health : ? positive impact on bone mass.

- It is necessary for making osteocalcin

Recommended daily intake : It is 120mcg/day for men and 90mcg/day for women.

Sources :

- Fermented soy and dairy products.
- Cabbage
- Olive oils
- Liver

- Soyabeans
- Eggs
- Plant oils

Deficiency : It may be associated with :

- Hemorrhages
- Osteoporosis
- Atherosclerosis

K. Vitamin C :

Role :

- Antioxidant
- Collagen synthesis
- Wound healing
- Immune system
- Iron absorption

Recommended dietary intake : It is 90mg/day for men and 75mg/day for women with tolerable upper limit of 2000 mg/day.

Impact on bone health : Higher intake of vitamin C may reduce hip fracture risk and increase BMD in postmenopausal women (dose of 100-125 mg/day)

Source :

- Citrus fruits
- Cantaloupes
- Berries
- Mangoes
- Melons
- Tomatoes
- Potatoes
- Cabbage
- Broccoli

Deficiency :

- Scurvy
- Bleeding gums
- Loosening of the teeth
- Capillary haemorrhages
- Pseudoparalysis
- Poor wound healing

L. Vitamin E

Role :

- Cellular membrane stability
- Antioxidant
- RBC protection
- Improved immune response
- Useful as antioxidant in coronary heart disease, prostate cancer and alzheimer's disease.

Source :

- Vegetable and seed oils

- Whole grains
- Nuts
- Green leafy vegetables

Deficiency :

- Muscle wasting
- RBC damage
- Hemolytic anaemia
- Haemorrhages
- Neurological abnormalities

M. Phytoestrogens and omega-3 fatty acids:

Phytoestrogen have mild estrogenic and antiestrogenic effects on specific tissues in the body, depending on factors such as gender, age and hormonal status.

Dietary recommendations :

A consumption of 50mg/day may have a beneficial effect on cardiovascular and muscloskeletal system in postmenopausal women.

Source :

- Soy
- Chick peas
- Legumes

N. Omega 3 – fatty acids:

These may have a beneficial effect on bone mass.

Sources:

Plant source

- Soybeans
- Walnuts
- Flaxseed

Animal source

- Fish oil

As retinol is found in high concentration in fish oil; vegetable sources are preferable.

1.6 A SUMMARY OF BONE EFFECT OF VARIOUS MICRONUTRIENTS:

Established beneficial effect	Potential beneficial effect	Potential harmful effect
Calcium	Boron	Vitamin A from retinal
Vitamin D	Copper.	High intake of fluoride
Protein	Magnesium.	High intake of oxalate
	Fluoride (in low doses)	High intake of phosphorus
	Manganese	High intake of strontium
	Omega 3 fatty acids	
	Soy	
	Vitamin C	
	Vitamin K	
	Zinc	

	Strontium	
	Phosphorus.	

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