Calcium and phosphates

Clinical Biochemistry

Dept. Biochemistry 2008 (J.S.)

Calcium distribution and turnover

Daily intake
about. **25 - 30 mmol** / d

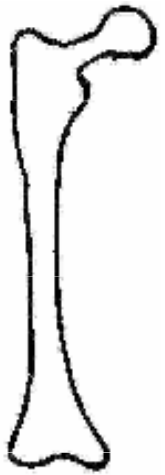
(phosphates, oxalates, phytates)

absorption
< 15 mmol / d

secretion
up to 7 mmol / d

17 – 25 mmol / d

Mineral deposits
25 mol Ca
(rapidly exchangeable 100 mmol)



bone remodelation
7,5 mmol / d

rapid exchange
500 mmol / d

ECT
2,5 mmol / l
total only 35 mmol

ICT
signaling function
0,1 µmol / l

filtration
240 mmol / d

tubular resorption
230 mmol / d

Excretion fraction max. 5 %
decreased by PTH,
enlarged by calcitonin and
high supply of Na^+

5 – 6 mmol / d

Calcium homeostasis

PARATHYRIN (PTH, the older name parathormone currently taken as an improper one) has 84 amino acid residues (prepro-PTH 115 AK, pro-PTH 90 AK). Biological activity is exhibited by the N-terminal sequence, the initial 28 residues.

PTH secretion is regulated by plasma Ca^{2+} concentration – hypocalcaemia stimulates the synthesis and secretion, hypercalcaemia (and calcitriol) inhibits, phosphates don't have a direct influence. The calcium sensor is a receptor cooperating with G_q–proteins (however, an increase in intracellular Ca^{2+} **inhibits secretion** in contradistinction to other cell types).

Intact PTH is degraded rapidly (half-live 3 – 5 min) by splitting off the long C-terminal fragment.

In the bones, PTH initiates bone resorption through differentiation and activation of osteoclasts. This effect is mediated by osteoblasts that, after PTH binding, expose one of the ODF proteins. Those proteins are recognized by the receptors of osteoclasts and initiate the activation.

In the renal tubules, PTH increases the Ca^{2+} reabsorption increasing so calcaemia and decreasing calciuria (but at a high Ca^{2+} supply is the maximal transport capacity of the tubular cells exceeded and excretion of Ca^{2+} increases);
decreases the HPO_4^{2-} reabsorption and increases excretion of phosphate;
in hyperparathyroidism, PTH **decreases the HCO^{3-} reabsorption** (may be the cause of mild hyperchloridaemic metabolic acidosis).

The indirect effect of PTH on intestinal mucosa (**increased Ca absorption**) results from renal production of calcitriol (**1α-hydroxylation of calcidiol**).

CALCIOL (calciferol, vitamin D_3) and ercalciol (ergocalciferol, D_2, of plant origin) are present in the diet and, in addition, calciol is synthesized from 7-dehydrocholesterol in the skin due to UV irradiation.

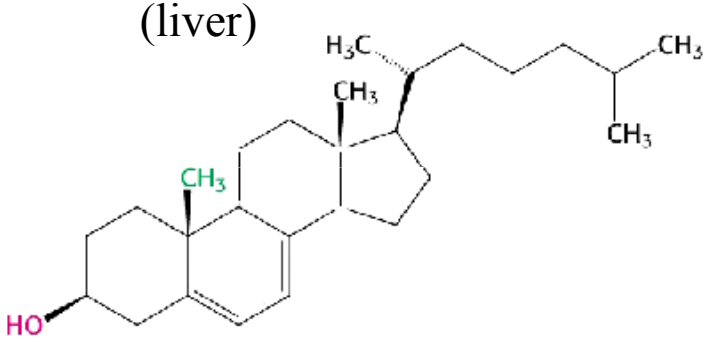
Physiologic dose is in the range 5 – 20 μg/d (200 – 800 units/d).

In the blood plasma, calciol is transported bound to the specific protein DBP (D-binding protein, its concentration less than 10 μmol/l and saturation by calciol not more than 1 – 2%).

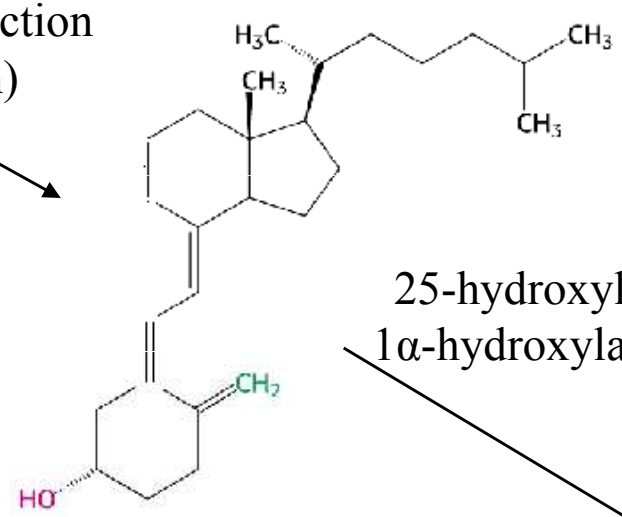
Calciol is hydroxylated to **calcidiol** (25-hydroxycalciol) *in the liver*, controlled by calcidiol negative feed-back. Calcidiol is the major metabolite of calciol circulating in the blood (biological half-life 20 – 30 days). Calcidiol concentration *informs of tissue calciol saturation* (seasonal variations).

In the proximal *renal tubules*, calcidiol is transformed by means of controlled 1α-hydroxylation into the **real steroid hormone calcitriol** (1α,25-dihydroxycalciol). The hydroxylation is inhibited by high calcitriol level, calcitonin, and high dietary calcium. 1α-Hydroxylation is stimulated by PTH (in hypocalcaemia) and growth hormone. The biological half-life of calcitriol is short.

7-Dehydrocholesterol
(liver)

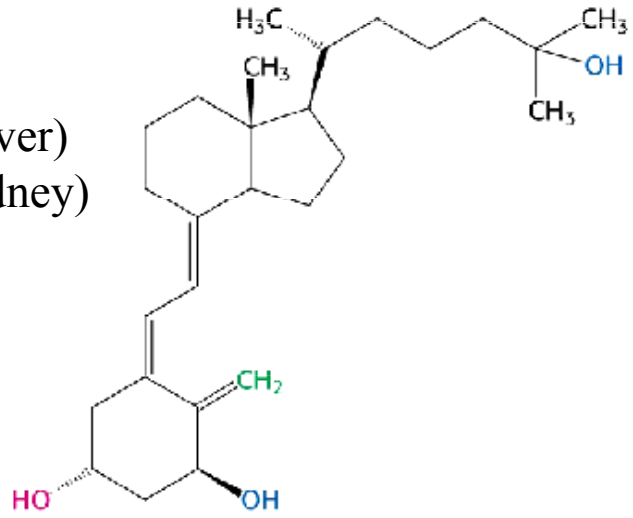


photoreaction
(skin)



Calciol
(cholecalciferol, D_3)

25-hydroxylation (liver)
1α-hydroxylation (kidney)



Calcitriol
(1α,25-dihydroxycalciol)

Actions of calcitriol:

In the enterocytes, it increases resorption of calcium into ECF in all three distinct steps:

- Entrance into the cell. There are no special transporters for calcium, calcitriol induces a **change in the binding of calmodulin to brush border myosin** (it forms a complex with a special type of myosin that is bound to membrane actin). The complex may remove calcium from the brush border after it crosses the membrane. Changes in the phospholipid composition of the brush border also may explain the flux of calcium across this membrane after calciol administration.
- Calcitriol **induces the synthesis of cytosolic protein calbindin** (CaBP, calcium-binding protein) after a lag-period about 20 h. CaBP seems to prevent rapid increase in Ca^{2+} by mediating rapid Ca^{2+} transport in cytoplasm.
- Calcitriol **induces the synthesis of Ca^{2+}-ATPase** that removes Ca^{2+} from the cell into ECF.

In bone, calcitriol **controls both resorption and formation** by means of, že induction of the synthesis of numerous proteins in osteoblasts and odontoblasts. The control mechanism is not yet quite clear.

The effect of calcitriol depends on the degree of osteoblast differentiation:

- In immature osteoblasts, calcitriol **supports differentiation pathway to mature, fully functional osteoblasts.**
- In mature osteoblasts, calcitriol **induces the synthesis of osteocalcin** and (besides PTH and certain cytokines) **production of osteoclast differentiation factors** (ODF) so that it also take part in maintaining of physiologic levels of Ca^{2+} and phosphates in ECF.

Osteocalcin (BGP - bone Gla-protein) is a small conservative protein (49 AA residues) that has three γ-carboxyglutamate centres (Gla) able to bind Ca^{2+}. Its function (γ-carboxylation)depends on the presence of vitamin K.

Bone contains 50 - 100 μg osteocalcin per gram of dry mineralized tissue (more than 20 % non-collagen bone proteins).

Osteocalcin concentration in plasma is 5 - 10 μg / l, its biological half-life only 4 – 5 min.

Osteocalcin takes part in the **control of bone mineralization.** It seems to **slow down mineralization** of the bone matrix by binding onto collagen (preventing so Ca^{2+} and phosphate leakage from plasma into bone), on the other hand osteocalcin **retards bone resorption** in epiphysis.

Concentration of plasma osteocalcin is one of the biochemical **markers of bone remodelation** (turnover), perharps also of osteoblast activity in formation of organic bone matrix. In sole osteoresorption, increase in osteocalcin concentration is not observed.

CALCITONIN

is a 32 amino acid peptide secreted by the C-cells of the thyroid gland.
It is classified as a neuropeptide, because it played a role of neurotransmitter during the evolution. In humans, the thyroid gland only synthesizes calcitonin.

Under normal conditions, calcitonin exhibits a **limited role in calcium metabolism control.** Its effect on hypercalcaemia is not long, but acute, and it results in blockade of bone resorption.

> No disturbance of calcium metabolism occurs following removal of the thyroid gland.

Calcitonin has a special role **in pregnancy**. Plasma concentration increases till the end of the 2nd trimester up to the double normal values and is maintained till the end of lactation.

> Calcitonin primarily supports formation of calcium deposits in the bones of mother (formation of stores for the foetus), later on the effect is diminished due to increased secretion of PTH (increasing the calcium accessibility to the foetus).

Secretion of calcitonin is controlled by calcaemia. The calcium sensor is of the same kind as in parathyroid glands, though in the C-cells the increase in intracellular Ca^{2+} is the signal for secretion.

> Calcitonin is a marker of the medullary thyroid carcinoma (about 10 % of all thyroid malignancies), increased even at normal or decreased calcaemia.

Calcitonin counteracts PTH in the control of Ca metabolism.
Estrogens stimulate the effects of calcitonin.
In bone, calcitonin **inhibits bone resorption** (if increased) by suppressing the activity of osteoclasts and **supports both synthesis of organic matrix** and **mineralization of osteoid** by a rapid activation of osteoblasts.

In renal tubules, calcitonin **inhibits reabsorption of both Ca and phosphates** increasing their excretion in this way.

¨ **In GIT,** it inhibits the secretion of HCl, pepsin, and pancreatic enzymes. Calcitonin has an **analgetic effect** (bone pain in the course of metabolic osteopathies or bone metastases).

Calcitonin structure differs in animal species. Rat calcitonin resembles human one (only 2 different amino acids), the most effective calcitonin is that of salmon (50 % homology with the human calcitonin).

In the therapy of metabolic osteopathies, the salmon, human, or the dicarba analogue of eel calcitonin are used.

Procalcitonin (116 AK, calcitonin sequence in position 60-91) is split to calcitonin and catacalcin in the C-cells and not secreted normally.

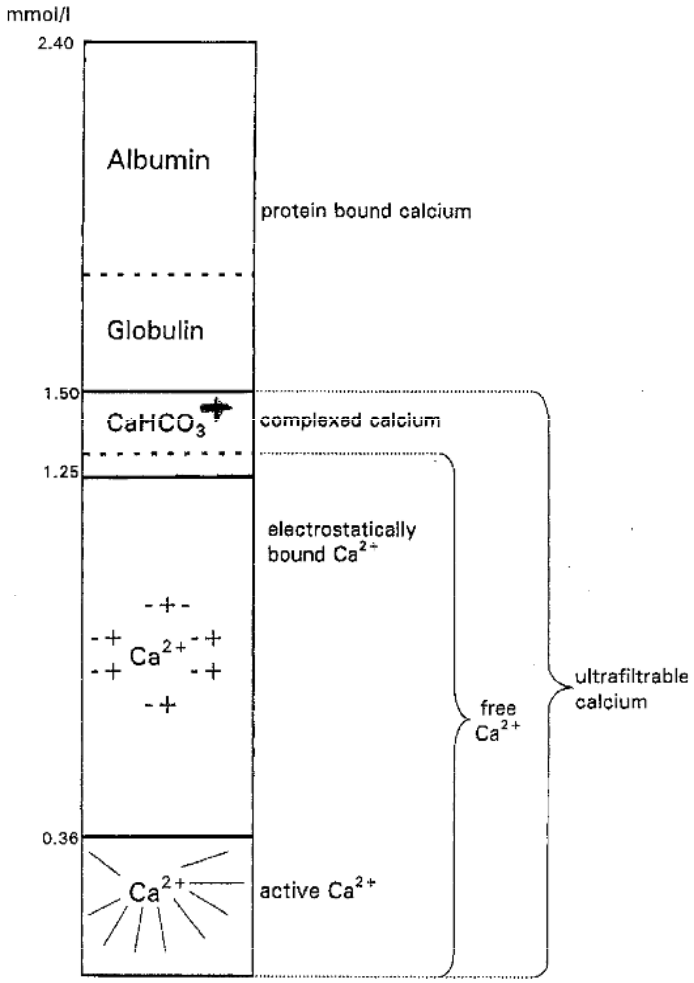
In sepsis (not in localized infections), procalcitonin is secretes in considerable amount (from neuroendocrine cells of the lung and intestine and mononuclear leukocytes obviously), without an increase in calcitonin.

Plasma procalcitonin is a reliable marker of sepsis.

Basal biochemical investigation

Blood serum calcium concentration

The biologically active fraction is ionized Ca^{2+} (free Ca^{2+} ions)..



Chemical methods (photometry, AAS) estimate **total serum calcium**..

Approximately one half (~ 46 %) of Ca^{2+} is bound to carboxyl groups of proteins - **non-diffusible calcium**..

A small fraction (2 - 5 - 10 %) of calcium is bound in chelate complexes with carboxylate anions (citrate, lactate, oxalate, etc.), slightly also with HCO_3^- and phosphates - **non-ionized diffusible calcium** (usually not taken into account).

Free Ca^{2+} ions - **ionized calcium** - are decisive for an assessment of the calcium accessibility.

Total serum calcium S-Ca – mean value 2,5 mmol/l,
reference range **2,25 - 2,75 mmol/l** .

Ionized calcium S-[Ca^{2+}] – mean value 1,25 mmol/l,
reference range **1,0 – 1,4 mmol/l** .

Ion-selective electrodes are used for determination of ionized calcium. The measured electrode potential depends on the Ca^{2+} *activity*, which is usually only about 0,38 mmol/l (at ionic strength of blood serum the activity coefficient of Ca^{2+} equals about 0,30). Therefore, the results of measurements is edited in the form of *adjusted activity concentration* (real Ca^{2+} concentration of the calibrator relevant to serum, the Ca^{2+} activity of which is the same as the activity of the measured sample)-

Conclusions deduced from the values of total calcium *may be erroneous,* unless account to protein (namely albumin) concentration and abnormal pH value is taken. **Normal total Ca concentration doesn't exclude a change in ionized Ca^{2+}, abnormal total Ca can be accompanied by a normal value of ionized Ca^{2+}.**

Sampling of the blood for calcium determination can influence the result. Change in patient position (orthostatic water shift) may increase protein concentration by more than 5 %, venous stasis of the arm at venepuncture can decrease the pH value, as well as increase the albumin concentration.

Protein-bound calcium increases if plasma protein concentrations, particularly of albumin, are high.

Influence of pH can be understood as a competition for Ca^{2+} binding sites between H^+ and Ca^{2+}ions. pH value changed by 0.1 corresponds to the change in $[Ca^{2+}]$ by approximately 0,02 mmol/l.

Various formulae have been proposed to correct the total calcium concentrations for the effect of albumin and pH. However, corrected formulae give only an approximation of Ca^{2+} levels and for an accurate assessment of this, ionized calcium determination is required.

Serum phosphate (inorg.) fS-phosphate – assessment in a particular part.

Serum alkaline phosphatase activity S-ALP – increased activity can be the sign of high calcium turnover in bone (although the most common requirement for this test is a suspicion of cholestasis).
Isoenzymes of ALP – hepatic, bone, intestinal, placental. **Bone isoenzyme** is a marker of osteoblast activity.

Total ALP activity in adults **0,8 - 2,3 μkat/l**,
children **1,0 - 8,0 μkat/l** (high activity of bone isoenzyme)

Serum creatinine and **urea** – renal failure may be a cause of changed calcaemia

Special tests

Urinary calcium excretion dU-Ca or fU-Ca / creatinine ratio
is **not a basal test** – calciuria occurs usually in all hypercalcaemias.

Plasma intact PTH (1-84) with the reference range **1 - 5 pmol/l** , if the reliable two-site immunometric method is used. Intact PTH has a half-life $t_{1/2}$ 3 - 5 min, hepatic cells rapidly split inactive C-terminal segments from it.

The concentration of *C-terminal segments* in plasma is several times higher than that of intact PTH (namely in renal diseases).
Similarly, the *biologically active N-terminal sequence 1-34.*

The sequence 1-13 is present also in PTHrP (PTH-related proteins) produced by some malignances (even if intact PTH is low, 0.1 – 0.7 pmol/l).

High level of intact PTH is usual in primary hyperparathyroidism (the secretion is not suppressed by hypercalcaemia), although it might be in the normal range.

Serum calcidiol is the best method how to assess the accessibility of calciol.
The average concentration during summer about **75 nmol/l** (30 μg/l),
in winter about **37 nmol/l** (15 μg/l, i.e. a mild deficit)
Values < 20 nmol/l have to be taken as a sign of serious calciol deficiency.
In enormous exposition to sunlight – up to 250 nmol/l,
in hypervitaminosis D up to 1250 - 2500 nmol/l.

Serum calciol 2.6 - 13 nmol/l,
serum calcitriol in children about 160 pmol/l, in adults 3 - 580 pmol/l .

Intestinal calcium resorption can be estimated by Sr^{2+} resorption: after ingestion of 2.5 mmol $SrCl_2$, the serum concentration of Sr^{2+} is measured immediately (c_0) and after 240 min (c_{240}); the amount of absorbed Fc_{240} is then calculated (normal range **20 – 22 %** ingested Sr^{2+}).

Plasma calcitonin about **13 - 27 pmol/l** (50 - 100 ng/l), in women older than 50 years of age the values are lower (what can explain the high risk of osteoporosis). Calcitonin is a tumour marker of medullary thyroid carcinoma.

Serum bone isoenzyme of ALP – reference range **0.2 – 0.6 μkat/l,** marker of osteoblast activity.

Plasma osteocalcin 5 - 10 μg/l.

More about markers of osteoblasts and osteoclasts – see Osteoporosis.

Hypercalcaemia

Mild hypercalcaemia (< 3 mmol/l) has usually no clinical symptoms, at **higher values** not inevitably associated with fatigue, depression, anorexia, vomiting or constipation.

Defects of renal tubular functions, particularly polyuria, occur and ECG abnormalities are also seen – characteristically, a short QT interval.

Hypercalcaemia > **3.5 - 4 mmol/l is a serious menace**.

If hypercalcaemia is chronic, **renal functions are impaired oft**, soft tissue calcification and renal tract stones develop.

In turn, **chronic renal failure disturbs calcium homeostasis**.

Decrease in GFR to about one third is the cause of phosphate retention and hyperphosphataemia, FE(Ca) increases, calcium loss follows, calcidiol is hydroxylated insufficiently, and intestinal calcium resorption diminished.

The consequence is hypocalcaemia that induces **secondary hyperparathyroidism**.

The commonest cause of hypercalcaemia (in about 90 %) is primary hyperparathyroidism, the second most frequent being malignant disease.

Primary hyperparathyroidism is usually caused by a solitary adenoma, although occasionally it results from a carcinoma or from hyperplasia affecting all four glands.
It is detected mostly (up to 50 %) as c*alcium urolithiasis* or *nephrocalcinosis, polyuria, osteopathy* (osteodystrophy, pathological bone fractures), etc.
The diagnosis is supported by *hypophosphataemia* resulting from enormous phosphate losses (fasting serum ratio Cl^- / phosphate > 120), mild metabolic *acidosis* from losses of HCO_3^-. **Increased PTH is conclusive**,

Secondary hyperparathyroidism is the **consequence of hypocalcaemia**.
Either from chronic **renal failure** (decrease in phosphate excretion, hyperphosphataemia, insufficient hydroxylation of calcidiol, even increased PTH is not able to release enough calcium from bones), or from **impaired intestinal resorption of calcium or calciol** (unless renal functions are impaired, high excretion of phosphates occurs, phosphataemia normal or decreased).

Tertiary hyperparathyroidism occurs in about 5 % of patients during final phases of **chronic renal failures**. Hypercalcaemia is the consequence of long-lasting hypocalcaemia, when some of hyperplasic parathyroid glands is transformed to **functionally independent adenoma**; in contradistinction to primary hyperparathyroidism, **hyperphosphataemia** occurs.

Syndrome of multiple endocrine neoplasia (MEN, also ME adenomatosis), namely the **type I** (Wermer syndrome) is associated with increased secretion of not only PTH, but also pancreatic (insulin, gastrin, VIP, glukagon) and pituitary secretion (ACTH, STH, prolactin). PTH in the **type IIa** (called also type 2) in increased rarely, not at all in the **type IIb** (type 3).

Malignancy-related hypercalcaemia

– **osteolytic metastases** of solid tumours (lung, breast, kidney) resorb bone either directly, or (breast carcinoma) activate osteoclasts by means of local release of humoral factors (e.g. PGE, transformation growth factor α a β, PDGF);

– multiple **myeloma** (and some lymphomas) can induce local bone resorption also by release of factors that activate osteoclasts (TNF, TGF, lymphotoxin) or through the ectopic synthesis of calcitriol;

– **tumours producing PTHrP** (namely squamous cell carcinomas of the bronchus) – the term *humoral hypercalcaemia in malignancy* is used. Serum PTHrP always > 1.5 pmol/l, but intact PTH is low, 0.1 – 0.7 pmol/l.

Other causes of hypercalcaemia:

Calciol intoxication in excessive intake of calciol (e.g. long-lasting treatment of hypoparathyroidism, calciol overdose is released from the adipose tissue slowly, in the course of several weeks) or when **calcitriol** is used (immediate effect, a narrow therapeutic range).

Long-lasting treatment by **lithium salts** or **thiazide diuretics.**

Long-lasting immobilization intensifies osteoresorption, namely in teenagers due to high bone turnover. A complete immobilization can cause about 30 % loss of bone minerals.

Familiar hypocalciuric hypercalcaemia is the inherited defect (autosomal dominant) of calcium receptors in parathyroid glands that don't react to calcaemia. Life-long hypercalcaemia, calcium excretion is normal (FE(Ca) less than 1 %) – similar to primary hyperparathyroidism. This defect occurs in about 2 % of hypercalcaemias.

Hypocalcaemia

Clinical symptoms appear when *total* calcium is lower than approx. **1.9 mmol/l** (*ionized* Ca lower than **0.9 - 1.0 mmol/l**).

Hypocalcaemia is accompanied by typical neuromuscular manifestations – *paresthesiae* in the fingers and about the mouth, increased neuromuscular excitability – *carpal spasms* (Trousseau's sign), *Chvostek´s sign* (a contraction of facial muscles elicited by tapping the facial nerve), *tetany* (carpopedal spasms up to a state of spontaneous tonic muscular contraction).

Spurious hypocalcaemia (due to hypoalbuminaemia or haemodilution) should be excluded primarily.

Causes of hypocalcaemia:

Alkalosis – hyperventilation (hysteric reaction, artificial hyperventilation), too rapid infusion of HCO_3^-.

Acute complexation or deposition of calcium

- multiple blood transfusions (citrate or EDTA form complexes with Ca^{2+}), as a prevention, 10 ml of Ca-gluconate should be applied after approx. two transfusions;
- rapid, excessive skeletal mineralization,
- acute pancreatitis (released fatty acids bind Ca^{2+}),
- acute hyperphosphataemia.

Reduced parathyroid hormone action

– Rare **primary hypoparathyroidism** – congenital aplasia or part of the DiGeorge syndrome (with an immunodeficit), a familial form (mutations in the parathyroid calcium-sensing receptors).

– **Secondary hypoparathyroidism** – surgery on the neck is the most common form (destruction of the glands or transient ischemia); pluriglandular autoimmune endocrinopathies (circulating parathyroid antibodies), severe magnesium depletion (it disables the mechanism of stimulation of PTH secretion by hypocalcaemia).

Diagnosis – hypocalcaemia, hyperphosphataemia (if a renal insufficiency is excluded), ALP activity is usually low,
conclusive is **low** (< 10 ng/l) **or absent plasma PTH**.

– **Pseudohypoparathyroidism** is a heritable disorder of PTH-receptors in target- tissue (renal tubules and/or bones). Past history of the patient‘s family, skeletal abnormalities occur (short stature, metacarpals. and metatarsals – phenotype of Albright‘s hereditary osteodystrophy), Hypocalcaemia, hyperphosphataemia, conclusive is **excessive plasma PTH**, and **a blunted response of urinary cAMP** to the administration of PTH.

Insufficient supply of calcium, impairment of intestinal calcium absorption, failure to produce calcitriol or **resistance to calcitriol:**

– **inadequate dietary intake** of calcium or calciol,
– **limited exposure to sunlight** (this sometimes occurs in elderly subjects, high sensitivity in immigrants from Asian tropical zones),
– **malabsorption** of liposoluble compounds (diseases of pancreas, insufficient bile production, intestinal by-pass, coeliakia),
– **liver diseases** (low bile acid secretion, impairment of calciol 25-hydroxylation),
– **chronic renal failure** (insufficient 1α-hydroxylation of calcidiol).

In adults, insufficient calcium intake results in **osteomalacia** (bands of decalcification, affecting particularly the pelvis, femur, and scapula – Looser´s zones), in children in **rickets** (**rachitis**, skeletal deformities and muscle weakness). The most common biochemical features are

low-normal calcaemia (unless compensated by PTH),
elevated PTH level,
decreased urinary excretion of calcium, increased excretion of phosphate,
hypophosphataemia (as far as renal tubular function is normal),
increased serum ALP activities;
low serum calcidiol (determination should be repeated, seasonal variations).

Rare causes of rickets or osteomalacia:

Vitamin D-resistant rachitis type I (pseudovitamin D-deficient rickets) is a rare inherited defect of renal 1α-hydroxylation of calcidiol.
The treatment by calcitriol is efficient.

Vitamin D-resistant rachitis type II (rare inherited calcitriol-resistant rickets) – defect of intracellular calcitriol receptors – end-organ unresponsiveness to calcitriol.

Hypophosphataemic vitamin D-resistant rachitis (muscle weakness is not observed in this form) is an inherited defect in renal tubular phosphate reabsorption, occurring either as an isolated abnormality or as part of the Fanconi syndrome..

Calciuria increases in – high monosaccharide intake,
– deficits of Mg and phosphates, and
– in starving.

Hypercalciuria – excretion > 7.5 mmol / d (300 mg / d) in men,
> 6.2 mmol / d (250 mg / d) in women

is the natural consequence of all hypercalcaemias.

It may also occur at normal calcium levels or in hypocalcaemia.

– Primary hyperparathyroidism

– "Idiopathic" hypercalciurias (at normal calcaemia, inherited?)
- either a hyperabsorptive type
- or a renal type

(More about calciuria – see Renal stones)

Phosphate distribution and turnover

Daily intake
about. **30 - 80 mmol** / d

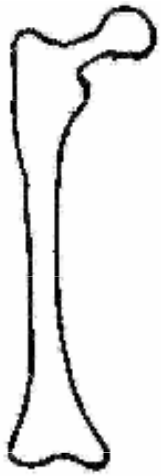
Al^{3+} and calcium

absorption
15 - 55 mmol / d

ECT (total only 55 mmol)
1.0 mmol / l inorg. phosphate
2.5 mmol / l phosphate esters

ICT (15 – 19 % of total content)
1 mmol / l inorg. phosphate
100 mmol / l organic esters

Mineral deposits
15 - 25 mol PO_4^{3-}
(80 – 85 % total P)



exchange
8 - 15 mmol / d

filtration
> 130 - 200 mmol / d

tubular reabsorption
> 110 - 150 mmol / d

Excretion fraction < 25 %
(children < 15 %)
increased by PTH or calcitonin

10 – 40 mmol / d

13 – 40 mmol / d

Phosphate homeostasis

is **maintained by urinary excretion of phosphate** that **depends on renal tubular reabsorption of phosphate**.

Filtration of free P_i (about 90 %) is complete, from which 50 – 70 % is reabsorbed in the proximal tubule (specific active co-transport with Na^+, inhibited by PTH), about 10 – 20 % in the distal parts of the nephron.

Of the 240 mmol of phosphate filtered daily, about 85 % (80 – 97 %) is reabsorbed. **Excretion fraction** $EF_{(phosphate)}$ – in adults **less than 20 %**, in children less than **15 %**.

Increased phosphate excretion (decrease of tubular phosphate reabsorption) is caused by **PTH** or **calcitonin**, as well as by **acidosis** (hyperphosphataemia, protons excreted as $H_2PO_4^-$), and indirectly (through hypocalcaemia that stimulates PTH secretion) by **alkalosis** or **increased ECF volume**.

Certain role is presumed to the putative as yet unidentified hormone **phosphatonin** secreted by osteoclasts (besides inhibition of bone mineralization, it may also inhibit tubular phosphate reabsorption).

Retention (*decreased excretion*) *of phosphates* (increased tubular phosphate reabsorption) is caused by **somatotropin** (decrease in ECF volume), **corticosteroids**, to a lesser degree, by **calcitriol** and **phosphate deficiency**.

Basal investigation

Fasting serum inorganic phosphate in adults **0.8 – 1.3 mmol/l**,
in children **1.6 - 2.2 mmol/l**.

Plasma concentration is 0.06 – 0.10 mmol/l lower than that of serum due to the release of intracellular phosphate from platelets and erythrocytes during clotting: **serum should be separated from the coagulum without delay.**

In healthy adults, there is a marked diurnal variation (range 12 – 22 %) in phosphataemia – lowest being in the morning and highest during the night (this variation is abolished by fasting). Postprandially, phosphate concentration tends to decrease presumably because of insulin release (anabolic action of insulin). **Samples** for serum phosphate estimation **should be taken in the fasting state in the morning.**

Great and rapid changes in serum phosphate concentration may have their causes in the shifts of phosphates between ICF and ECF that depend on the nutritional status (energy charge of the cells).

Urinary phosphate excretion depends considerably on dietary intake.
At the average daily intake of phosphate (about 50 mmol/d),
dU-P_i is in the range **13 - 29 mmol/d** in healthy adults.

Special tests

Standardized phosphate clearance in adults equals **0.18** (**0.09 – 0.27**) **ml / s** ,
in children up to 1 year **0.1 – 0.62 ml / s** .

About 80 – 97 % filtered phosphate is reabsorbed.

Excretion fraction EF(phosphate) – in adults **less than 20 %**,
in children up to 1 year **7 %** (**1.5 – 15 %**).

Maximal tubular reabsorption of phosphate **TmP** (maximal transfer) is
1.1 – 3.2 μmol / s ; it is decreased, among others, in hyperglycaemia.

Measurements of tubular phosphate reabsorption are important in assessing parathyroid function (diagnosis of primary hyperparathyroidism and other hypophosphataemias).

The **threshold phosphate concentration** is thought to be the best parameter – the theoretical phosphate concentration in glomerular filtrate (equal to plasma) below which all phosphate is reabsorbed and above which phosphate is excreted in urine:
the **ratio of maximal tubular reabsorption** to **glomerular filtration rate**
TmP / GFR (normal range **0.74 – 1.2 mmol / l**).

It can be easily determined using fasting urinary and serum concentrations of phosphate and creatinine and the Walton's nomogram.

In primary hyperparathyroidism, TmP/GFR is considerably lower than 0.6 mmol/l, and higher than 1.2 mmol/l in hypothyroidism.

Hyperphosphataemia

Even a mild form (> 1.6 mmol/l) is rather a rare finding among hospital patients (1.5 %).

Consequences – Acute hyperphosphataemia can lead to hypocalcaemia and hypotension. In chronic hyperphosphataemia, the low production of calcitriol may lead to bone changes (osteomalacia or rickets); the other major complication is soft tissue calcification in myocardium, lungs, and the liver (namely in concomitant hypercalcaemia).

Artificial hyperphosphataemia may result from haemolysis
due to the release of intracellular phosphate from red blood cells and platelets.

Causes of hyperphosphataemia

Reduced urinary excretion – Renal failure accounts for more than 90 % of cases in hospital patients; retention of phosphate occurs when the GFR falls below 20–30 % of normal and may be a cause of secondary hyperparathyroidism.

– **Hypoparathyroidism** – increased tubular phosphate reabsorption.

Excess phosphate administration to patients on parenteral and enteral nutrition, also in sucklings nourished with undiluted cow's milk.

Shift of intracellular phosphate into the ECF, leading to hyperphosphataemia, is seen in

– **hypercatabolic states** and in **metabolic acidosis** (untreated lactic acidosis and diabetic ketoacidosis) due to increased ATP breakdown and tissue hypoxia.

– **Tumour lysis syndrome** and **rhabdomyolysis**, excessive **haemolysis**.

Hypophosphataemia

is much more frequent than hyperphosphataemia.

Mild and moderate hypophosphataemia (**0.6 -0.8 mmol/l**) is quite common in hospital patients (14 – 39 %, namely in acutely ill, malnourished, or with diabetic ketoacidosis), but it is rather exceptional in out-patients (incidence 0.9 %).

In acute mild hypophosphataemia, it is unusual to see clinical manifestations.
In mild and moderate hypophosphataemia of long duration, **skeletal changes** (i.e., rickets or osteomalacia) are the only consistent abnormalities.

Severe hypophosphataemia (<0.6 mmol/l, in particular **< 0.3 mmol/l**) is relatively rare (<1 % of hospital patients), but if it is of at least four days duration, important consequences may be seen: **haemolysis** due to decreased 2,3-BPG and ATP in erythrocytes, muscular weakness that may result in **respiratory failure**, **impaired cardiac contractility**, **nervous system disorders** ranging from irritability to confusion, convulsion, and coma.

Causes of hypophosphataemia:

Hypophosphataemia does not always indicate intracellular phosphate deficiency, phosphate depletion may be present with normal or even increased phosphataemia.

Haemodilution – water retention, parenteral nutrition.
Reduced absorption – Low dietary intake is an unusual cause (strict vegetarians) as phosphate occurs widely in foods, **– oral phosphate-binding agents** (Al^{3+}-containing or other antacids in excessive quantities), **– diarrhoea** or long-lasting **vomiting**,
– malabsorption. ·/.

Increased uptake of phosphate into cells

– **Administration of glucose** (both intravenous and enteral) or hyperalimentation leads to increased insulin concentration, increased formation of phosphorylated intermediates leads to the shift of phosphate into the cells. Life-threatening hypophosphataemia may occur in rapid realimentation of starved patients ("**refeeding syndrome**"), treatment of diabetic ketoacidosis, alcoholics during alcohol withdrawal (and glucose refeeding).

– **Hyperventilation and respiratory alkalosis** in severe liver disease, septicaemia, head injury, mechanically ventilated patients (alkalosis stimulates glycolysis and intracellular utilization of phosphate, as well se increase in PTH).

– **Rapidly growing tumours** (lymphomas and other haematologic malignancies).

Increased phosphate elimination

– **Decreased renal tubular reabsorption** due to high **PTH** secretion in hyperparathyroidism, rickets and osteomalacia.

– **Renal tubular defects** (e.g. Fanconi´s syndrome),

– **Hereditary hypophoaphataemic rickets** ("phosphate diabetes", a form of vitamin D-resistant rachitis) with phosphate commonly below 0.8 mmol/l and enormous renal phosphate wasting and hypercalciuria – a defect of phosphate tubular reabsorption and perharps in intestinal transport and calciol hydroxylation, too.

– **Haemodialysis**.