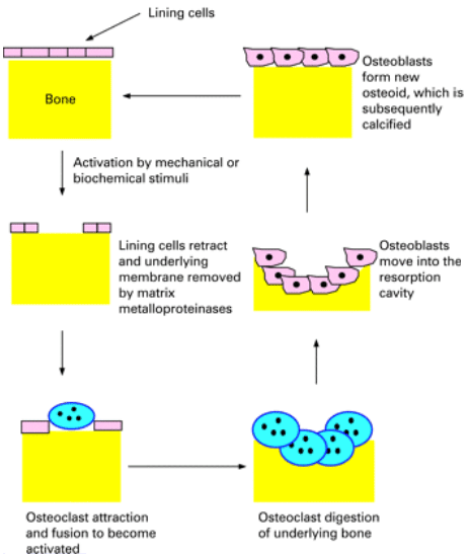
CB-2-C-5: Perform the analyses to laboratory standard operating procedures on:

- ***Bone metabolism (calcium, phosphate, magnesium and alkaline phosphatase)***
- ***Vitamin D and its metabolites***
- ***Parathyroid hormone (PTH)***
- ***Markers of bone turnover.***

Bone turnover:

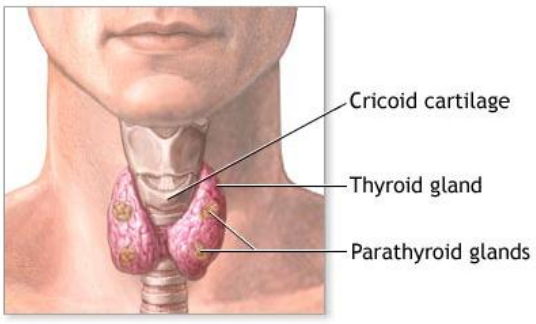
Bone remodelling is a lifelong process in which mature bone is removed (bone resorption) and new bone is formed (ossification). This process occurs in response to injury, for example fractures and micro-damage, functional demands and physical loading. It is also highly important in controlling the concentration of calcium in blood. In adults, approximately 10% of the skeleton is remodelled each year and imbalances between the two processes of bone remodelling can lead to a range of metabolic bone diseases, for example osteoporosis.



The two main cells involved in bone remodelling are osteoclasts which break down bone and osteoblasts which secrete new bone. Regulation of these cells is controlled by several complex pathways involving many different signalling molecules such as parathyroid hormone, vitamin D, calcium, RANK and RANKL and various cytokines and growth factors. When stimulated via appropriate pathways osteoblasts become activated. This causes them to express RANKL which binds RANK receptors on osteoclasts, in turn activating them and causing bone resorption. Osteoblasts also express osteoprotegerin which inhibits RANK/RANKL interaction by binding RANKL and stopping osteoclast over activation. Following bone resorption, osteoblasts secrete new bone, called osteoid, which is subsequently calcified.

Parathyroid hormone (PTH):

PTH is a hormone secreted by chief cells of the parathyroid glands, four small endocrine glands situated on the thyroid gland in the neck. PTH contains 84 amino acids and interacts with its receptors via 34 N-terminal residues. It has a half-life of approximately 4 minutes. Parathyroid secretion of PTH is important in the regulation of blood calcium and becomes clinically significant in hyper and hypo-parathyroidism.



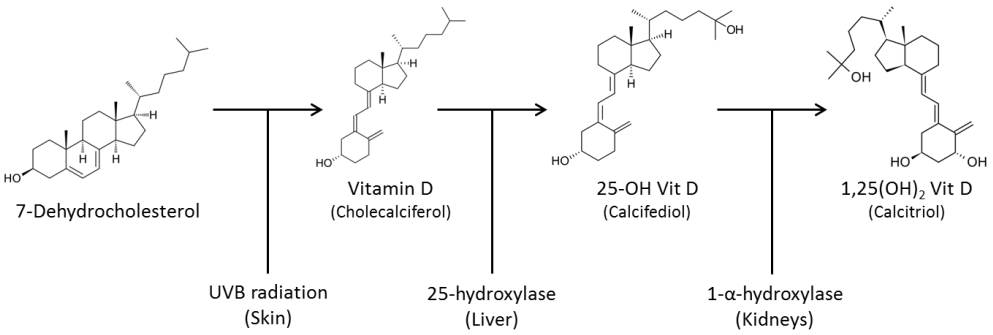
Ionised or free blood calcium is the biologically active form of calcium in the body and is tightly regulated (1.13-1.32mmol/L). Falls in calcium are detected by the parathyroid glands which synthesise and secrete PTH into the circulation. PTH then acts on the kidney and skeleton to cause the following effects:

- Kidney;
    - Increased tubular reabsorption of calcium
    - Increased 1-α-hydroxylase activity
- Bone;
    - Increased bone resorption

By increasing calcium resorption in the kidney less calcium is excreted whilst increasing bone break down releases calcium into the blood. Both of these processes increase blood calcium which in turn has a negative feedback effect on the secretion of PTH as the calcium concentration returns to normal.
PTH is measured in the lab is patient plasma collected into a lithium heparin tube using a sandwich immunoassay with an approximate reaction time of 18 minutes. Samples must be transported to the laboratory as quickly as possible due to the liable nature of the analyte. There is no agreed normal reference range for PTH and interpretation should be performed in conjunction with a patient's calcium results.

Vitamin D:

Vitamin D is a group of fat soluble secosteroids that are either made in the skin or ingested in food. The most important compounds in this group are vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol). Cholecalciferol and ergocalciferol can be ingested from the diet and supplementation though few foods contain much vitamin D. Synthesis of vitamin D, specifically cholecalciferol, in the skin is the major natural source. Dermal synthesis of vitamin D is dependent on UV-B radiation from sun exposure causing the conversation of 7-dehydrocholesterol to previtamin D_3. Once vitamin D_2 or D_3 enters the circulation, it is transported to the liver and hydroxylated to give 25-hydroxyvitamin D_2 or 25-hydroxyvitamin D_3, the main circulating form of vitamin D. The 25-hydroxy form of the vitamin is then converted to the biologically active 1,25-dihydroxy vitamin in the kidneys via the action of 1-α-hydroxylase.
Although 25-hydroxyvitamin D is much less biologically active than 1,25, the measurement of circulating 25-hydroxyvitamin D provides the best information to determine if a patient is vitamin D deficient, insufficient, sufficient or intoxicated. The half-life of 25-OHD is approximately 3 weeks in contrast to the half-life of previtamin D which is 24 hours and the half-life of 1,25 which is approximately 4-6 hours. Thus, 25-hydroxyvitamin D provides an indication of total vitamin D stores obtained from UV light and also dietary intake over long periods.



Vitamin D levels indicative of vitamin D deficiency, insufficiency, sufficiency and toxicity are shown in the table below:

Total vitamin D (nmol/L)	Indication
≤15	Severe deficiency
>15 and ≤30	Deficiency
>30 and ≤50	Insufficiency
>50	Adequate
>150	Toxicity

Measurement of serum vitamin D can be useful in the investigation, monitoring and diagnosis of vitamin D deficiency and supplementation, osteomalacia and rickets, hyper and hypocalcaemia and vitamin D toxicity. Vitamin D is also often measured and replaced if deficient before starting treatment with bisphosphonates and similar drugs to reduce the incidence of post treatment hypocalcaemia.

Vitamin D appears to have effects on immune function and has been postulated to play a role in influenza with lack of vitamin D synthesis during the winter as a possible explanation for high rates of infection during the winter. Low levels of vitamin D also appear to be a risk factor for tuberculosis. Low serum vitamin D levels have also been associated with falls and low bone mineral density in elderly patients.

Vitamin D overdose can cause hypercalcemia and the main symptoms of vitamin D toxicity are those of hypercalcemia such as nausea and vomiting, polyuria, polydipsia, weakness, insomnia and nervousness / anxiety.

Vitamin D is commonly given in several different supplements including:

Vitamin D:	Supplement:	Dose:
Alfacalcidol (1-α-hydroxycolecalciferol)	Alfacalcidol (non-proprietary): - 250ng capsules (10 units) - 500ng capsules (20 units) One-Alpha® (LEO): - 250ng capsules - 500ng capsules	Initial: - 1µg daily Maintenance: - 0.25-1µg daily
Calcitriol (1,25-dihydroxycolecalciferol)	Calcitriol (non-proprietary): - 250ng capsules - 500ng capsules	Initial: - 250ng daily / on alternate days if serum calcium normal or slightly reduced Maintenance: - Increase in 250ng steps at 2-4 weeks intervals to 0.5 – 1µg
Colecalciferol	Adcal-D_3® (ProStrakan): - 1.5g calcium carbonate (600mg calcium) + 10µg colecalciferol (400 units) tablets Calcichew-D3 / Forte® (Shire): - 1.25g calcium carbonate (500mg calcium) + 5µg colecalciferol (200 units) tablets - Forte; 1.25g calcium carbonate + 10µg colecalciferol tablets	2 tablets per day, one each morning and evening.

As colecalciferol needs to be hydroxylated in the liver and kidneys to be activated, the pre-hydroxylated vitamin D supplements (alfacalcidol and calcitriol) should be given to patients with significant impairment of liver and or kidney function who require supplementation. Vitamin D is measured in patient serum sent in plain (no gel) clot activating tubes. Samples are analysed routinely throughout the week in batches using a LC-MS method detecting both D_2 and D_3 to give total vitamin D status.

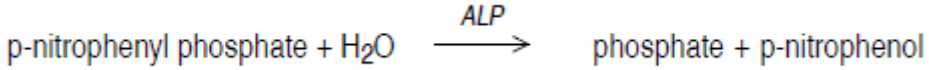
Alkaline Phosphatase (ALP):

ALP is a hydrolase enzyme responsible for removing phosphate groups (dephosphorylation) from many types of molecules, including nucleotides, proteins, and alkaloids and is most effective in an alkaline environment.
ALP is present as several isoenzymes including liver, bone, placental, intestinal and kidney. Serum ALP is principally liver and bone isoforms derived from the biliary canicular membrane and osteoblasts respectively and as such primarily reflects changes in bone and liver function.
ALP can be raised in a number of conditions including hepatobiliary disease (often seen with elevated gamma-GT), bone disease associated with increased osteoblast activity (Paget's disease, osteomalacia), disorders of calcium homeostasis and malignancy. When interpreting a raised ALP the age of patient should be considered as higher levels can be observed in childhood due to growth, pregnancy and old age. If significantly raised (>200U/L) with no apparent cause ALP isoenzymes can be useful in determining an origin. This test uses electrophoresis to separate out the different isoforms of ALP.

Hepatobiliary disease	Higher levels indicative of cholestasis. Intra-hepatic include: - Hepatitis - Drug reactions - Liver metastasis - PBC Extra-hepatic causes include: - Gallstones - Carcinoma of head of pancreas
Bone disease	Markedly high levels often associated with Paget's disease. Moderate increases occur in osteomalacia, metastatic bone disease and hyperparathyroidism.
Pregnancy	Modest increases (2-3x ULN) may occur due to ALP of placental origin.
Growth	Modest increases seen during periods of rapid bone growth in childhood and adolescence.
Thyrotoxicosis	Can result in a minor increase in ALP.

Decreased levels of ALP can be seen in malnutrition, vitamin B12 deficiency, magnesium deficiency, zinc deficiency, gross anaemias and post cardiac surgery. Decreased levels will also be seen in hypophosphatasia, an autosomal recessive inherited disorder associated with low ALP activity and characterised by dental and bone abnormalities. Clinical symptoms range from the rapidly fatal perinatal variant with profound skeletal hypomineralization and respiratory compromise to a milder, progressive osteomalacia later in life due to tissue non-specific alkaline phosphatase deficiency in osteoblasts and chondrocytes impairing bone mineralization.

ALP is measured in patient serum collected using a colt activating tube using the colourimetric assay shown below:



In the presence of magnesium and zinc ions, p-nitrophenyl phosphate is cleaved by ALP into phosphate and p-nitrophenol. The p-nitrophenol released is directly proportional to the catalytic ALP activity and is determined by measuring the developing yellow colour of p-nitrophenol at 405nm.
The normal reference range for ALP is 30-130 U/L.

CTX:

C-terminal telopeptide of type 1 collagen (ß-CTX) is a peptide fragment from the carboxy terminal end of the collagen protein matrix and a biomarker of the rate of bone turnover. It is more specific to bone resorption than any other test currently available. It can be useful in assisting clinicians to monitor a patient's response to anti-resorptive therapies, such as bisphosphonates and hormone replacement therapy. It is also useful in postmenopausal women and people with low bone mass (osteopenia), as well as in evaluating a patient's risk of developing complications during healing following surgical intervention and estimating risk of future osteoporotic fracture.
CTX reflects resorption of mature collagen by osteoclasts. Any process increasing osteoclast activity results in an increase in CTX. Values 3-4 times the upper limit of normal are observed in immobilisation, metastatic cancer, severe thyrotoxicosis and in some patients with Paget's disease. Values twice the upper limit of normal are often seen in Paget's disease, osteoporosis, primary hyperparathyroidism, thyrotoxicosis and osteomalacia.

CTX (µg/L):	Indication:
<0.5	- Normal for males/pre-menopausal females
0.5 – 1.0	- Osteoporosis - Up to 3-6 months post fracture depending on severity - Osteomalacia - Primary hyperparathyroidism - Mild Paget's disease - Some malignancies
1.0 – 1.5	- Severe osteoporosis and fractures - Malignancy with metastatic spread - Untreated thyrotoxicosis - Myeloma - Paget's disease
>1.5	- Metastatic disease - Severe Paget's disease - - Fybrous Dysplasia

When using ß-CTX to assess response to bisphosphonate treatment the expected observations 3 months post starting treatment in patients with baseline CTX measurement would be:

- Decrease from baseline of at least 50%
- Excellent response = 70% reduction

If no baseline CTX was taken prior to treatment the expected observations 3 months post treatment would be:

- CTX should be <0.3 µg/L
- If poor response to therapy check compliance

CTX is measured in patient plasma collected into an EDTA tube via a sandwich principle immunoassay with an approximate reaction time of 18 minutes. A fasting morning sample (09:00-12:00) is preferred.

Calcium:

Adjusted calcium reference range: 2.20 – 2.60mmol/L
Ionised calcium reference range: 1.13 – 1.32mmol/L

Calcium performs a large number of roles within the body and is involved in a diverse range of processes including bone growth and remodelling, secretion (exocytosis), excitation-contraction coupling, stabilization of membrane potentials, enzyme co-factor, second messenger and intracellular signalling. The amount of calcium in the blood is tightly controlled by signalling pathways and molecules, including PTH and vitamin D, and a wide range of symptoms and disorders can result from interruption of these methods.

Signs and symptoms:	
Hypocalcaemia:	Hypercalcaemia:
Tetany	Nausea
Carpopedal spasm	Depression
Muscle cramps	Renal failure
Seizures	Renal stones
Prolonged QT interval	Polyuria
Bronchospasm	Constipation
Laryngospasm	Polydipsia

Hypercalcaemia:

- Increased GI absorption;
    - Elevated vitamin D;
        - Excess exogenous (therapeutic)
        - Excess endogenous
        - Elevated PTH
        - Hypophosphataemia
- Increased bone resorption;
    - Increased net bone resorption;
        - Elevated PTH
        - Malignancy
    - Increased bone turnover;
        - Paget's disease
        - Hypothyroidism
- Decreased bone mineralisation;
    - Elevated PTH
    - Aluminium toxicity
- Decreased urinary excretion;
    - Thiazide diuretics
    - Elevated vitamin D
    - Elevated PTH

Common causes:

- Primary hyperparathyroidism – 99% of ambulant patients:
    - Single adenoma – 80%
    - Hyperplasia – 15%
    - Double adenoma – 2%
    - Carcinoma – <1%
- Malignant disease – 99% of ill patients:
    - Metastases and myeloma
    - PTHrp secreting tumours
    - Lymphoma

Uncommon causes:

- Vitamin D excess
- Tertiary hyperparathyroidism
- Hyperthyroidism

Rare causes:

- Familial hypocalciuric hypercalcaemia

Hypocalcaemia:

- Decreased GI absorption;
    - Poor dietary intake
    - Impaired absorption;
        - Vitamin D deficiency
        - Decreased conversion of vitamin D;
            - Liver failure
            - Kidney failure
            - Low PTH
- Decreased bone resorption / increased bone mineralisation;
    - Hypoparathyroidism
    - PTH resistance
    - Vitamin D deficiency
    - Hungry bone syndrome
    - Osteoblastic metastases
- Increased urinary excretion;
    - Low PTH;
        - Thyroidectomy
        - Parathyroidectomy
        - I^{131} treatment
        - Hypoparathyroidism
    - PTH resistance
    - Vitamin D deficiency

Parathyroid causes:

- Parathyroid agencies:
    - DiGeorge syndrome
    - Parathyroid destruction:
    - Surgery
    - Radiation
    - Infiltration (Wilson's, haemochromatosis)
- Autoimmune:
    - Isolated
    - Polyglandular
- Reduced parathyroid function:
    - PTH gene defects
    - Hypomagnesaemia
    - Hungry bone disease
    - Neonatal hypocalcaemia

Non-parathyroid causes:

- Vitamin D deficiency
- PTH resistance
- Bisphosphonates
- Acute pancreatitis
- Acute rhabdomyolysis
- EDTA contamination
- Extensive transfusion with citrated blood products

The most common causes of hypocalcaemia are acute or chronic renal failure, hypoparathyroidism, hypomagnesaemia and vitamin D deficiency.

Magnesium

Magnesium reference range: 0.70 – 1.00mmol/L

Magnesium has a diverse range of roles within the human body and is an important cofactor for over 300 enzymes. Other functions include acting as a co-substrate for many ATP requiring enzymes, roles in DNA replication, transcription and translation, structural maintenance of DNA, proteins and ribosomes, regulation of membrane permeability and excitability and interactions with calcium and calcium homeostasis.

Signs and symptoms:	
Hypomagnesaemia:	Hypermagnesaemia:
Loss of appetite	Nausea and vomiting
Nausea and vomiting	Neuromuscular symptoms
Fatigue	Low blood pressure
Tingling and numbness	Heart block
Cramps	
Seizures	
Personality changes	

Hypermagnesaemia

- Impaired renal function
- Large magnesium load;
    - IV
    - Post cardiac surgery
    - Enema / laxative abuse
- Tissue breakdown
- Lithium therapy
- Hypothyroidism
- Addison's disease
- Familial hypocalciuric hypercalcaemia

Hypomagnesaemia

- Decreased intake or absorption;
    - Starvation
    - Malabsorption syndrome
    - PPIs
- Loss from body;
    - Diarrhoea
    - Laxative abuse
    - Gut fistula
- Renal;
    - Alcoholism
    - RTA
    - Diuresis and loop diuretics
    - Hypercalcaemia

Phosphate

Phosphate reference range: 0.80 – 1.50mmol/L

Phosphate is predominately located within the bones and teeth (85%) with the remainder present as an intracellular ion (14%) and within the extracellular fluids (1%). It is present in both organic (phospholipids, phosphoprotiens) and inorganic (phosphate) forms. One of the main roles of phosphate is in the formation of high energy molecules and compounds such as ATP. It also plays an important role within second messenger molecules such as cAMP and IP_3, is a component of DNA, RNA, bone and cell membranes and is required for the regulation of enzymes via phosphorylation.

Hyperphosphataemia

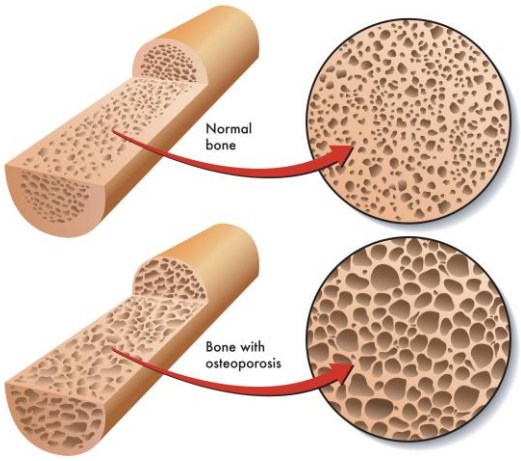
- Pseudo causes;
    - Haemolysis
    - Myeloma
    - Age of sample
- Increased input;
    - IV phosphate
    - Rectal phosphate
    - Cell death;
        - Tumour lysis syndrome
        - Rhabdomyolysis
        - Heat stroke
- Reduced excretion;
    - Acute and chronic renal failure
    - Increased reabsorption
    - Reduced PTH or PTH resistance
    - Vitamin D toxicity
    - Thyrotoxicosis

Hypophosphataemia

- Inadequate intake or absorption;
    - Low dietary intake
    - Phosphate binders
- Abnormal loss;
    - Hyperparathyroidism
    - Diuretics and diuresis
    - Genetic conditions, e.g. X-linked hypophosphataemia
- Phosphate shifts into cells;
    - Recovery from DKA
    - Refeeding syndrome
    - Respiratory alkalosis
    - Salicylate toxicity
    - Hepatic encephalopathy
    - Acute leukaemia

Metabolic bone disorders

Osteoporosis:



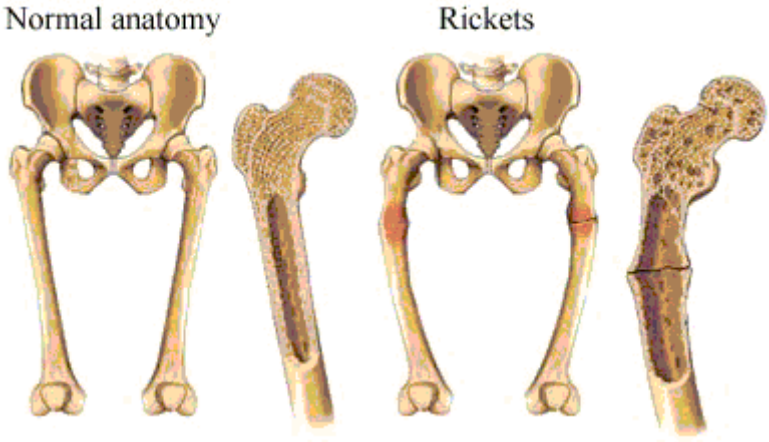
Osteoporosis is a disease characterised by decreased bone strength and in turn increased risk of fractures even after only minor stress. Osteoporosis is the most common cause of fractures in the elderly, commonly in the bones of the forearm, hip, wrist and back. Patient's may often show no symptoms until a fracture occurs and often experience decreased quality of life due to a reduced ability to carry out normal activities post fracture. Osteoporosis is estimated to affect approximately three million people in the UK with more than 300,000 people receiving hospital treatment for fragility fractures. It is also more common in women. Initial assessment for osteoporosis may involve FRAX or Q-Fracture assessment and referral for a bone mineral density scan. The main method, and gold standard, for measuring bone mineral density is via duel energy X-ray absorptiometry (DXA). Done mineral density is compared to a normal value derived from a healthy reference population and expressed as a T score. The WHO diagnostic guidelines state:

Category:	T score:
Normal	≥ -1.0
Osteopenia	> -2.5, < -1.0
Osteoporosis	≤ -2.5
Severe osteoporosis	≤ -2.5 with fragility fracture

Osteoporosis may be due to lower than normal peak bone mass and greater than normal bone loss. Bone loss increases after menopause due to lower levels of oestrogen, hence why it is more common in women. Osteoporosis may also occur due to a number of conditions including alcoholism, anorexia, hyperthyroidism, surgical removal of the ovaries, and kidney disease. Certain medications increase the rate of bone loss including some anti-seizure medications, chemotherapy, proton pump inhibitors, selective serotonin reuptake inhibitors and steroids. Lack of exercise and smoking are also risk factors. Other factors that increase the risk of developing osteoporosis include inflammatory conditions such as rheumatoid arthritis, Crohn's disease and chronic obstructive pulmonary disorder (COPD), hyperthyroidism and hyperparathyroidism. A family history of osteoporosis, particularly history of hip fracture in a parent, is also a risk factor.

Prevention of osteoporosis includes a proper diet during childhood and efforts to avoid medications that cause the condition. Those at risk of developing osteoporosis can also take steps to reduce this risk including regular exercise, healthy eating including foods rich in calcium and vitamin D and lifestyle changes such as giving up smoking and reducing alcohol consumption. Medications of the bisphosphonate type, for example zoledronic acid, which reduce bone resorption are useful in patients with osteoporosis.

Osteomalacia and rickets:
Osteomalacia, also known as rickets in children, is a condition caused by defective bone mineralisation due to inadequate levels of calcium and/or phosphate or overactive resorption of calcium from bones, for example due to hyperparathyroidism. This results in a softening of the bones.The most common cause of osteomalacia is vitamin D deficiency and efforts to prevent it can be made using calcium and vitamin D supplementation. Nursing home patients and the housebound elderly are highly at risk of vitamin D deficiency as they often have very reduced sun exposure.
The signs and symptoms of osteomalacia include diffuse joint pain, muscle weakness, hypocalcaemia, bending of the bones and difficulty walking characterised by a waddling gait. Biochemical findings in osteomalacia include low serum and urinary calcium, low serum phosphate, elevated serum ALP and elevated PTH.



Paget's disease of bone:
Paget's disease is chronic disorder resulting in the formation of enlarged and misshapen bones caused by the excessive breakdown and formation of bone and disorganised bone remodelling. Affected bone is typically weaker and results in pain and fractures. The most commonly affected bones include the femur, pelvis and lower lumbar vertebrae, often localised to certain areas in each patient. Paget's disease is rare in those under 55 years of age and generally affects men more than women. Anti-resorption medication, such as bisphosphonates, can help reduce pain and other symptoms and keep the condition under control. Medications are especially successful when started before any complications have developed. Two genes, SQSTM1 and RANK, are associated with Paget's disease of bone. Approximately 40-50% of people with inherited Paget's disease have a mutation in the gene SQSTM1, which encodes the protein p62 involved in regulating the function of osteoclasts. 10-15% of people that develop the disease without any family history also have a mutation in the SQSTM1 gene. Due to the importance of early diagnosis and intervention the siblings and children of affected individuals are offered screening via di-annual serum ALP measurement after the age of 40. If ALP is elevated further tests including bone scans, X-rays and bone specific ALP can be performed.