Paper 1

Basic Sciences

Calcium Metabolism

- Over 99% of the 1–2 kg of calcium present normally in the adult human body resides in the skeleton, where it provides mechanical stability and serves as a reservoir sometimes needed to maintain extracellular fluid (ECF) calcium concentration.

- Skeletal calcium accretion first becomes significant during the third trimester of fetal life, accelerates throughout childhood and adolescence, reaches a peak in early adulthood, and gradually declines thereafter at rates that rarely exceed 1–2% per year.

- These slow changes in total skeletal calcium content contrast with relatively high daily rates of closely matched fluxes of calcium into and out of bone (~250–500 mg each), a process mediated by coupled osteoblastic and osteoclastic activity.

- Another 0.5–1% of skeletal calcium is freely exchangeable (e.g., in chemical equilibrium) with that in the ECF.

- The concentration of ionized calcium in the ECF must be maintained within a narrow range because of the critical role it plays in a wide array of cellular functions, especially those involved in neuromuscular activity, secretion, and signal transduction.

- Intracellular cytosolic free calcium levels are ~100 nmol/L and are 10,000-fold lower than ionized calcium concentration in the blood and ECF (1.1–1.3 mmol/L). This steep chemical gradient promotes rapid calcium influx through various membrane calcium channels that can be activated by hormones, metabolites, or neurotransmitters, swiftly changing cellular function.

- In blood, total calcium concentration is normally 2.2–2.6 mM (8.5–10.5 mg/dL), of which ~50% is ionized. The remainder is bound ionically to negatively charged proteins (predominantly albumin and immunoglobulins) or loosely complexed with phosphate, citrate, sulfate, or other anions.

- Alterations in serum protein concentrations directly affect the total blood calcium concentration, even if the ionized calcium concentration remains normal.

- An algorithm to correct for protein changes adjusts the total serum calcium (in mg/dL) upward by 0.8 times the deficit in serum albumin (g/dL) or by 0.5 times the deficit in serum immunoglobulin (in g/dL). Such corrections provide only rough approximations of actual free calcium concentrations, however, and may be misleading, particularly during acute illness.

- Acidosis also alters ionized calcium by reducing its association with proteins. The best practice is to measure blood ionized calcium directly by a method that employs calcium-selective electrodes in acute settings during which calcium abnormalities might occur.

- Control of the ionized calcium concentration in the ECF ordinarily is accomplished by adjusting the rates of calcium movement across intestinal and renal epithelia. These adjustments are mediated mainly via changes in blood levels of the hormones PTH and 1,25(OH)2D. Blood ionized calcium directly suppresses PTH secretion by activating parathyroid calcium-sensing receptors (CaSRs). Also, ionized calcium indirectly affects PTH secretion via effects on 1,25(OH)2D production. This active vitamin D metabolite inhibits PTH production by an incompletely understood mechanism of negative feedback.

DIETARY INTAKE AND ABSORPTION

- Normal dietary calcium intake varies widely, ranging from 10–37 mmol/d (400–1500 mg/d). Intestinal absorption of ingested calcium involves both active (transcellular) and passive (paracellular) mechanisms. Passive calcium absorption is nonsaturable and approximates 5% of daily calcium intake, whereas the active mechanism, controlled principally by 1,25(OH)2D, normally ranges from 20–70%.

- Active calcium transport occurs mainly in the proximal small bowel (duodenum and proximal jejunum), although some active calcium absorption occurs in most segments of the small intestine. Optimal rates of calcium absorption require gastric acid. This is especially true for weakly dissociable calcium supplements such as calcium carbonate. In fact, large boluses of calcium carbonate are poorly absorbed because of their neutralizing effect upon gastric acid. In achlorhydric subjects or for those taking drugs that inhibit gastric acid secretion, supplements should be taken with meals to optimize their absorption. Use of calcium citrate may be preferable in these circumstances.

- Calcium absorption may also be blunted in disease states such as pancreatic or biliary insufficiency, in which ingested calcium remains bound to unabsorbed fatty acids or other food constituents.

- At high levels of calcium intake, synthesis of 1,25(OH)2D is reduced, which decreases the rate of active intestinal calcium absorption. The opposite occurs with dietary calcium restriction. Some calcium, ~2.5–5.0 mmol/d (100–200 mg/d), is excreted as an obligate component of intestinal secretions and is not regulated by calciotropic hormones.

- The feedback-controlled hormonal regulation of intestinal absorptive efficiency results in a relatively constant daily net calcium absorption of ~5–7.5 mmol/d (200–400 mg/d), despite large changes in daily dietary calcium intake.

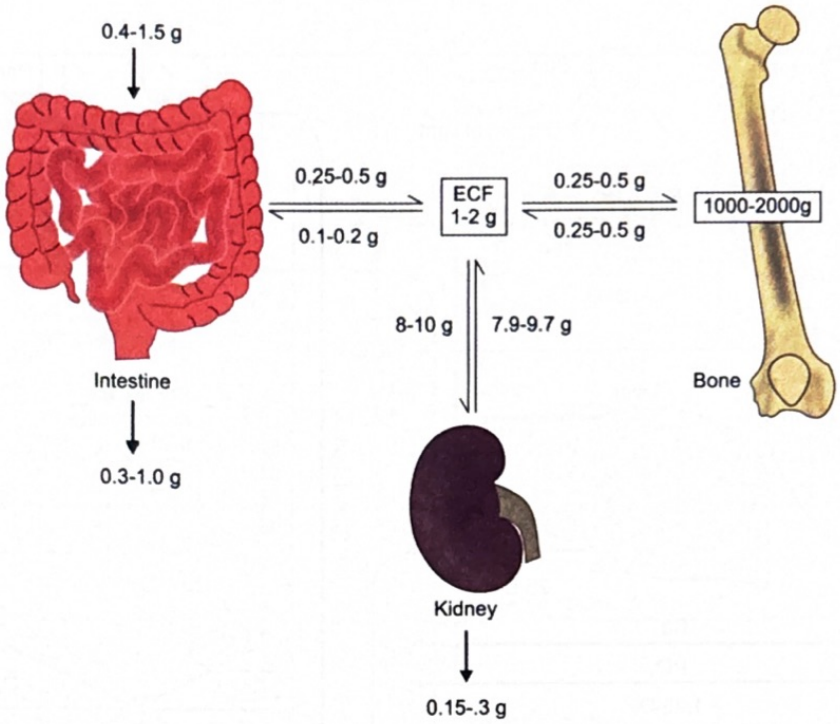


Fig: Calcium content of extracellular fluid (ECF) and bone as well as of diet and feces. Magnitude of calcium flux per day at sites of transport in intestine, kidney, and bone

RENAL ABSORPTION/ EXCRETION

- The daily load of absorbed calcium is excreted by the kidneys in a manner that is also tightly regulated by the concentration of ionized calcium in the blood.
- Approximately 8–10 g/d of calcium are filtered by the glomeruli, of which only 2–3% appears in the urine.
- Most filtered calcium (65%) is reabsorbed in the proximal tubules via a passive, paracellular route that is coupled to concomitant NaCl reabsorption and not specifically regulated.
- The cortical thick ascending limb of Henle's loop (cTAL) reabsorbs roughly another 20% of filtered calcium, also via a paracellular mechanism. Calcium reabsorption in the cTAL requires a tight-junctional protein called paracellin-1 and is inhibited by increased blood concentrations of calcium or magnesium, acting via the CaSR, which is highly expressed on basolateral membranes in this nephron segment. Operation of the renal CaSR provides a mechanism, independent of those engaged directly by PTH or 1,25(OH)2D, whereby serum ionized calcium can control renal calcium reabsorption.
- Finally, ~10% of filtered calcium is reabsorbed in the distal convoluted tubules (DCT) by a transcellular mechanism. Calcium enters the luminal surface of the cell through specific apical calcium channels, whose number is regulated. It then moves across the cell in association with a specific calcium-binding protein (calbindin-D28k) that buffers cytosolic calcium concentrations from the large mass of transported calcium. Ca2+-ATPases and Na+/Ca2+ exchangers actively extrude calcium across the basolateral surface and thereby maintain the transcellular calcium gradient.
- All of these processes are stimulated, directly or indirectly, by PTH.
- The DCT is also the site of action of thiazide diuretics, which lower urinary calcium excretion. Conversely, dietary sodium loads, or increased distal sodium delivery caused by loop diuretics or saline infusion, reduce DCT calcium reabsorption.
- The homeostatic mechanisms that normally maintain a constant serum ionized calcium concentration may fail at extremes of calcium intake or when the hormonal systems or organs involved are compromised.
- Thus, even with maximal activity of the vitamin D–dependent intestinal active transport system, sustained calcium intakes <5 mmol/d (<200 mg/d) cannot provide enough net calcium absorption to replace obligate losses via the intestine, kidney, sweat, or other secretions. In this case, increased blood levels of PTH and 1,25(OH)2D activate osteoclastic bone resorption to obtain needed calcium from bone, which leads to progressive bone loss and negative calcium balance.
- Increased PTH and 1,25(OH)2D also enhance renal calcium reabsorption, and 1,25(OH)2D enhances calcium absorption in the gut.
- At very high calcium intakes [>100 mmol/d; >4 g/d], passive intestinal absorption continues to deliver calcium into the ECF, despite maximally downregulated intestinal active transport and renal tubular calcium reabsorption. This can cause severe hypercalciuria, nephrocalcinosis, progressive renal failure, and hypercalcemia (e.g., "milk alkali syndrome").
- Deficiency or excess of PTH or vitamin D, intestinal disease, and renal failure represent other commonly encountered challenges to normal calcium homeostasis

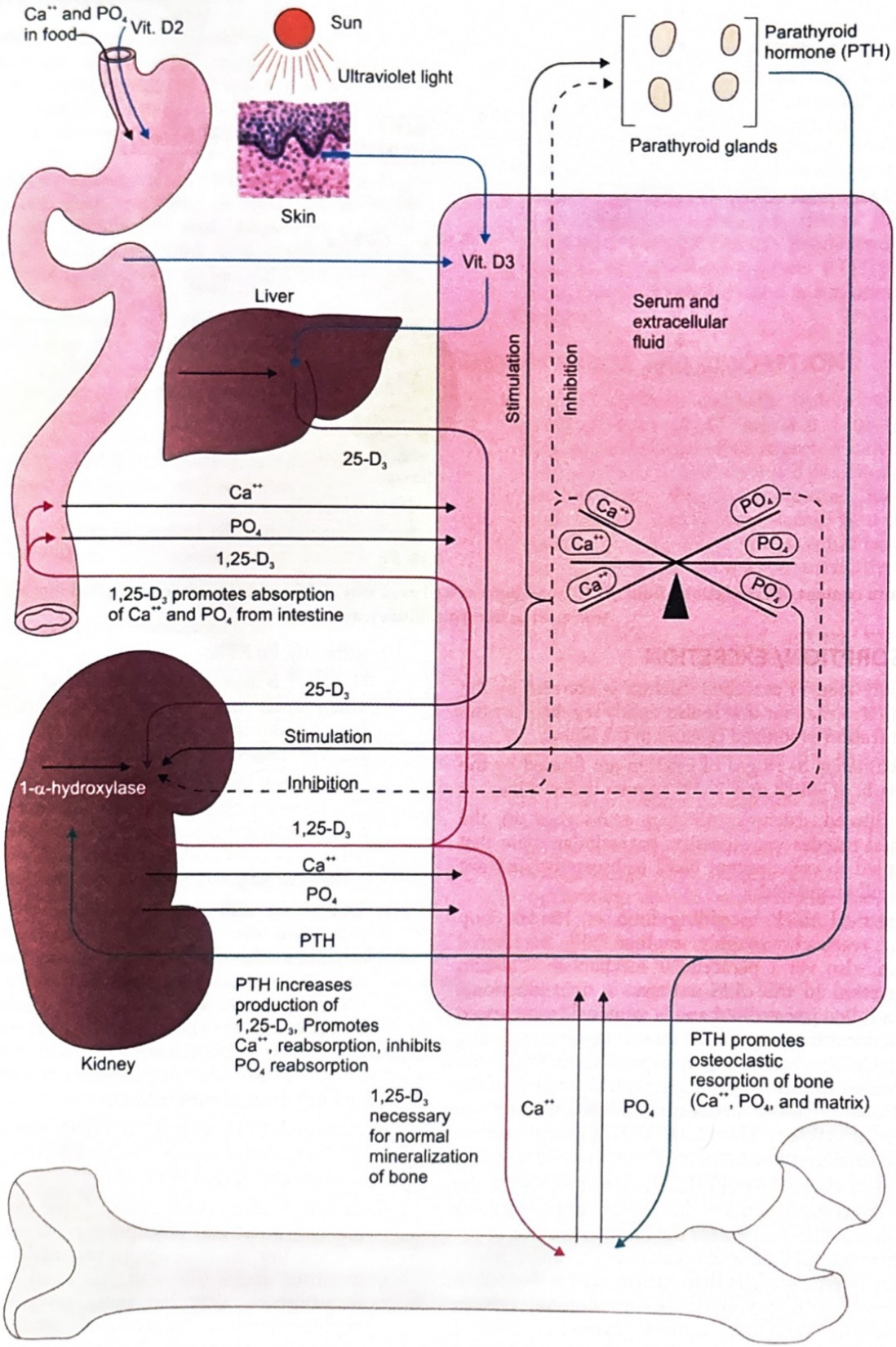


Fig: Normal Calcium and Phosphate Metabolism

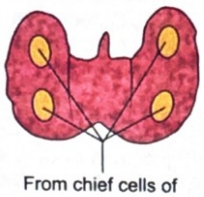
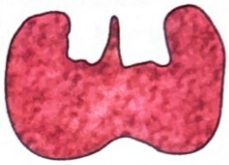
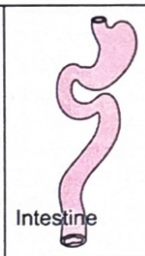
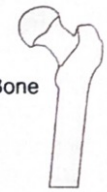
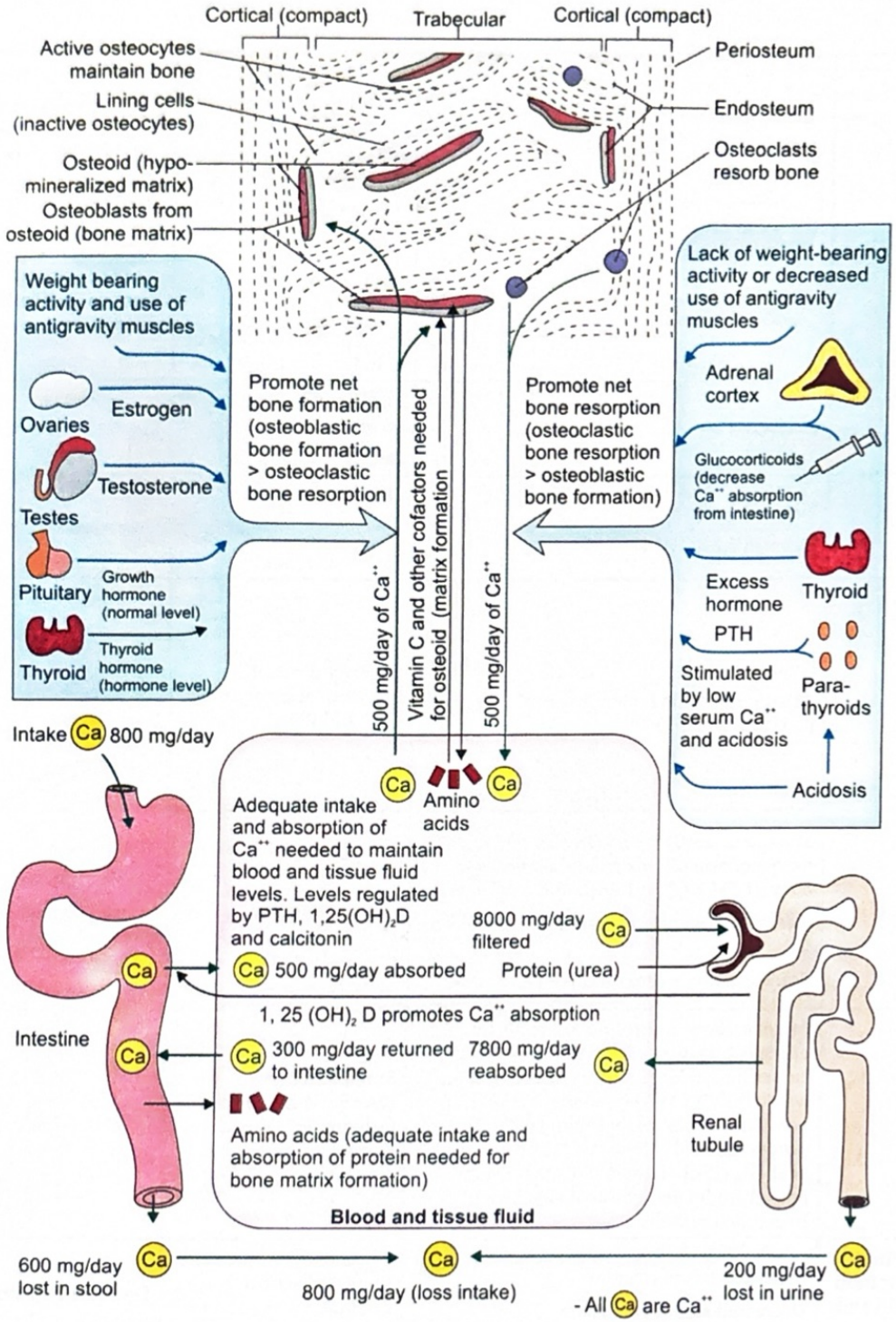
		Parathyroid hormone (PTH) (peptide)	$1,25\text{-}D_3$ (Steroid)	Calcitonin (peptide)
Hormone		 From chief cells of parathyroid glands	 From proximal tubule of kidney	 From parafollicular cells of thyroid gland
Factors stimulating production		Decreased serum Ca^{++}	Elevated PTH decreased serum Ca^{++} Decreased serum Pi	Elevated serum Ca^{++}
Factor inhibiting production		Elevated serum Ca^{++} Elevated $1,25(OH)_2D$	Decreased PTH Elevated serum Ca^{++} Elevated serum Pi	Decreased serum Ca^{++}
End organs from hormne action	 Intestine	No direct effect Acts indirectly on bowel by stimulating production of $1,25(OH)_2D$ in kidney	Strongly stimulates intestinal absorption of Ca^{++} and Pi	
	 Kidney	Stimulates 25(OH)D-1α-OH_{ase} in mitochondria of proximal tubular cells to convert 25(OH)D to $1\text{-}25(OH)_2D$ Increases fractional reabsorption of filtered Ca^{++} Promotes urinary excretion of Pi		Increases renal calcium excretion
	 Bone	Increases bone resorption indirectly by upregulating osteoblast production of autocrine cytokines such as interleukin-6, which results in increased production of paracrine cytokines that stimulate osteoclast production and activity. PTH also has an anabolic effect on osteoblasts that results in overproduction of osteoid in chronic hyperparathyroidism	Stimulates bone resorption in a similar fashion to PTH and also other membrane receptors	Inhibits bone resorption by direct inhibition of osteoclast differentiation and activity
Net effect on calcium and phosphate concentration in extracellular fluid and serum		Increased serum calcium Decreased serum phosphate	Increased serum calcium	Decreased serum calcium (transient)

Fig: Regulation of Calcium and Phosphate Metabolism



Dynamics of bone homeostasis

Parathyroid hormone

- The four parathyroid glands are located posterior to the thyroid gland produce parathyroid hormone (PTH), which is the primary regulator of calcium physiology.
- PTH acts directly on bone, where it induces calcium resorption; and on the kidney, where it stimulates calcium reabsorption and synthesis of 1,25-dihydroxyvitamin D [1,25(OH)2D], a hormone that stimulates gastrointestinal calcium absorption.
- Serum PTH levels are tightly regulated by a negative feedback loop. Calcium, acting through the calcium-sensing receptor, and vitamin D, acting through its nuclear receptor, inhibit PTH release and synthesis.

Parathyroid Hormone - Physiology

- The primary function of PTH is to maintain the extracellular fluid (ECF) calcium concentration within a narrow normal range.
- The hormone acts directly on bone and kidney and

indirectly on intestine through its effects on synthesis of $1,25(OH)_2D$ to increase serum calcium concentrations; in turn, PTH production is closely regulated by the concentration of serum ionized calcium. This feedback is the critical homeostatic mechanism for maintenance of ECF calcium.

- Any tendency toward hypocalcemia, as might be induced by calcium-deficient diets, is counteracted by an increased secretion of PTH. This in turn
  (1) increases the rate of dissolution of bone mineral, thereby increasing the flow of calcium from bone into blood;
  (2) reduces the renal clearance of calcium, returning more of the calcium filtered at the glomerulus into ECF; and
  (3) increases the efficiency of calcium absorption in the intestine by stimulating the production of $1,25(OH)_2D$.
- Immediate control of blood calcium is due to PTH effects on bone and, to a lesser extent, on renal calcium clearance. Maintenance of steady-state calcium balance, on the other hand, probably results from the effects of $1,25(OH)_2D$ on calcium absorption. The renal actions of the hormone are exerted at multiple sites and include inhibition of phosphate transport (proximal tubule), increased reabsorption of calcium (distal tubule), and stimulation of the renal 25(OH)D-1-hydroxylase. As much as 12 mmol (500 mg) calcium is transferred between the ECF and bone each day (a large amount in relation to the total ECF calcium pool), and PTH has a major effect on this transfer. The homeostatic role of the hormone can preserve calcium concentration in blood at the cost of bone destruction.
- PTH has multiple actions on bone, some direct and some indirect. PTH-mediated changes in bone calcium release can be seen within minutes. The chronic effects of PTH are to increase the number of bone cells, both osteoblasts and osteoclasts, and to increase the remodeling of bone; these effects are apparent within hours after the hormone is given and persist for hours after PTH is withdrawn. Continuous exposure to elevated PTH (as in hyperparathyroidism) leads to increased osteoclast-mediated bone resorption.
- However, the intermittent administration of PTH, elevating hormone levels for 1–2 h each day, leads to a net stimulation of bone formation rather than bone breakdown. Striking increases, especially in trabecular bone in the spine and hip, have been reported with the use of PTH in combination with estrogen.
- Osteoblasts (or stromal cell precursors), which have PTH receptors, are crucial to this bone-forming effect of PTH; osteoclasts, which mediate bone breakdown, lack PTH receptors. PTH-mediated stimulation of osteoclasts is believed to be indirect, acting in part through cytokines released from osteoblasts to activate osteoclasts.

Structure

- PTH is an 84-amino-acid single-chain peptide.
- The amino acid portion, PTH(1–34), is highly conserved and is critical for the biologic actions of the molecule.
- Modified synthetic fragments of the amino-terminal sequence as small as PTH(1–11) are sufficient to activate the major receptor.
- The carboxyl-terminal region of PTH binds to a separate receptor (cPTH-R), but it has not yet been cloned. Fragments shortened at the amino terminus bind to cPTH-R and inhibit the actions of the full-length PTH(1–84) or the PTH(1–34) active fragments.

Synthesis

- Parathyroid cells have multiple methods of adapting to increased needs for PTH production. Most rapid (within minutes) is secretion of preformed hormone in response to hypocalcemia. Second, within hours, PTH mRNA expression is induced by sustained hypocalcemia. Finally, protracted challenge leads within days to cellular replication to increase gland mass.
- Transcriptional suppression of the PTH gene by calcium is nearly maximal at physiologic calcium concentrations. Hypocalcemia increases transcriptional activity within hours.
- $1,25(OH)_2D_3$ strongly suppresses PTH gene transcription.
- In patients with renal failure, IV administration of supraphysiologic levels of $1,25(OH)_2D_3$ or analogues of the active metabolite can dramatically suppress PTH overproduction, which is sometimes difficult to control due to severe secondary HPT.
- Regulation of proteolytic destruction of preformed hormone (posttranslational regulation of hormone production) is an important mechanism for mediating rapid (minutes) changes in hormone availability. High calcium increases and low calcium inhibits the proteolytic destruction of hormone stores.

Regulation of PTH Secretion

- PTH secretion increases steeply to a maximum value of five times the basal rate of secretion as calcium concentration falls from normal to the range of 1.9–2.0 mmol/L (7.5–8.0 mg/dL) (measured as total calcium).
- The ionized fraction of blood calcium is the important determinant of hormone secretion.
- Severe intracellular magnesium deficiency impairs PTH secretion
- ECF calcium controls PTH secretion by interaction with a calcium sensor, a G protein–coupled receptor (GPCR) for which Ca2+ ions act as the ligand. Stimulation of the receptor by high calcium levels suppresses PTH secretion. The receptor is present i n parathyroid glands and the calcitonin-secreting cells (C cells) of the thyroid, brain and kidney. Mutations associated with loss of function cause a syndrome FHH resembling hyperparathyroidism but with hypocalciuria. On the other hand, gain of function mutation cause hypocalcemia resembling hypoparathyroidism.

Vitamin D

Synthesis and Metabolism

- 1,25-dihydroxyvitamin D $[1,25(OH)_2D]$ is the major steroid hormone involved in mineral ion homeostasis regulation.

- Vitamin D and its metabolites are hormones and hormone precursors rather than vitamins, since in the proper biologic setting, they can be synthesized endogenously.
- In response to ultraviolet radiation of the skin, a photochemical cleavage results in the formation of vitamin D from 7-dehydrocholesterol.
- Cutaneous production of vitamin D is decreased by melanin and high solar protection factor sunblocks, which effectively impair skin penetration of ultraviolet light. The increased use of sunblocks and a reduction in the magnitude of solar exposure of the general population over the past several decades has led to an increased reliance on dietary sources of vitamin D.
- Dietary sources largely consist of fortified cereals and dairy products, in addition to fish oils and egg yolks.
- Vitamin D from plant sources is in the form of vitamin D2, whereas that from animal sources is vitamin D3. These two forms have equivalent biologic activity and are activated equally well by the vitamin D hydroxylases in humans.
- Vitamin D enters the circulation, whether absorbed from the intestine or synthesized cutaneously, bound to vitamin D–binding protein, an α-globulin synthesized in the liver.
- Vitamin D is subsequently 25-hydroxylated in the liver by cytochrome P450–like enzymes in the mitochondria and microsomes. The activity of this hydroxylase is not tightly regulated, and the resultant metabolite, 25-hydroxyvitamin D [25(OH)D], is the major circulating and storage form of vitamin D.
- Approximately 88% of 25(OH)D circulates bound to the vitamin D–binding protein, 0.03% is free, and the rest circulates bound to albumin. The half-life of 25(OH)D is approximately 2–3 weeks; however, it is dramatically shortened when vitamin D–binding protein levels are reduced, as can occur with increased urinary losses in the nephrotic syndrome.
- The second hydroxylation, required for the formation of the mature hormone, occurs in the kidney.
- The 25-hydroxyvitamin D-1-hydroxylase is a tightly regulated cytochrome P450–like mixed function oxidase expressed in the proximal convoluted tubule cells of the kidney. PTH and hypophosphatemia are the major inducers of this microsomal enzyme, whereas calcium, FGF23, and the enzyme's product, $1,25(OH)_2D$, repress it.
- In addition to being present in the trophoblastic layer of the placenta, the 1-hydroxylase is produced by macrophages associated with granulomata and lymphomas. In these latter pathologic states, the activity of the enzyme is induced by interferon and TNF- but is not regulated by calcium or 1,25(OH)2D; therefore, hypercalcemia, associated with elevated levels of 1,25(OH)2D, may be observed. Treatment of sarcoidosis-associated hypercalcemia with glucocorticoids, ketoconazole, or chloroquine reduces 1,25(OH)2D production and effectively lowers serum calcium.
- The major pathway for inactivation of vitamin D metabolites is an additional hydroxylation step by the vitamin D 24-hydroxylase, an enzyme that is expressed in most tissues. $1,25(OH)_2D$ is the major inducer of this enzyme; therefore, this hormone promotes its own inactivation, thereby limiting its biologic effects. Polar metabolites of $1,25(OH)_2D$ are secreted into the bile and reabsorbed via the enterohepatic circulation. Impairment of this recirculation, seen with diseases of the terminal ileum, leads to accelerated losses of vitamin D metabolites.

Actions of $1,25(OH)_2D$

- 1,25(OH)2D mediates its biologic effects by binding to a member of the nuclear receptor superfamily, the vitamin D receptor (VDR).
- The affinity of the VDR for 1,25(OH)2D is approximately three orders of magnitude higher than that for other vitamin D metabolites.
- Under normal physiologic circumstances, these other metabolites are not thought to stimulate receptor-dependent actions. However, in states of vitamin D toxicity, the markedly elevated levels of 25(OH)D may lead to hypercalcemia by interacting directly with the VDR and by displacing 1,25(OH)2D from vitamin D–binding protein, resulting in increased bioavailability of the active hormone.
- The VDR is expressed in a wide range of cells and tissues.
- The molecular actions of 1,25(OH)2D have been most extensively studied in tissues involved in the regulation of mineral ion homeostasis.
- This hormone is a major inducer of calbindin 9K, a calcium-binding protein expressed in the intestine, which is thought to play an important role in the active transport of calcium across the enterocyte. The two major calcium transporters expressed by intestinal epithelia, TRPV5 and TRPV6 (transient receptor potential vanilloid), are also vitamin D responsive. By inducing the expression of these and other genes in the small intestine, 1,25(OH)2D increases the efficiency of intestinal calcium absorption,
- It has also been shown to have several important actions in the skeleton. The VDR is expressed in osteoblasts and regulates the expression of several genes in this cell. These genes include the bone matrix proteins, osteocalcin and osteopontin, which are upregulated by 1,25(OH)2D, in addition to type I collagen, which is transcriptionally repressed by 1,25(OH)2D. Both 1,25(OH)2D and parathyroid hormone induce the expression of RANK ligand, which promotes osteoclast differentiation and increases osteoclast activity, by binding to RANK on osteoclast progenitors and mature osteoclasts. This is the mechanism by which 1,25(OH)2D induces bone resorption.
- The VDR is expressed in the parathyroid gland, and 1,25(OH)2D has been shown to have antiproliferative effects on parathyroid cells and to suppress the transcription of the parathyroid hormone gene. These effects of 1,25(OH)2D on the parathyroid gland are an important part of the rationale for current therapies directed at preventing and treating hyperparathyroidism associated with renal insufficiency.

Fracture Healing Stages

When a bone fractures there is stripping of the periosteum and the vessels

This leads to bleeding at the fracture site and causes hematoma formation

If the fracture is not reduced the hematoma remains and if is immobilized the hematoma causes inflammation and this leads to exposure of the hematoma to progenitor cells.

This helps in the formation of cartilage cells in this hematoma and it is called the soft callus

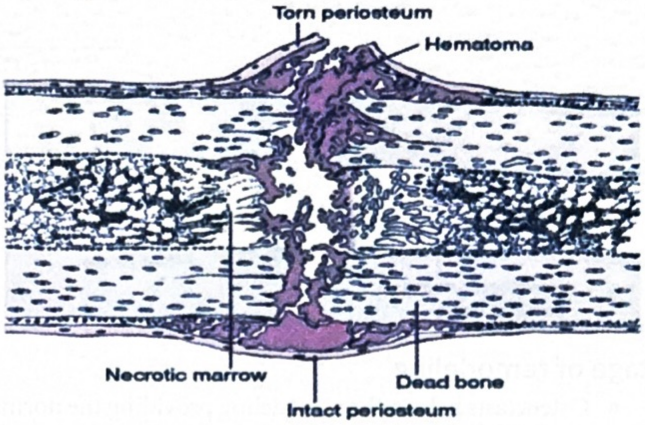
So the stages of healing are

1. Stage of hematoma
2. Stage of inflammation
3. Stage of soft callus
4. Stage of hard callus
5. Stage of remodeling

Stage of hematoma and inflammation:

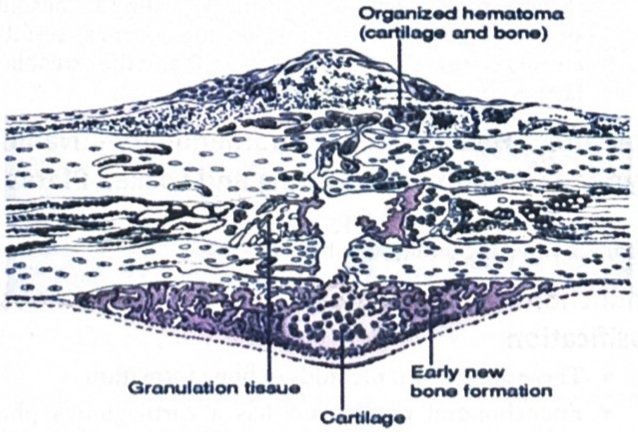
At the time of fracture the following happen-

- The periosteum ruptures
- The blood vessels rupture
- The marrow close to the fracture site becomes necrotic
- The fracture site bone becomes dead
- There is hematoma below the periosteum and the fracture site
- This initiates an inflammatory response and there is increased in many mediator cells and chemicals in this fracture site.
- There is a complex interaction of inflammatory signals, modulators, and cellular components.
- Neutrophils are the earliest and most active cell populations and they remove the debris through their phagocytic mechanism.
- Inflammatory cytokines increase in concentration in early phases, including IL-1β, IL-6, IL-8, IFNγ, and TNFα
- It is always the progenitor cells that help in healing than the already existing mature cells.
- The healing of bone is different from that of any other tissue as the populations of cells that appear in the fracture site and the time at which each individual population appears is important in the final outcome of the union
- Depletion of macrophages or macrophage receptors in the fracture hematoma has deleterious effects on the healing process.
- Initially granulocytes, monocytes, hematopoietic stem cells, and lymphocytes are seen
- Once the above B cell population regress then there is activation of T- cell population in the fracture site
- The periosteum has an inner and an outer layer. The inner layer has periosteal progenitor cells are located
- This is also a source of fibroblasts, osteoblasts, chondrocytes, and a vascular network.
- The periosteum provides an early source of osteoblasts and mesenchymal cells that can directly contribute to calcium deposition and bone formation.
- The periosteal vascular network is responsible for approximately one-third of the cortical blood supply.



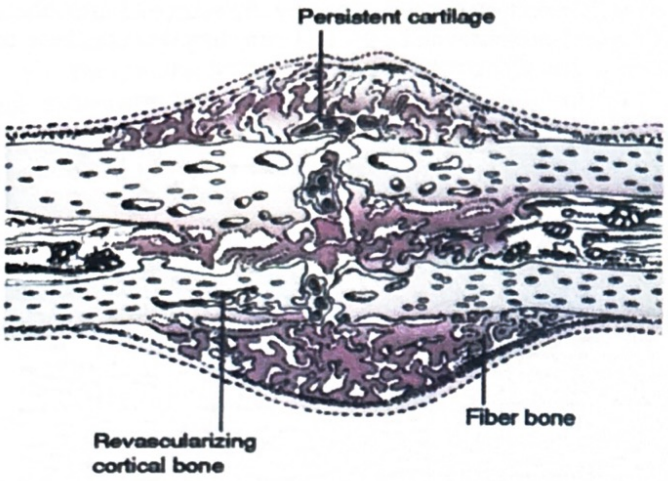
Stage of soft callus

- The mesenchymal cells are derived from the marrow, the endosteal layer of the cortex and peripheral blood. Another potential source of progenitor cells are the pericytes. And are a local cell population that resides in the perivascular space.
- The hematoma when exposed to the inflammatory cells and Sox transcription factor there is formation of chondrocytes which lay down cartilage. Remember Sox9 helps in hypertrophy of the chondrocytes and these remain beneath the periosteum and gets mobilized early once the fracture occurs.
- This chondral deposition is regulated by the TGF -beta and hedgehog signaling
- The chondrocytes get hypertrophied and later undergoes apoptosis and get converted into osteoblasts.



Stage of hard callus

- Osteoblasts lay down calcium and osteoid to transition from soft to hard callus
- These osteoblasts lay done new bone formation
- However due to the high catabolic activity there is osteoclastic activity as well which helps in remodeling



Stage of remodeling

- Osteoclasts help in the remodeling providing the normal shape of the bone
- These cells derive from the hematopoietic cell lineage found on the bone surface and form pits in the surface called Howship lacunae
- This activity is the reason why the bone tissue heals without any scarring

Endochondral ossification and intramembranous ossification

- These are the two methods of bone formation
- Endochondral ossification has a cartilaginous phase before getting converted into the osteogenic phase. This is seen in development of most of the long bones and in fractures which attempt to heal with relative stability
- Membranous ossification does not have a cartilaginous phase and hence skips the stage of soft callus. This is seen in development of flat bones and also fractures that attempt to heal with primary healing or absolute stability. Remember that this healing occurs as result of cutting cone action by the osteoclasts and the osteoblasts lay new bone in this cone.

Fracture Healing in Intramedullary Nailing, Dynamic Compression Plating and Locked Plates

One should know the types of bone healing- primary and secondary healing and how it happens

Endochondral ossification and intramembranous ossification

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- Endochondral ossification has a cartilaginous phase before getting converted into the osteogenic phase. This is seen in development of most of the long bones and in fractures which attempt to heal with relative stability or secondary healing. The endochondral ossification has an anabolic phase where there is increase in chondroblasts and a catabolic phase when there is increased activity of osteoclasts and osteoblasts.
- Membranous ossification does not have a cartilaginous phase and hence skips the stage of soft callus. This is seen in development of flat bones and also fractures that attempt to heal with primary healing or absolute stability. Remember that this healing occurs as result of cutting cone action by the osteoclasts and the osteoblasts lay new bone in this cone.

Fracture healing by intramedullary nailing

- Intramedullary nail is an implant which provides relative stability allowing some micromotion at the fracture site.
- Once the fracture is reduced one can pass the implant intramedullary
- It is a load sharing device
- It has the advantages of not affecting the soft tissue envelope when done closed
- The recent generation nails give the options of fixing the nail with proximal and distal locking and also allows various planes of fixation giving more purchase options
- The nails also give options of dynamization to improve the healing at the fracture site
- As the relative stability is being provided, there is healing through secondary healing and has the cartilagenous phase initially before union.

Factors that affect this healing

1. Size of the nail
2. Contact of the nail with the bone
3. Reamed/unreamed nail
4. Fracture reduction
5. Fracture pattern
6. Soft tissue cover

Size of the nail

- If a smaller size nail is used then there is more unstable fixation and that can lead to callus formation but this callus will not be able to bridge the fracture gap leading to a hypertrophic non union.
- Smaller nails have less bony contact and hence longer working lengths
- If a larger size nail is used then the nail might distract the fracture and lead to a gap non union

Contact of nail with bone

- Reaming increases the contact providing more stability
- The size of the nail also matters to provide contact with bone
- If a small nail is placed in a wider canal then the only contact of nail with the bone will be at the site of screw insertion
- Also know the concept of working length of the nail
- It is the distance between the two bony contacts of the nail proximal and distal to the fracture site.
- The fracture stiffness is indirectly proportional to the working length
- So when the working length is calculated between the nearest two screws in a case of thinner nail the working length increases and hence the stiffness reduces.
- When reaming is done, the point of contact of nail with

bone will be near the fracture site and hence reducing the working length.

Reaming

- Reamed material can act as an internal bone graft.
- Reaming increases the contact of bone with the nail.
- It reduces the working length.
- It initiates an inflammatory response as well which increases chances of union.
- Excessive reaming should not be done as it can either cause an uncontrolled pattern of fracture shattering and also it can cause excessive disruption of the endosteal blood supply
- Chances of thermal necrosis (when there is temperatures more than 50 degree Celsius) also can hinder healing.
- Remember adequate reaming even though initially reduces the endosteal supply, later it leads to an increase in the blood supply after 6 to 8 weeks and 6 times increase in periosteal blood supply.
- Present day concept is to have an optimal reaming for all cases.

Fracture reduction

- Gap more than 2 mm usually hinders healing. Attempt to healing will be noticed in the form of excessive callus.
- Gaps can be attempted to be closed utilizing dynamization principle

Fracture patterns

- Comminuted fractures can benefit with relative stability
- Well reduced long spiral fractures have more contact and helps in better healing.

Soft tissue cover

- An intact soft tissue cover helps in improving the local healing potential as there is intact source of mesenchymal cells to the fracture environment

Fracture healing in a locked plate.

- Locking plates act as an internal fixator while allowing the fracture to heal.
- The screw is fixed on to the bone as well as the plate at the hole where threads are present on both the screw head as well as the wall of the plate hole
- There was better purchase on the bone as well as there were more number of threads on the screw
- While applying a compression screw, it can also push the plate towards the bone and interrupt the periosteal blood supply
- If a locking screw is placed then the plate can be placed away from the bone and there is no disruption of periosteal blood supply
- It has advantages of no screw back out and also gives better stability
- So once a plate is fixed to the bone only with locking screws then it acts as like an internal fixator and provides stability
- So, without providing compression at the fracture site if locking screws alone are placed then the healing happens through enchondral ossification.
- Here healing follows all the stages of healing and this is more biological way of healing.
- This again shows the importance of the bony contact to form union.
- In cases where comminution is present and a bridge plating has to be provided, then avoid providing more screws and prevent a very rigid fixation. Rigid fixation prevents micromovement at the fracture site and leads to non-union along with creating stress risers at the screw neck leading to failure.

Fracture healing in a dynamic compression plate

- In this scenario there is absolute stability provided at the fracture site
- After reduction of the fracture there is additional compression also that is provided at the fracture leading to no possible gap.
- The compression if achieved leads to fracture healing through intramembranous mechanism
- Here the osteoblasts start acting at the time of fixation, skipping the activity of chondrocytes
- The osteoclasts act by making cutting cones through the fracture site
- These cones are filled with new bone by activity of osteoblasts which follow the osteoclasts
- Here there is no chance of external callus formation due to absolute stability provided.

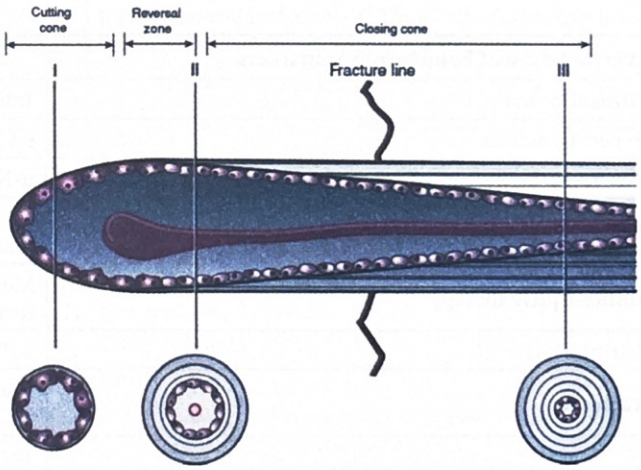
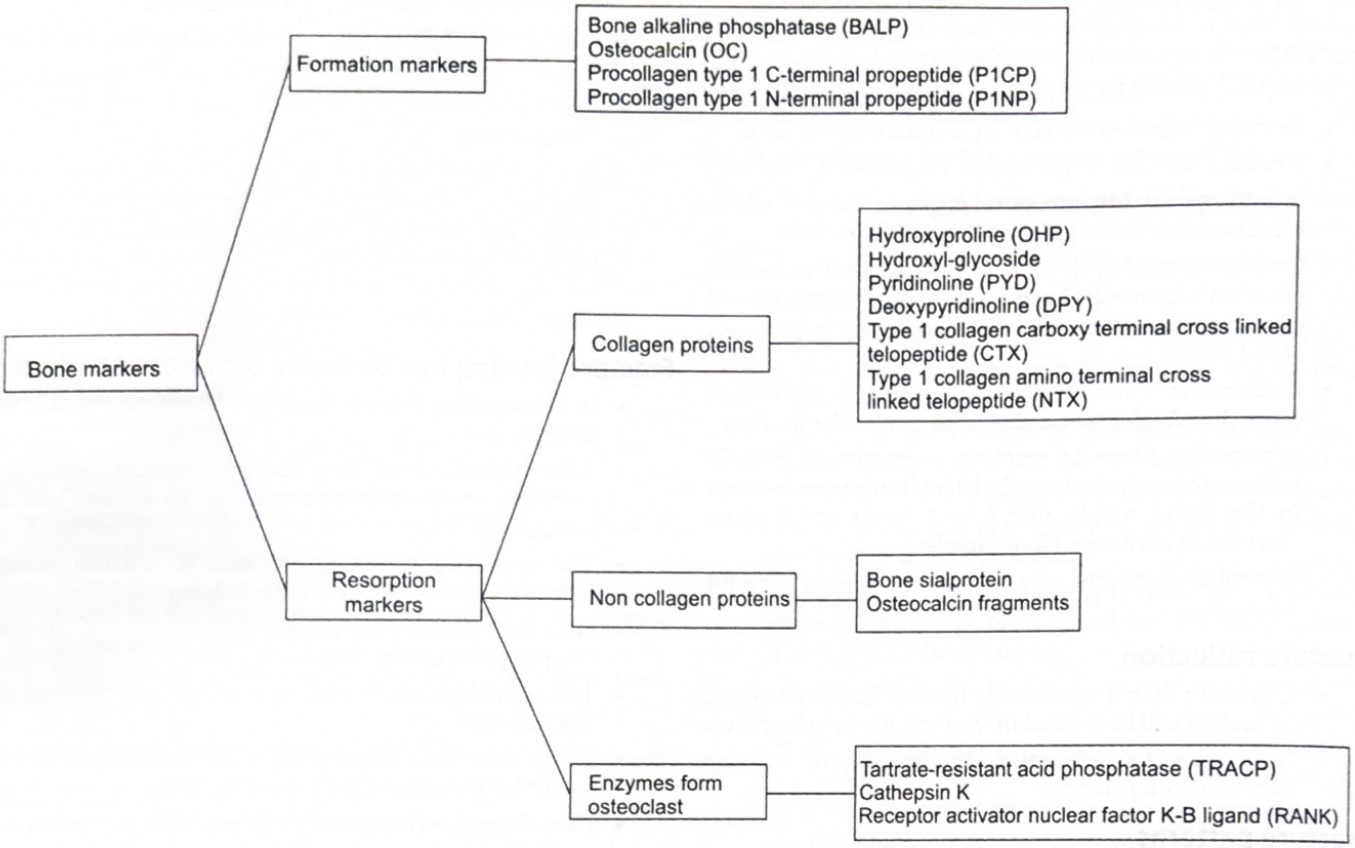


Fig. Primary bone healing utilizes an osteoclastic cutting cone crossing the fracture gap (I) followed bone reconstitution by the trailing osteoblasts (II, III)

- The same can be achieved by an interfragmentary compression screw
- While applying a compression screw, it can also push the plate towards the bone and interrupt the periosteal blood supply which can also affect healing.

One has to remember that new plates are available now which can provide both options of compression and locking so that the compression achieved at the fracture site can be protected better with locking principle. This plate is called a

combi-hole plate.



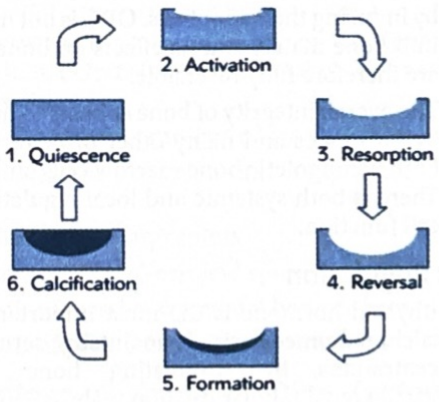
Interpretation of bone turnover markers	
Clinical status	**Interpretation of bone turnover markers**
Hyperthyroidism	s-CTX↑
Hyperparathyroidism	u-NTX↑
Postmenopausal women, paget disease or bone metastasis	Most marker levels ↑; u-NTX excretion and s-BSAP and s-PINP are very sensitive serum osteocalcin levels may be in normal range
Antiresorptive therapy	Most bone marker levels ↓ during antiresorptive therapy, depending on treatment and bone marker
Osteoporosis	s-PINP and s-CTX↑
Fracture	Most bone markers ↑after a fracture, maximal at 2-12 weeks, but effect lasts for up 1 year
Fracture risk	The association between bone formation markers and fracture risk was not statistically significant (especially for OC, BALP, PICP and PINP)
Chronic kidney disease	s-OC, s-CTX, s-BSP ↑
Liver disease	BALP, PICP, PINP, OHP, PYD, DPY, CTX, BSP↑
Hemolysis	s-TRACP↑
Drugs	Glucocorticoids reduced bone turnover marker levels Anticonvulsants increased bone turnover marker levels Oral contraceptive reduce bone turnover marker levels with > 35 years of use

BALP: Bone-specific alkaline phosphatase, BSP: Bone sialoprotein, CTX: Type 1 collagen carboxy terminal cross-linked telopeptide; DPY: Deoxypyridinoline;
NTX: Type 1 collagen amino terminal cross-linked telopeptide; OC:osteocalcin; OHP: Hydroxyproline; P1CP: Procollagen type1 C-terminal propeptide; P1NP: Procollagen type 1 N-teminalpropeptide; PYD: Pyridinoline; S:Serum; TRACP: Tartrate-resistant acid phosphatase; U:Urine.

Preanalytical factors for bone turnover markers		
Marker	**Source**	**Interpretation**
Bone alkaline phosphatase (BALP)	Osteoblast membrane-bound tetramer enzyme	It is bone-specific but can cross-react with liver isoforms up to 10%. The results can be adversely affected by liver alkaline Phosphatase level. The effect of circadian rhythm is very low.
Procollagen type 1 N-terminal propeptide (P1NP)	Precursor molecules of collagen type 1 synthesized by osteoblasts	Tests have been developed for intact or total forms. The effect of circadian rhythm is very low. It is the most sensitive marker of bone formation and is particularly useful in monitoring bone formation and anti-resorptive therapies. The biological and analytical variability of serum P1NP has been well documented
Procollagen type 1 C-terminal propeptide (P1CP)	Precursor molecules of collagen type 1 synthesized by osteoblasts	It is mostly derived from bone collagen type 1 (90%) The effect of circadian rhythm is very low.
Type 1 collagen amino terminal cross-linked telopeptide (NTX)	Serum NTX formed by osteoclastic hydrolysis of type 1 collagen.	The effect of circadian rhythm is high.
Type 1 collagen carboxy terminal cross-linked telopeptide (CTX)	Serum CTX is always β-isomerized. It is formed by osteoclastic hydrolysis of collagen. Cathepsin K releases CTX	Test samples require prior fasting The level is influenced by kidney and liver functions The effect of circadian rhythm is high. The biological and analytical variability of s-CTX has been well documented EDTA is preferred for plasma samples
Urinary Deoxypyridinoline (DPY)	Proteolytic hydrolysis of collagen	Present in mature collagen only It is independent of dietary intake It is influenced by UV radiation and circadian rhythm
Urinary pyridinoline (PYD)	Bone, cartilage, tendon, blood vessels	Present in mature collagen only It is independent of dietary intake It is influenced by liver function, active arthritis and UV radiation The effect of circadian is high Results should be provided as the ratio of creatinine
Serum tartrate-resistant acid phosphatase	Platelets, erythrocytes osteoclasts	It is influenced by hemolysisThe effect of circadian is high Sample stability: 2 years at -80° C
Serum/urine osteocalcin	Osteoblasts and odontoblasts;	It is influenced by the kidneys The effect of circadian is high

Bone remodelling

- Bone is a living organ that undergoes remodelling throughout life.
- Remodelling results from the action of osteoblasts and osteoclasts, and defects such as microfractures are repaired by their coupling.
- In a homeostatic equilibrium resorption and formation are balanced so that old bone is continuously replaced by new tissue so that it adapts to mechanical load and strain.
- Osteoclasts and osteoblasts closely collaborate in the remodeling process in what is called a basic multicellular unit (BMU).
- The organization of the BMUs in cortical and trabecular bone differs (mainly morphological rather than biological).
- In cortical bone the BMU forms a cylindrical canal and gradually burrows through the bone with a speed of 20–40 micrometre/day.
- During a cycle 10 osteoclasts dig a circular tunnel in the dominant loading direction and then they are followed by several thousands of osteoblasts that fill the tunnel.
- In this manner between 2% and 5% of cortical bone is being
- Th actively remodelled than larger surface to volume ratio.
- Osteoclasts travel across the trabecular surface with a speed of approximately 25 micrometre/day, digging a trench.
- The remodelling cycle consists of three consecutive phases:
  1. Resorption,
  2. Reversal, and
  3. Formation.



- Resorption (2 weeks) -begins with the migration of partially differentiated mononuclear preosteoclasts

to the bone surface where they form multinucleated osteoclasts.

- Reversal (4 or 5 weeks)- After the completion of osteoclastic resorption, there is a reversal phase when mononuclear cells appear on the bone surface. These cells prepare the surface for new osteoblasts to begin bone formation and provide signals for osteoblast differentiation and migration.
- Formation phase (4 months)- follows with osteoblasts laying down bone until the resorbed bone is completely replaced by new. When this phase is complete, the surface is covered with flattened lining cells and a prolonged resting period begins until a new remodelling cycle is initiated.
    - The bone remodelling cycle begins with activation mediated by cells of the osteoblast lineage.
    - Activation may involve the osteocytes, the lining cells, and the pre-osteoblasts in the marrow.
    - These cells undergo changes in their shape, they secrete enzymes that digest proteins on the bone surface and express a peptide (member of the tumor necrosis factor (TNF) superfamily) called receptor activator of NF-kappa B ligand (RANKL).
    - RANKL interacts with a receptor on osteoclast precursors called RANK.
    - The RANKL/RANK interaction results in activation, differentiation, and fusion of hematopoietic cells of the osteoclast lineage so that they begin the process of resorption.
    - Furthermore, it also prolongs osteoclast survival by suppressing apoptosis.
    - This interaction indicates that bone resorption and bone formation are coupled among others through RANKL.
    - The effects of RANKL are blocked by osteoprotegerin (OPG), a secretory dimeric glycoprotein belonging to the TNF receptor family. OPG acts as a decoy receptor (a soluble receptor acting as antagonist) for RANKL and it is mainly produced by cells of the osteoblast lineage, but it can also be produced by the other cells in the bone marrow.
    - OPG regulates bone resorption by inhibiting the final differentiation and activation of osteoclasts and by inducing their apoptosis. OPG is not incorporated into bone matrix and its effects on bone resorption are therefore fully reversible.
    - The overall integrity of bone appears to be controlled by hormones and many other proteins secreted by both hemopoietic bone marrow cells and bone cells. There is both systemic and local regulation of bone cell function.

Systemic Regulation

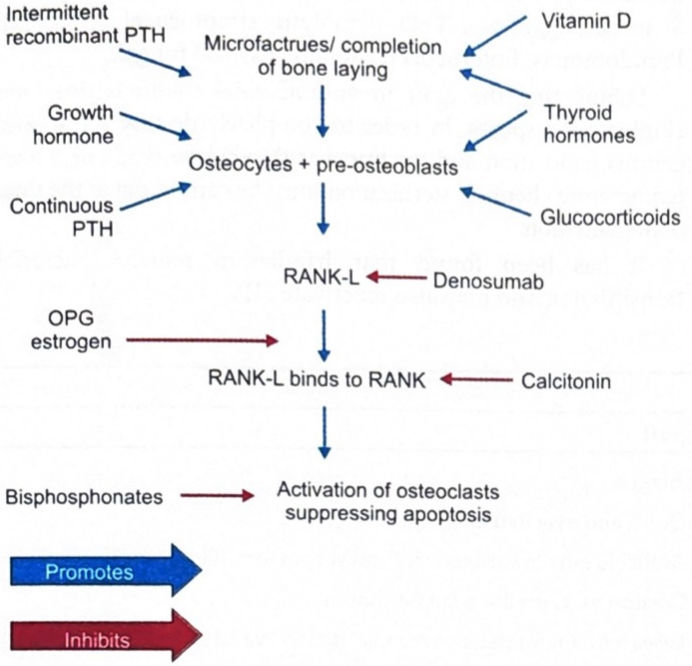
- Parathyroid hormone is the most important regulator of calcium homeostasis. It maintains serum calcium concentrations by stimulating bone resorption, increasing renal tubular calcium reabsorption and renal calcitriol production. PTH stimulates bone formation when given intermittently and bone resorption when secreted continuously.
- Calcitriol is essential in enhancing intestinal calcium and phosphorus absorption, and in this way it promotes bone mineralization. In addition, vitamin D3 possesses important anabolic effects on bone, thus exerting a dual effect on bone turnover.
- Calcitonin, in pharmacologic doses, mediates loss of the ruffled border, cessation of osteoclast motility, and inhibition of the secretion of proteolytic enzymes through its receptor on osteoclasts. This effect, however, is dose limited and its physiologic role is minimal in the adult skeleton.
- Growth hormone (GH)/IGF-1 system and IGF-2 are important for skeletal growth, especially at the cartilaginous end plates and during endochondral bone formation. They are among the major determinants of adult bone mass through their effect on regulation of both bone formation and resorption.
- Glucocorticoids exert both stimulatory and inhibitory effects on bone cells. They are essential for osteoblast maturation by promoting their differentiation from mesenchymal progenitors but they decrease osteoblast activity. Furthermore, glucocorticoids sensitize bone cells to regulators of bone remodeling and they augment osteoclast recruitment.
- Thyroid hormones stimulate both bone resorption and formation. Thus, bone turnover is increased in hyperthyroidism and therefore bone loss can occur.
- Estrogens decrease the responsiveness of the osteoclast progenitor cells to RANKL, thereby preventing osteoclast formation. Furthermore, besides reducing osteoclast life span, estrogens stimulate osteoblast proliferation and decrease their apoptosis. They affect gene coding for enzymes, bone matrix proteins, hormone receptors, transcription factors, and they also upregulate the local production of OPG, IGF I, IGF II, and TGF-ALPHA.
- Androgens are essential for skeletal growth and maintenance via their effect on androgen receptor, which is present in all types of bone cells.

Local Regulation

- RANKL, expressed on the surface of preosteoblastic/ stromal cells binds to RANK on the osteoclastic precursor cells and is critical for the differentiation, fusion into multinucleated cells, activation, and survival of osteoclastic cells. OPG inhibits the entire system by blocking the effects of RANKL.
- Macrophage colony-stimulating factor (M-CSF), which binds to its receptor, c-Fms, on pre-osteoclastic cells, appears to be necessary for osteoclast development
- A number of cytokines such as TNF-alpha and IL-10 modulate this system primarily by stimulating M-CSF production and by directly increasing RANKL expression.
- IL-6, a pleiotropic cytokine secreted by osteoblasts, osteoclasts, and stromal cells, appears to be an important regulator of bone remodelling by stimulating osteoclastic bone resorption but also by promoting osteoblast generation in conditions of high bone turnover.
- Recent studies have also suggested that osteoblast-

derived PTHrP promotes the recruitment of osteogenic cells and prevents the apoptotic death of osteoblasts, thus being an important regulator of bone cell function.

- Abnormalities of bone remodeling can produce a variety of skeletal disorders.



ALLOGRAFTS

- A graft may be **orthotopic** (when it is transplanted to the exact same site in recipient that it occupied in the donor, e.g. proximal tibia to proximal tibia); **heterotopic** (when it is transplanted to a different site but one occupied by the same tissue as in the donor, e.g. fibula to spine); or **ectopic** (transplanted to a site normally occupied by a different type of tissue, e.g. fascia lata as a tendon graft).
- Allograft Supply in our country is limited due to lack of awareness and availability of minimal efficient banking facilities.
- **Allografts or allogenic bone grafts** are available in various forms:
  - **Fresh**—
    - It has High immunogenic potential
    - No known clinical use
  - **Fresh-frozen**—
    - It is Less immunogenic but needs an additional procedure for sterilization.
    - Advantage is that it preserves Bone morphogenic protein.
  - **Freeze-dried (lyophilized)**:
    - Bone must be extracted from donor within 8–12 hours of death.
    - Intracellular and extracellular enzymes cause enzymatic autodigestion and lead to loss of inductive factors and BMP of bone.
    - The type that retains inductive factors is known as Antigen-extracted allogeneic (AAA) bone.

1. Chloroform-methanol is used to extract lipids and cell membrane lipoproteins (4 hours);
2. HCl extracts acid-soluble proteins and requires 24hrs to demineralize the surface; and
3. Neutral phosphate buffer is used to remove endogenous intracellular and extracellular transplantation antigens. The bone is then frozen and freeze dried and thereafter stored at −60°C.

Demineralized bone matrix (or bone matrix gelatin):

- It is used as bone graft extender
- It is a digested source of BMP.
- DBM is available in two forms, dry or injectable.
- DBM has to be mixed with a carrier.
- Carriers (which are inert with regards to bone generation) include hyaluronic acid, glycerol, collagen, gelatin, and actual derivatives of DBM itself.
- Second generation DBM putties (where the carrier is loaded with BMP) have higher concentrations of BMP and are possibly better.

Osteochondral allografts:

- It is a bony chunk alongwith cartilage cover
- Used for large articular defects in osteochondroses of knee.

Shell allografts:

- Devascularized osteoarticular graft with a small bony component is used for
- Biologic resurfacing of articular defects.

Large composite allografts:

- Usually required for excision of large tumors or reconstruction of defects in revision arthroplasty after freeze thawing.

Indications for use of allograft:

- Reconstruction of bone defects as a result of primary joint arthroplasty osteolysis
- Reconstruction of skeletal defects following tumor resection
- Reconstruction of congenital or developmental bone and joint defects (protrusioacetabuli, dysplastic hip) and deformities.
- Repair of fresh comminuted fractures with bone loss
- Treatment of nonunion and complicated osteoporotic fractures
- Arthrodesis of large joints
- Treatment of scoliosis and spinal fusion
- Repair of massive segmental bone defects
- Repair of periodontal osseous defects.

Complications of bone grafting (autograft and allograft):

- Development of incisional hernia
- Vascular injury
- Neurological injury: posterior iliac grafting—cluneal nerves, anterior iliac grafting—lateral femoral cutaneous grafting
- Fracture of donor bone
- Hematoma and seroma formation
- Cosmetic concern and chronic pain
- Transmission of infection (allograft)
- Very rarely tumor cell transplantation.

Infections associated with bone allografts:

Allografts act as porous, acellular and avascular foreign bodies that are liable for bacterial adhesion.

After attachment bacteria become unapproachable to immune surveillance and local cellular defence mechanisms by secreting a thick glycocalyx matrix.

Varied infections have been reported form implanted allograft bone including viral [hepatitis B and C, human immunodeficiency virus (HIV)], bacterial [Clostridium species, S aureus, (group-A beta hemolytic streptococci (GABHS), Pseudomonas, Enterococcus organisms] and fungal.

Submerging the graft in antimicrobial solutions does not eliminate the spores. In order to completely destroy the spores, gamma irradiation and treatment with ethylene oxide or a low-temperature chemical sterilization must be carried out at the time of implantation.

It has been found that Irradiation reduces bacterial transmission and may also inactivate HIV.

Advantages and Disadvantages of autograft versus allograft

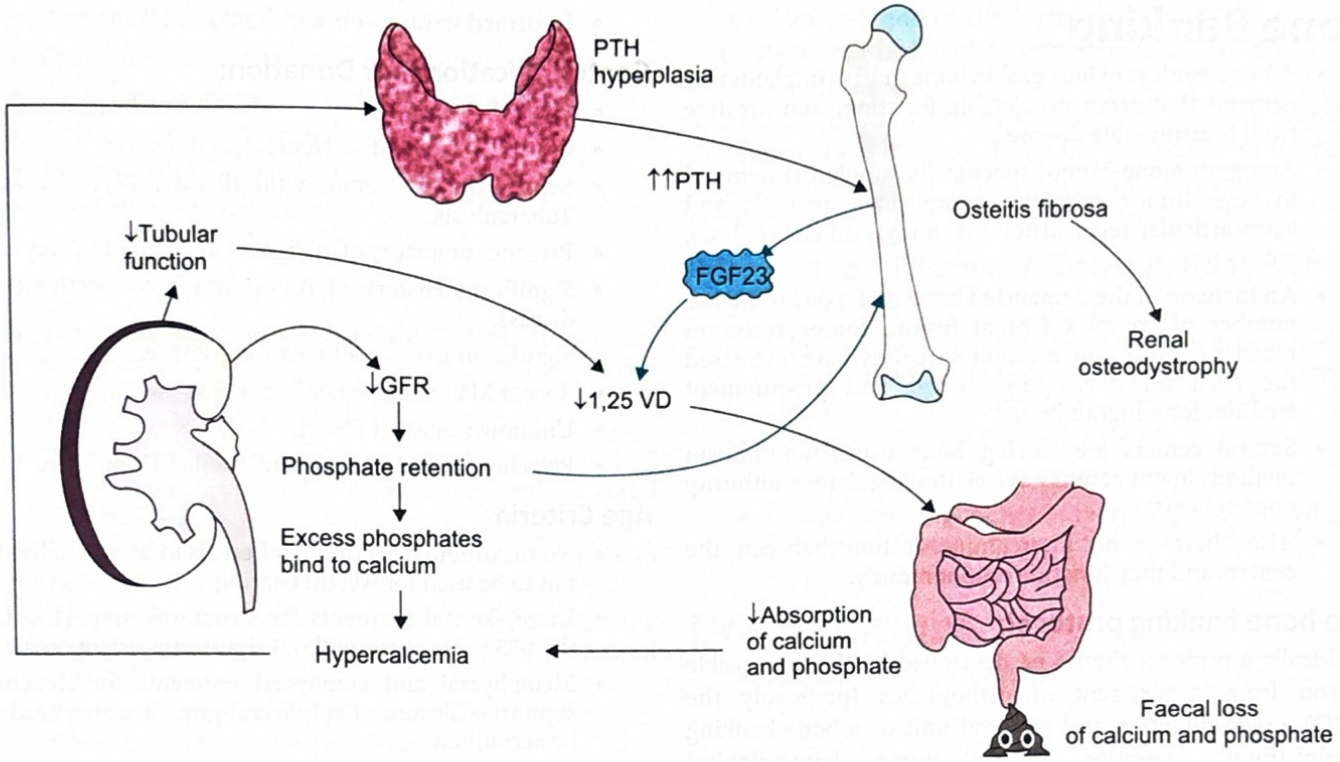
Autograft	Allograft
Advantages: • Readily available • Quick and reliable incorporation • No immunogenicity • No clearance hassles for authority • No risk of "transmitting" infections as with allografts • Different forms—cortical, cancellous, combined available for various use	**Advantages:** • Quick and easy to use • Available now in various sizes and shapes for different use • Cortical vs. cancellous can be chosen • Large amount for massive reconstruction available • Shortens operative time
Disadvantages: • Increase time of surgical procedure • Limited in amount for large reconstructions and defects • Pain at operative site, morbidity and increased length of stay • Increased cost of surgery • "Second" surgery and potential risks like infection • Chronic pain may persist • Possibility of hernia and secondary complications	**Disadvantages:** • Requires set-up and maintenance • Not readily available in India • Various regulatory clearances needed • Risk of infection especially the clostridial and viral infections has still not been eliminated • Weak osteogenic potential though provides good structural support (osteoconductive) • Procurement, processing and preservation need expertise and dedication

Effect of CKD on bones in adults, diagnosis and management

- Chronic kidney disease can occur due to both congenital and acquired conditions
- Chronic kidney disease leads to changes on bones in adults; known as renal osteodystrophy
- It is characterized by the presence of secondary hyperparathyroidism
- There can be two types of disease which can be seen with renal osteodystrophy
  - High turnover bone disease
    - Also known as osteitis fibrosa cystica
    - 2º hyperparathyroidism → osteoclast activation and bone resorption
  - Low turnover bone disease
    - Seen due to
      - control of renal disease with transplant or dialysis
      - High doses of calcium and phosphate binding agents
      - Parathyroidectomy
    - Adynamic form of renal osteodystrophy

Pathophysiology

- Inability of the damaged glomerulus to excrete phosphorus→Hypocalcemia
- Raised FGF23 → decreased production of active vitamin D →Hypocalcemia
- Hypocalcemia→ 2º hyperparathyroidism → bony changes



Diagnosis

- Clinical features
  - Resembles rickets/osteomalacia
  - Wide physis, rachitic rosary
  - Periarticular enlargement of long bones
  - Short stature and long bone bowing
    - Lower limb involvement > upper limbs
  - Bone pain / Fractures
  - Genu valgum
  - SCFE
- Radiological findings
  - Due to vitamin D deficiency
    - Generalized osteopenia
    - Thinning of cortices / Ground glass appearance
    - Wide physis
  - Due to 2° hyperparathyroidism
    - Resorption of terminal tufts of the distal phalanges
    - Subperiosteal resorption of bone
    - SCFE
    - Salt and pepper skull
    - Osteosclerosis
    - Rugger jersey spine
    - Brown tumours
  - Due to generalized disease/treatment with steroids for renal failure
    - Pathological fractures / Corticosteroid induced AVN

Management

- Medical management
  - Treatment of the underlying renal disease
  - Medical treatment
    - Vitamin D
      - Active form
      - Calcitriol therapy
    - Treatment of acidosis
    - Phosphate binding agents
    - Growth hormone
- Orthopaedic management
  - Bracing is not helpful
  - Surgery in cases of
    - Angular deformity of the lower extremities
      - Corrective osteotomies
        - Multilevel deformity correction
    - SCFE
      - Correction of metabolic disease
      - Screw epiphysiodesis
    - Avascular necrosis
      - Symptomatic treatment
      - Total hip replacement
    - Pathologic fractures / Brown tumours
      - Biopsy + internal fixation

Bone Banking

- A bone bank provides usable bone grafts (implants) on demand that serve predictable function, and are free from transmissible disease.
- Autograft alone cannot specifically supply the demand in large tumor excisions, complicated articular and extra-articular reconstructions in revision arthroplasty, etc.
- An increase in the demand of bone grafts due to higher number of complex (spinal fusion, tumor resection reconstruction) and revision surgeries have increased the need for developing storage and procurement facilities for allograft bone.
- Several centers are storing bone using nonuniform methods in our country as a central regulatory authority does not exist.
- Also there is no intercommunication between the centers and they function autonomously.

The bone banking protocol:

Ideally a protocol should be developed by the responsible person from department of orthopedics (preferably the HOD), organ donation and retrieval unit or a bone banking administrator, operation theatre nurse, hematological laboratory technician, pathologist and microbiologist in a combined effort depending on the needs and availability of resources for that center and surroundings.

They also comprise the team or organizational unit of bone bank. The bone banking administrator need not be a medical personnel but should have adequate training to handle the bone bank.

Methods for Bone Banking:

1. Freezing: -200C to -800C
2. Defatting →Freeze Drying →ETO sterlisation
3. Autoclaving and Boiling : Not truly recommended as they impair mechanical properties of grafts ,destroy osteo-inductive capacity of bone and diminish Bone graft incorporation
4. Ionizing radiation: provide freeze dried, gamma irradiated bone allograft for transplantation

Bone Donation:

- Cadavers or live donors
- In cadavers should be retrieved as soon as possible after death
- Tissues from live donors are obtained as surgical residues consequent to surgical procedures. Ex: Femoral Head grafts from THR, tibial shavings from TKR, Bone Wedges from Tibial osteotomies.

Ethical Aspects:

- When Bone is obtained specially for therapeutic purposes donor must be informed.
- All samples should be screamed for HIV, Hepatitis and syphilis.
- Informed written consent should be taken.

Contraindications for Donation:

- HIV,HBV,HCV
- Central Degenerative Neurological disease
- Septicaemia, systemic viral disease, Mycosis, active Tuberculosis.
- Presence or history of malignant disease with exceptions.
- Significant History of Autoimmune connective tissue disease.
- Significant Exposure to toxic substance.
- Recent Major surgery or burns.
- Unknown cause of Death.
- Presence or evidence of irradiation at Donor site.

Age Criteria

- No maximum age criteria if Bone is to be morsellised or not to be used for weight bearing.
- Large skeletal segments for structural support → Less than 55 years of age without significant osteoporosis.
- Metaphyseal and epiphyseal segments for structural support→Closure of epiphyseal growth plates needs to be accounted.
- Viable Cartilage or Osteochondral allograft or meniscus donor →Less than 45 years.
- Tendons or fascia lata→<65 Year

Types of Grafts

- Deep Frozen Allografts
- Irradiated Frozen Bone
- Processed Bone
- Lyophilized ,irradiated bone
- Demineralised Bone

Alloprosthetic Composite

- Over a period of time the osteoarticular allografts show degeneration of articular cartilage causing collapse and fragmentation
- Hence bone length should ideally be reconstructed with bone and joint surface with a prosthesis whenever the entire bone is to be replaced
- In the hip this is useful where Bone stock can be restored with Allograft and Hip joint function with a prosthesis.
- The Allograft also permits biological attachment for muscles especially the abductors.
- Around the knee the alloprosthetic composite may last longer than the prosthesis or allograft alone.
- This method helps the surgeon to combine the advantages of both the methods of reconstruction
- Given below are the individual advantages of allograft and endoprosthesis which tend to get combined in a composite of allograft and endiprosthesis

Advantages of allografts:

- Quick and easy to use
- Available in various sizes and shapes for all kinds of use
- Cortical vs cancellous can be chosen
- Large amount for massive reconstruction available
- Shorten the operative time
- No donor site morbidity

Avdantages of endoprosthesis:

- For metaphyseal lesions that are difficult to reconstruct using conventional methods because of extensive bone destruction, it is possible to provide a functional limb with modular endoprosthetic systems.
- This is successfully used for the proximal femur and distal femur, proximal tibia and proximal or distal humerus.
- Though uncommon but lesions around ankle are also amenable to this
- They are highly effective in maintaining function having a low failure rate (primarily due to limited patient survival)
- But cost is a prohibiting factor in the developing nations where health insurance penetration is low.
- Also, dedicated centers regularly treating such patients are missing.
- The high success rate for any arthroplasty primarily depends on the volume of treated cases, and hence the expertise developed through experience.

Phemister BG Bone Grafting:

In 1947 Phemister published a technique of onlay bone grafting for use in the treatment of nonunited fractures of the long bones. The operative procedure is so designed that the site of nonunion is approached through healthy tissue, the periosteum being stripped from about one side or surface of the fragments at the site of nonunion. The bony bed is prepared only enough to allow the graft to lie in close apposition to both fragments, and the graft, instead of being fixed with screws or ties, is held in place by the periosteum and soft tissues which are sewn snugly over it. A cast is applied for immobilization.

With the Phemister technique the fibrous union is not disturbed, and the old practiceof removing the fibrous union of pseudarthrosis, sawing the ends of the bone off clean and drilling the medullary canal, is unnecessary. Rigid fixation of the onlaygraft with screws or by other methods is also unnecessary and in fact may be contraindicated in some cases. Fixation requires more exposure and soft-tissue stripping, thus decreasing the circulation at the fracture site; it prevents the osteogenic effect of compression at the fracture site by muscular contraction and increases the chances of fracture of the bone graft by holding it completely rigid.

The bonegraft, whether in one or more pieces, is relatively strong. It bridges the fracture, splints the fragments, and contains osteogenic cells which survive along its periosteal and endosteal surfaces. Stripping of periosteum and other soft parts attached to one side of the bone, chiseling off prominences to create a level bed, and accurate application of the graft to the fragments set up osteogenesis from both shaft and graft. This results in formation of callus, which unites the graft to the fragments of the shaft. Rigid immobilization of the site of fracture then occurs, the fibrous union undergoes ossification, and the fragments are bridged with bone.

Principles of Phemister bone grafting can be summarized as:

- Fibrous union should not be broken down by refreshing or resection as it disturbs the ongoing healing process and increases instability.
- Non union heals if induced to do so.
- Rigid immobilization is unnecessary.
- Can be used in presence of recent sepsis provided graft is inserted through normal tissues away from fracture site.

Bone cement

- Commonly used cement is Polymethyl methacrylate (PMMA)
- "Cement" is a misnomer
- This is because PMMA doesn't bind two things together
- It acts as a space-filler
- Once the cement sets in, it creates a tight space which can hold implants against the bone
- It hence acts as a grout
- PMMA is viscoelastic and hence useful in prosthesis survival.
- They do not have any adhesive properties but they provide a close mechanical interlock between the bone surface and the prosthesis.
- The bone surface is irregular and the cement has option to move into these crevices when it is in the sticky phase
- Once it becomes hard the crevices in the bone prevents the bone cement from sliding down and hence forms a mechanical bond
- Other types - calcium phosphate cements (CPCs) and Glass polyalkenoate (ionomer) cements (GPCs) are successfully used in a variety of orthopaedic applications.
- CPCs are bio resorbable and biocompatible, but are mainly used in cranial and maxillo-facial surgeries because of their low mechanical strength.

History

- Themistokles Gluck in 1870- fixed a total knee prosthesis made of ivory using cement made of plaster and colophony.
- Otto Rohm and Kulzer -early pioneers who worked on physical properties and uses.
- John Charnley – 1958
  1. The first surgeon to use PMMA in orthopedics practice
  2. He used cold cured PMMA
  3. He attached an acrylic cup to the femoral head and

fixed a metallic femoral prosthesis.

4. He was the first to use PMMA to fill the medullary canal and found that it was easy to blend the cement with the bone morphology.

- 1970- FDA approval for use of PMMA in replacements

Constituents

- PMMA is an acrylic polymer
- Formed by mixing two sterile components
- A liquid MMA monomer and a powered MMA-styrene co-polymer.
- While mixing both the components the liquid part which is a monomer polymerizes around the pre polymerized powdered particles and leads to formation of a hardened PMMA.
- While this happens there is heat generation and hence it is an exothermic reaction.
- If the PMMA is exposed to light or high temperatures there can be premature polymerization of the liquid component.
- To prevent this, an additional component- Hydroquinone is added.
- This acts as a stabiliser or inhibitor and helps to prevent premature polymerization.
- Di-benzoyl peroxide (BPO)in the powder acts as an initiator
- N, N dimethyl-p-toluidine (DmpT) acts as an accelerator. This promotes the polymer and monomer to polymerise at room temperature- cold curing cement.
- Zirconium dioxide (ZrO2) or barium sulphate (BaSO4) in the cement makes it radiopaque and therefore acts as a contrast agent
- Zirconium dioxide does not affect the mechanical properties of the cement as it is less soluble than barium sulphate
- Polymerization temperatures
- When the polymerization happens there is around 82 to 86 degree Celsius heat that is formed
- While inside the body there is not much heat achieved as the thickness of bone cement between the implant and the bone is very thin. There is a larger surface area of bone cement and hence the sudden rise in temperature is dissipated fast. The blood flow beneath these cement mantle also dissipates the heat.
- This doesn't cause much damage to the native bone or surrounding soft tissues.
- When used to fill cavities should be careful to protect the tissues surrounding or use methods to reduce the temperature of the bone cement.
- This heat is also utilized to destroy any tumorous cells following curettage as well.

Constituents of bone cement.	
Powder	Liquid
I) Polymer: Polymethyl methacrylate/co-polymer (PMMA)	I) Monomer: Methyl methacrylate (MMA)
II) Initiator: Benzoyl peroxide (BPO)	II) Accelerator: N, N-Dimethyl para- toluidine (DMPT)/diMethyl para-toluidine (DMpt)
III) Radio-opacifier: Barium sulphate (BaSO4)/Zirconia (ZrO2)	III) Stabilizer: Hydroquinone
IV) Antibiotics (e.g. Gentamycin)	

Curing process

4 stages:

a) Mixing
b) Sticky/ waiting
c) Working
d) Hardening.

Mixing

- Done by hand or with the aid of centrifugation or vacuum technologies
- As the bone cements are highly heat sensitive, there are various factors affecting the time taken for the cement to set.
- Temperature- 23 Degree Celsius is ideal and any other temperature can affect the handling characteristics and setting time of the cement.

1. Manual handling
2. Body temperature
3. Humidity- hence setting time can vary in winter and summer
4. Type of mixing- hand, vacuum etc
5. Pre-opening treatment of cement- unopened cement components to be stored at 23 Degree Celsius for a minimum of 24 h before use. This can increase the setting time.

Methods of application

Digital

- All antibiotic bone cements can be applied digitally.
- The cement is mixed thoroughly but carefully to minimize the entrapment of air.
- The mixing is done till the dough is formed well and one has to wait to see if the cement is sticking to the glove or not.
- Once the cement doesn't stick to the glove then the cement has entered into the next phase of working.