INÊS LOPES CARDOSO
FERNANDA LEAL

PATHOLOGIES AND CLINICAL CASES IN BIOCHEMISTRY

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1. CARBOHYDRATE METABOLISM

CHAPTER CONTENTS

1.1. **Glycolysis, Gluconeogenesis and Pentoses-phosphate pathway**

1.1.1. **Short review**

Carbohydrate metabolism focuses on the use of plasma glucose by all cells for adenosine triphosphate (ATP) production. There are two different types of glucose transporters in cells: one dependent of insulin that is present in most tissues like muscles and adipose tissue and another independent of insulin in liver cells and in cells that are not able to store glycogen such as brain cells and erythrocytes. The entrance of glucose in cells always occurs according with its gradient, by a facilitated process. The gradient of glucose is maintained since as soon as it enters the cell, glucose is immediately phosphorylated by the enzymes glucokinase (in liver) or hexokinase (in other tissues), being converted in glucose-6-phosphate. This reaction consumes 1 ATP and is an important regulatory point and for this reason it is an irreversible reaction. The hexokinase enzyme is inhibited by the end product, glucose-6-phosphate, while glucokinase is inhibited by fructose-6-phosphate.

In the phosphorylated form, glucose can no longer cross the plasma membrane. In this way, glucose is kept inside the cell and will be used in several metabolic pathways like glycolysis, glycogenesis and pentoses-phosphate pathway.

Glycolysis

Glycolysis is the first stage of respiration and occurs in the cytosol of cells (Fig. 1.1.1). Besides the reaction described above, glycolysis involves 9 additional steps:

1. Conversion of glucose-6-phosphate in fructose-6-phosphate by phosphoglucose isomerase.

2. Conversion of fructose-6-phosphate in fructose-1,6-biphosphate by phosphofructokinase, with the use of 1 ATP. This is the main regulatory point of glycolysis. This enzyme is activated by fructose-2,6-biphosphate, adenosine monophosphate (AMP) and inorganic phosphate (P_i) and inhibited by ATP, citrate and H^+ ions.

3. Breakdown of fructose-1,6-biphosphate in gliceraldahyde-3-phosphate and di-hydroxyketone phosphate (DHAP) by aldolase.

4. Triose phosphate isomerase converts DHAP in gliceraldahyde-3-phosphate. Now, glycolysis proceeds with 2 molecules of gliceraldahyde-3-phosphate.

5. Gliceraldahyde-3-phosphate dehydrogenase converts this last substrate in 1,3-biphosphoglycerate, with the production of 1 nicotinamide adenine dinucleotide in the reduced form (NADH). The phosphate group that is inserted in the substrate comes from P_i.

6. By the action of the enzyme phosphoglycerate kinase, 1,3-biphosphoglycerate is converted in 3-phosphoglycerate with the production of 1 ATP.

7. 3-Phosphoglycerate is then converted in 2-phosphoglycerate by phosphoglycerate mutase. During this conversion, 2,3-biphosphoglycerate is produced. This intermediate works as a modulator of oxygen fixation by haemoglobin, reducing its affinity to O_2.

8. Enolase performs the dehydration of 2-phosphoglycerate, leading to the production of phosphoenolpyruvate (PEP).

9. In the last step, PEP is converted into pyruvate by the action of pyruvate kinase, and the released phosphate group is used to produce 1 ATP. This is the third regulatory point of glycolysis. Pyruvate kinase is activated by fructose-1,6-biphosphate and inhibited by alanine and ATP.

The final product of glycolysis can suffer two different transformations depending on the availability of oxygen. If oxygen is absent, pyruvate is converted into lactate, by the enzyme lactate dehydrogenase (Fig. 1.1.1). This transformation allows to regenerate nicotinamide adenine dinucleotide in the oxidised form (NAD^+) required for the activity of the enzyme glyceraldahyde-3-phosphate dehydrogenase.

In aerobic conditions, pyruvate will be transferred to the mitochondrial matrix where it will be converted into acetyl-CoA for further use in the Krebs cycle (Fig. 1.2.1).

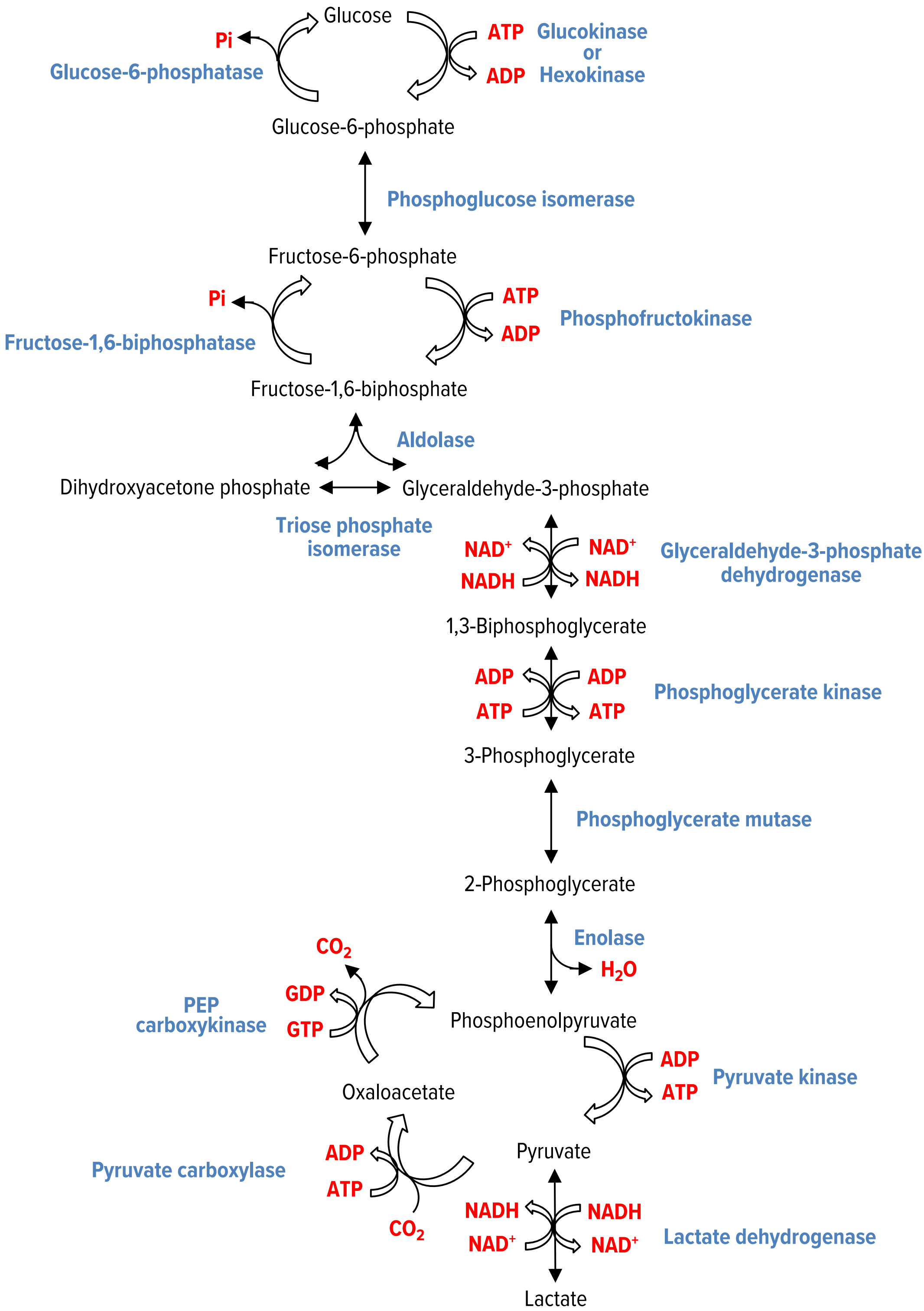

Fig. 1.1.1. Glycolysis and Gluconeogenesis.

Gluconeogenesis

This is a specialized metabolic pathway since it does not occur in all cells of the organism. Gluconeogenesis is one of the essential metabolic pathways for hepatic control of plasma glucose levels, meaning that it allows to send glucose into the bloodstream when hepatic glycogen stores are depleted.

In periods of starvation longer than 4 hours, this metabolic pathway is activated and can use several different substrates (non-carbohydrates) to produce glucose. Among these substrates is lactate produced in skeletal muscle in periods of intense physical exercise or in mature red blood cells that, for not having mitochondria, always convert pyruvate into lactate. Other possible substrates for gluconeogenesis can be alanine, glycerol and fatty acids with odd number of carbons.

Most steps of gluconeogenesis are the reverse of glycolysis steps since the reactions are reversible, except for the regulatory points (Fig. 1.1.1). The steps of gluconeogenesis are:

1. Lactate converted into pyruvate by lactate dehydrogenase (LDH); alanine converted into pyruvate through transamination by alanine transaminase (ALT), etc.

2. Pyruvate cannot be converted into PEP by pyruvate kinase, since this is an irreversible step. Instead, pyruvate must be converted into oxaloacetate (OAA) by pyruvate carboxylase with consumption of 1 ATP. This carboxylation uses biotin as a cofactor and is the only reaction of gluconeogenesis that takes place in the mitochondrial matrix. After that, OAA is transferred to the cytosol, via malate or aspartate shuttle, and is then transformed into PEP by the enzyme PEP carboxykinase with consumption of 1 guanosine triphosphate (GTP). This last reaction is a regulatory point of gluconeogenesis.

3. The enzyme enolase catalyses the conversion of PEP into 2-phosphoglycerate.

4. 2-Phosphoglycerate is converted in 3-phosphoglycerate by the enzyme phosphoglycerate mutase.

5. Through the action of the enzyme phosphoglycerate kinase 3-phosphoglycerate is converted into 1,3-biphosphoglycerate spending 1 ATP.

6. 3-Phosphate-gliceraldehyde dehydrogenase converts 1,3-biphosphoglycerate into gliceraldehyde-3-phosphate, consuming 1 NADH.

7. Two molecules of gliceraldehyde-3-phosphate are used to produce fructose-1,6-biphosphate by aldolase.

8. Conversion of fructose-1,6-biphosphate into fructose-6-phosphate is then catalysed by fructose-1,6-biphosphatase, with the release of P_i. This is also a regulatory point of gluconeogenesis. This enzyme is inhibited by fructose-2,6-biphosphate, AMP and P_i and activated by ATP, citrate and H^+ ions.

9. Conversion of fructose-6-phosphate into glucose-6-phosphate by phosphoglucose isomerase.

10. At last, glucose-6-phosphate will be dephosphorylated by an endoplasmic reticulum (ER) enzyme, glucose-6-phosphatase, releasing P_i. This is the last regulatory point of gluconeogenesis.

After being dephosphorylated, glucose can be released into the bloodstream, allowing the control of its plasma levels.

Gluconeogenesis is activated in fasting periods through the secretion of the hormone glucagon that activates the transcription of the gene coding for PEP carboxykinase.

Pentoses-phosphate pathway

The pentoses-phosphate pathway has the goal of producing two molecules: nicotinamide adenine dinucleotide phosphate in the reduced form (NADPH) and ribose. NADPH is essential for cells involved in lipogenesis such as adipose cells and liver. This NADPH also has an important indirect role in antioxidant protection, since its presence is required for glutathione recycling during peroxide destruction, essential in mature red blood cells. Ribose is required by dividing cells for the synthesis of nucleic acids and coenzymes.

This metabolic pathway uses glucose in the phosphorylated form (glucose-6-phosphate), as first substrate. The pentoses-phosphate pathway can be divided in two stages: the oxidative (Fig. 1.1.2) and the non-oxidative phases.

The oxidative stage involves the following steps:

1. The enzyme glucose-6-phosphate dehydrogenase that is a regulatory point, converts glucose-6-phosphate into 6-phosphogluconolactone, with the production of NADPH.

2. 6-Phosphogluconolactone suffers a hydration reaction, by 6-phosphogluconolactonase, being converted in 6-phosphogluconate.

3. 6-Phosphogluconate is then transformed into ribulose-5-phosphate, by 6-phosphogluconate dehydrogenase, with the production of NADPH and release of CO_2.

The product of the oxidative stage will enter the non-oxidative stage, where interconversion of hexoses in pentoses and vice-versa takes place, till the regeneration of glucose-6-phosphate that starts a new cycle.

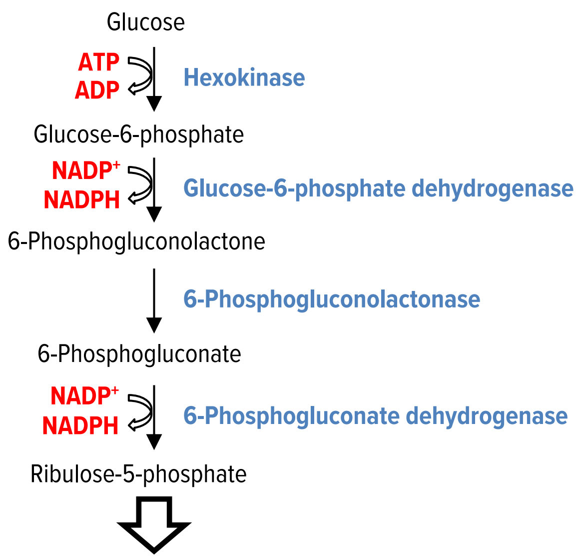

Fig. 1.1.2. Oxidative phase of the Pentoses-phosphate pathway.

1.1.2. **Pathologies**

Glucokinase deficiency

Glucokinase is the enzyme catalysing the phosphorylation of glucose in liver cells (Fig. 1.1.1). This reaction is essential for glucose retention inside the cell and further use through glycolysis for ATP production. This enzyme acts as a glucose sensor of the pancreatic β-cells, playing a role in the glucose-stimulated release of insulin.

The deficiency of glucokinase, resulting from inactivating mutations in the coding gene, leads to mild fasting hyperglycaemia in heterozygous individuals or to severe diabetes in homozygous cases. In this way, the resulting clinical phenotype of partial glucokinase deficiency is diabetes in youth, while complete deficiency of this enzyme leads to the clinical phenotype of permanent neonatal diabetes mellitus. In cases of permanent neonatal diabetes, patients exhibit intrauterine growth retardation and permanent insulin dependent diabetes from the first day of life.

Glucokinase deficiency leads to hyperinsulinism, caused by a lowered threshold for the release of insulin. This condition is characterized by an excessive and uncontrolled secretion of insulin that conducts to episodes of deep hypoglycaemia induced by fasting or protein rich meals. These episodes require fast intervention and intensive treatment to prevent neurological problems.

Phosphofructokinase (PFK) deficiency

The deficiency of phosphofructokinase is a metabolic disease transmitted in an autosomal recessive manner. This enzyme is responsible for the conversion of fructose-6-phosphate into fructose-1,6-biphosphate (Fig. 1.1.1), being the most important regulatory point in glycolysis. In this way, the deficiency of this enzyme leads to decreased production of ATP in cells under anaerobic conditions.

Phosphofructokinase is an enzyme organized in tetramers composed of three different subunits: PFKL (liver), PFKM (muscle) and PFKP (platelet). While muscular and liver PFK are composed of homogenous tetramers, PFK-M4 and PFK-L4, respectively, erythrocytes have a mixture of both previous tetramers.

Deficiency of the M subunit affects the capacity of cells such as erythrocytes and skeletal muscle cells to use carbohydrates as energy source. Consequently, muscles will have lack of available energy during intensive exercise, leading to cramps and pain.

This condition results from mutations in the gene coding for phosphofructokinase, that lead to the production of an enzyme with low or no function. In most cases, affected individuals show 50 to 65% of total normal phosphofructokinase activity.

This disorder can be divided in four types that can be distinguished by the age of development of symptoms and by the type of symptoms present: classic, late-onset, infantile and haemolytic.

The classic form is the most common type of this disorder, being characterized by exercise-induced muscle cramps and weakness, myoglobinuria and haemolytic anaemia (causing dark urine). Another common feature is hyperuricemia that results from the inability of kidneys to process uric acid. These patients can experience nausea and vomiting after strenuous exercise, as well as high levels of bilirubin that can lead to jaundice. In the classic form, symptoms usually appear in early childhood.

On the other hand, the late-onset form develops later in life, being characterized by myopathy, weakness and fatigue.

There is also a rare infantile form of this disease, where affected infants show hypotonia, arthrogryposis, encephalopathy and cardiomyopathy. Central nervous system can also be affected in the form of seizures. These patients can also have some sort of respiratory issue. Patients having this form of the disease have a low survival rate, being respiratory failure the most frequent cause of death.

Finally, the haemolytic form is characterized by haemolytic anaemia. In this form, muscle pain or weakness is not as common as in the previous forms.

Main symptoms of this condition resemble the ones present in other metabolic diseases, such as deficiencies of phosphoglycerate kinase, phosphoglycerate mutase, lactate dehydrogenase, among others. So, it is crucial a correct diagnosis to determine the treatment plan.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) deficiency

This enzyme is encoded by a single gene, producing a unique mRNA. No splice variants have been identified. However, there are two different forms of the enzyme glyceraldehyde-3-phosphate dehydrogenase: a cytoplasmic form and a nuclear form. Post-translational modifications occurring in the cytoplasmic form lead to the formation of the nuclear form that has non-metabolic functions.

The cytoplasmic form exists as a tetramer, being composed of four identical 37 kDa subunits, each with a thiol group, essential for its catalytic activity. This enzymatic form catalyses the conversion of glyceraldehyde-3-phosphate into 1,3-biphosphoglycerate, during glycolysis, allowing the use of carbohydrates for energy production (Fig. 1.1.1). This deficiency is particularly severe when it affects red blood cells, since glycolysis is the only metabolic pathway used in these cells for energy production. Therefore, it may lead to haemolytic anaemia.

Other functions of this enzyme, recently attributed to the nuclear enzymatic form, are several non-metabolic activities, such as transcription activation, initiation of apoptosis, vesicle transport from the ER to the Golgi apparatus, as well as axoplasmic transport. The nuclear enzyme has an increased isoelectric point (8.3-8.7). The thiol group present in the cysteine residue C152 is required for induction of apoptosis in oxidative stress.

Phosphoglycerate mutase deficiency

The deficiency of phosphoglycerate mutase is a rare condition, with only 15 affected individuals reported in literature.

This enzymatic deficiency results from mutations in the *PGAM2* gene, that codes for phosphoglycerate mutase, an enzyme catalysing one of the steps of glycolysis. This enzyme is responsible for the conversion of 3-phosphoglycerate in 2-phosphoglycerate (Fig. 1.1.1). The mentioned gene codes for the isoenzyme present in skeletal muscle cells. Mutations in this gene strongly reduce its enzymatic activity, disrupting energy production in these cells.

This defect is responsible for the muscle cramping and myoglobinuria observed after strenuous exercise in these patients. Symptoms develop in childhood or adolescence. Myoglobinuria develops due to abnormal breaks in muscular tissue that leads to the release of myoglobin into the bloodstream and its elimination in the urine. If not treated, myoglobinuria can lead to renal failure.

This defect has an autosomal recessive transmission. However, the presence of one mutated copy of the *PGAM2* gene can lead to certain phenotypes, including episodes of exercise-induced muscle cramps and myoglobinuria.

Pyruvate kinase deficiency

A similar picture occurs with the deficiency of the glycolytic enzyme pyruvate kinase. This enzyme catalyses the last step of glycolysis by converting PEP into pyruvate with ATP production (Fig. 1.1.1). In erythrocytes, glycolysis is the only source of energy. Therefore, this defect blocks ATP production in these cells. Without energy, red blood cells lyse, increasing bilirubin production. This defect increases the risk of erythrocyte haemolysis and consequent degradation of haemoglobin, subsequently increasing bilirubin plasma content.

Fructokinase deficiency (Fructosuria)

Fructokinase is an enzyme of the transferase's family, present in the liver, intestine and the cortex region of kidneys. This enzyme is involved in carbohydrate metabolism, specifically fructose and sucrose metabolism, catalysing the transfer of a phosphate group from ATP to fructose (Fig. 1.1.3), the first step in fructose degradation.

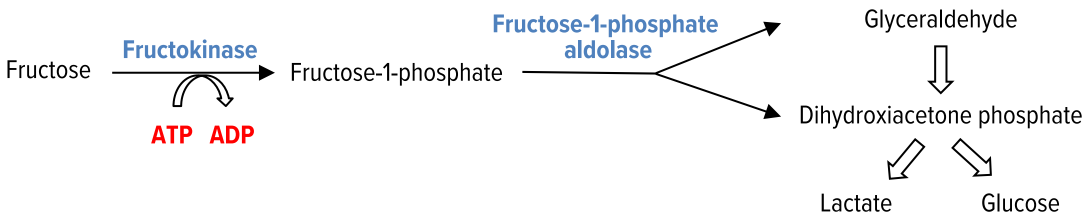

Fig. 1.1.3. Fructose metabolism.

Essential fructosuria or liver fructokinase deficiency is a rare benign condition, with an estimated incidence of 1:130,000. However, this incidence is probably higher since affected individuals are asymptomatic. This condition is characterised by the incomplete metabolism of fructose in liver cells, leading to its presence in blood and excretion in urine. These biochemical changes may lead to its incorrect diagnosis as diabetes mellitus.

This deficiency does not lead to any clinical symptoms, being fructose excreted without any modification or converted into fructose-6-phosphate by hexokinase in adipose tissue or muscles.

This condition is a genetic disorder with an autosomal recessive transmission and results from mutations in the *KHK* (ketohexokinase, also called fructokinase) gene present in chromosome 2 (p23.3-23.2).

Fructose-1-phosphate aldolase deficiency (Fructose intolerance)

In liver, ingested fructose suffers the action of the enzyme fructokinase that converts it into fructose-1-phosphate. The deficiency of fructokinase leads to essential fructosuria. This is a benign condition characterized by the excretion of fructose in the urine.

Fructose-1-phosphate is further metabolised by the enzyme aldolase B, being converted into dihydroxyacetone phosphate and glyceraldehyde (Fig. 1.1.3). The hereditary intolerance to fructose results from the deficiency of the aldolase B enzyme. This condition has an autosomal recessive transmission that results from mutations in the *ALDOB* gene, present in chromosome 9 (9q31.1).

Patients with this condition do not develop symptoms, unless they ingest fructose, sucrose or sorbitol. The intake of fructose leads to the accumulation of fructose-1-phosphate that affects gluconeogenesis and ATP production and can ultimately cause death of liver cells.

Main symptoms of this disorder include vomiting, convulsions, hypoglycaemia, jaundice, haemorrhage, hepatomegaly, hyperuricemia, and consequent kidney failure.

Diagnosis of this condition is based on dietary history, such as in children that develop symptoms after breast feeding. Diagnosis is then confirmed by molecular techniques.

Galactose-1-phosphate uridyltransferase deficiency (Galactosaemia)

Classical galactosaemia develops due to the absence of galactose-1-phosphate uridyltransferase. This enzyme catalyses one of the steps in the conversion of galactose into glucose (Fig. 1.1.4). This deficiency leads to the accumulation of galactose-1-phosphate.

The clinical symptoms of this disorder come directly from the toxicity of this metabolite. Moreover, plasma galactose levels increase, and this molecule starts to be excreted in the urine.

Children with this disorder show failure to thrive, vomiting, hepatomegaly and jaundice. The conversion of galactose to galactitol in the lens can lead to the development of cataracts. Patients may also show hypoglycaemia and impairment of renal tubular function.

The accumulation of galactose-1-phosphate resulting from this enzyme deficiency leads to the inhibition of phosphoglucomutase, the enzyme that catalyses the reversible conversion of glucose-6-phosphate into glucose-1-phosphate. This inhibition leads to a decreased production of glucose-6-phosphate from glycogen degradation and hence less glucose is formed by glucose-6-phosphatase and released into the bloodstream. Consequently, hypoglycaemia develops. At the same time, with the lack of phosphoglucomutase activity, glucose-6-phosphate is not converted to glucose-1-phosphate, preventing the formation of UDP-glucuronic acid, that is required in bilirubin conjugation (conversion of bilirubin into bilirubin diglucuronide). Bilirubin starts to accumulate in tissues, causing jaundice.

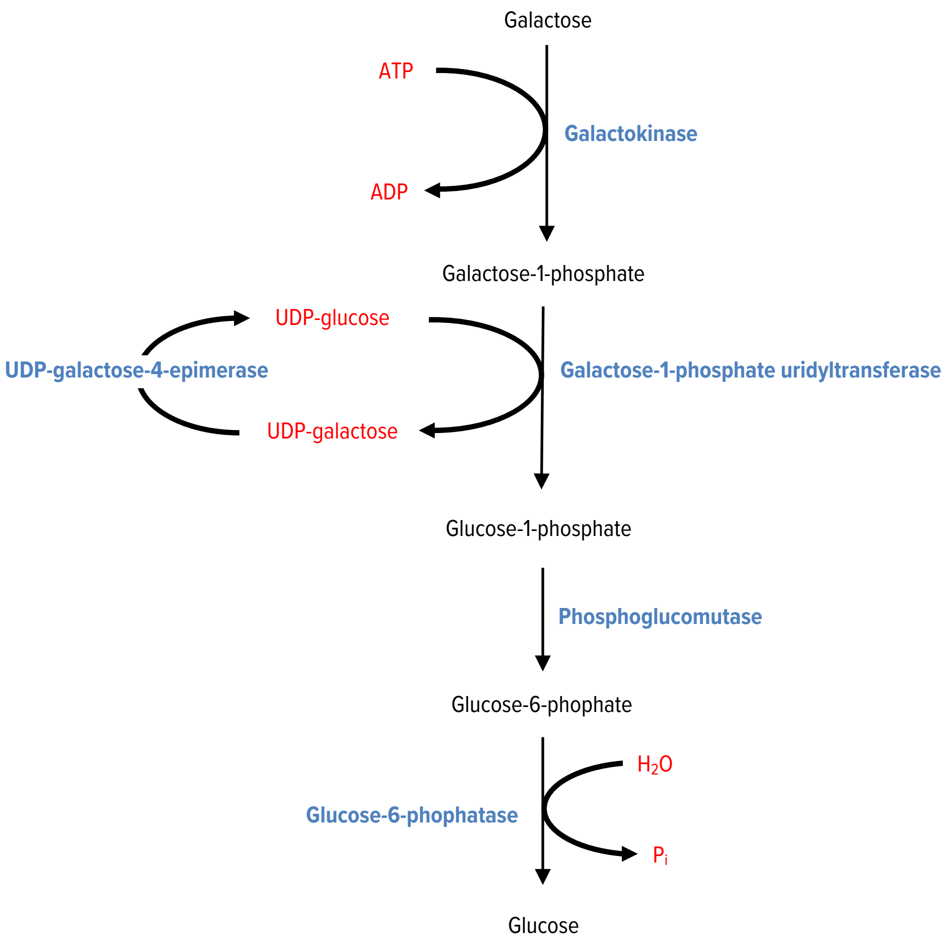

Figure 1.1.4. Galactose metabolism.

Two other enzyme deficiencies may lead to galactosaemia: a similar clinical syndrome but much less common is caused by the deficiency of UDP-galactose-4-epimerase (Fig. 1.1.4); the deficiency of galactokinase (Fig. 1.1.4) leads to increased plasma galactose levels and consequent galactosuria.

Pyruvate carboxylase deficiency

Pyruvate carboxylase is responsible for the conversion of pyruvate to OAA, with ATP consumption (Fig. 1.1.1). This enzyme requires the presence of the coenzyme biotin. This reaction is crucial for the correct functioning of gluconeogenesis, being one of the first steps of this metabolic pathway. Consequently, this deficiency leads to the reduced capacity of glucose production from non-carbohydrate compounds, leading to hypoglycaemia.

This reaction is also important for the proper functioning of the Krebs cycle, since it leads to the production of OAA, that will react with acetyl-CoA, yielding citrate, the first intermediate of the tricarboxylic acids cycle.

The deficiency of this enzyme is a hereditary condition with autosomal recessive transmission, causing accumulation of lactic acid in blood as well as other possibly toxic compounds that can damage organs and tissues, especially the nervous system.

Three different types of pyruvate carboxylase deficiency have been identified, which can be distinguished by the intensity of signs and symptoms.

Type A, identified in North America, exhibits severe symptoms that start to develop during infancy, including delay in development and lactic acidosis. Blood acidification gives rise to vomiting, abdominal pain, fatigue, muscle weakness and breathing problems. Children with this type A deficiency usually survive only until early childhood.

The type B deficiency, observed in Europe (specially in France), shows life-threatening signs, that develop soon after birth. Main symptoms are severe lactic acidosis, hyperammonaemia and liver failure. Patients develop neurological problems such as hypotonia, abnormal movements, seizures and coma. Children carrying this condition do not survive more than 3 months after birth in average.

The third form, type C, is a milder condition, characterised by slightly increased blood levels of lactic acid, together with minimal symptoms of the nervous system.

Pyruvate carboxylase deficiency results from mutations in the *PC* gene, having an incidence of 1 in 250,000 births worldwide.

Besides the lack of blood glucose and disturbances in ATP production, especially in brain cells, this enzyme is also involved in the formation of myelin and neurotransmitters, contributing to the neurologic signs of this disease.

Glucose-6-phosphatase deficiency (Von Gierke's disease)

Type I glycogen storage disease results from the deficiency of glucose-6-phosphatase. This enzyme plays a crucial role in the dephosphorylation of glucose-6-phosphate in the liver cells, to allow glucose release into the bloodstream. Glucose-6-phosphatase catalyses the last step of gluconeogenesis, that is also the last step of glycogenolysis (Fig. 1.1.5).

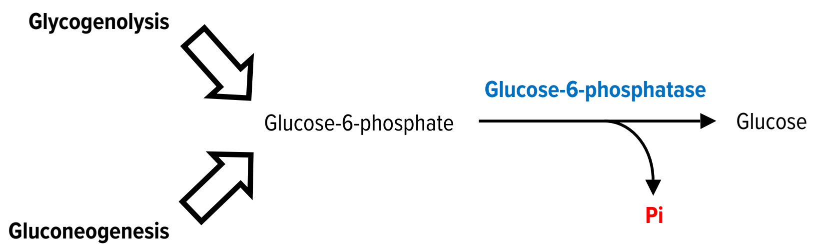

Fig. 1.1.5. Reaction catalysed by glucose-6-phosphatase.

In this way, in patients carrying this disease, the liver is not able to control plasma glucose levels by the action of glucagon or epinephrine.

Children with this disorder will show severe fasting hypoglycaemia. The liver continues to store glycogen that cannot be used up, so this can lead to hepatomegaly. Moreover, the blockage of gluconeogenesis results in the accumulation of lactate, leading to the development of lactic acidosis.

Glucose-6-phosphate dehydrogenase deficiency (Favism)

Glucose-6-phosphate dehydrogenase belongs to the pentoses-phosphate pathway, being responsible for the conversion of glucose-6-phosphate into 6-phospho-gluconolactone (Fig. 1.1.2). This pathway is essential for NADPH production, which is required for recycling of glutathione (involved in the destruction of reactive oxygen species (ROS) such as peroxides) and for lipid biosynthesis.

The gene coding for this enzyme (*G6PD* gene) is present in the X chromosome. Mutations in this gene lead to deficiency of the coded enzyme, glucose-6-phosphate dehydrogenase. Since this condition is associated with the X chromosome, it develops almost exclusively in males since they have only one copy of this chromosome. This condition is transmitted by mothers to their children, being expressed mainly in men.

This disease is more frequent in certain regions of Africa, Asia, the Mediterranean and Middle East. In the USA, it affects 1 in 10 African American males.

Cells most affected by this deficiency are erythrocytes, that need NADPH for peroxide destruction since they are constantly in contact with oxygen. This deficiency leads to premature breakdown of red blood cells (haemolysis). This haemolytic anaemia is evident by paleness, jaundice, dark urine, breathing problems, fatigue and fast heartbeat. This deficiency is responsible for mild to severe jaundice in newborns.

Affected individuals can be asymptomatic and the characteristic anaemia can be triggered by bacterial or viral infections or using specific pharmaceutical products such as drugs used in the treatment of malaria. Symptoms can also develop after eating fava beans. This reaction is called favism. All these circumstances increase the level of ROS that can damage red blood cells.

1.1.3. **Clinical Cases**

Clinical case 1.1.1. *Glucokinase deficiency*

Consider a patient with a genetic defect that stops glucokinase production.
a) After a carbohydrate meal, plasma glucose levels should be high, low or normal?
b) Which organ accumulates glycogen in these conditions?

Clinical case 1.1.2. *Phosphofructokinase deficiency*

A patient suffers from strong muscular pain after intense physical activity but can tolerate moderate exercise. After physical activity, extremely high levels of muscle proteins were observed in blood, as well as moderate haemolysis, that persist for several hours after the end of the exercise. Blood glucose levels were normal and increased after glucagon treatment. Table below shows results after biopsy:

Table 1.1.1. Observed results on patient biopsy.

	Glycogen (mg/g)	Glucose-6-phosphate (μmol/g)	Fructose-6-phosphate (μmol/g)	Fructose-1,6-biphosphate (μmol/g)
Patient values	43.8	9.2	1.6	0.02
Standard values	9.6±1.8	0.5±0.3	0.1±0.05	0.61±0.23

a) Why is lactate produced instead of pyruvate, in muscle working in anaerobic conditions?
b) What is the metabolic deficiency of this patient?
c) Explain the increase in glycogen levels observed in this patient.
d) Explain the presence of muscular proteins in blood after intense exercise.
e) Why is the patient able to tolerate moderate exercise?
f) If the patient receives fructose before intense exercise would be able to tolerate it better? Explain.

Clinical case 1.1.3. *Phosphofructokinase deficiency*

A 12-year-old girl suffers fatigue and muscular pain when trying to perform intense physical exercise, although feeling well while playing, walking, running slowly and in other situations that do not require vigorous and sudden exercise. Explain why a patient having deficiency in muscular phosphofructokinase has only limited capacity for intense muscular activity and accumulates glycogen with normal structure.

Clinical case 1.1.4. *Gliceraldehyde-3-phosphate dehydrogenase deficiency*

Huntington's disease is a neurodegenerative pathology that provokes involuntary movements and dementia. This disease is caused by the fact that mutant huntingtin (protein of Huntington's disease) contains a polyglutamine region with 40 to 120 glutamine residues. It is believed that that this region attaches to gliceraldehyde-3-phosphate dehydrogenase in an almost irreversible manner. Suggest the implications of this interaction in Huntington's disease, knowing that the brain uses almost exclusively glucose as energy source.

Clinical case 1.1.5. *Phosphoglycerate mutase deficiency*

A person goes to the doctor due to incapacity in performing intense physical activity for a long period of time. After examination, the doctor requested the analysis of several enzymes of the glycolytic pathway. The concentration of all enzymes was normal except for phosphoglycerate mutase.
a) Explain how the energy metabolism of cells with low levels of this enzyme, is affected.
b) Do you think that lactate production will be affected by the absence of this enzyme? Explain.

Clinical case 1.1.6. *Pyruvate kinase deficiency*

Haemolytic anaemia is caused by deficiency in the enzyme pyruvate kinase.
a) Explain how this deficiency affects red blood cells metabolism.
b) Explain the changes in ATP production.

Clinical case 1.1.7. *Fructokinase deficiency (Fructosuria)*

Indicate the deficient enzyme and the product that accumulates in fructosuria.

Clinical case 1.1.8. *Fructose-1-phosphate aldolase deficiency (Fructose intolerance)*

What is the deficient enzyme and the accumulated product in cases of hereditary fructose intolerance? Explain how this intolerance leads to hypoglycaemia after fructose ingestion.

Clinical case 1.1.9. *Galactose-1-phosphate uridyltransferase deficiency (Galactosaemia)*

A newborn showed frequent vomiting and diarrhoea from the 4^{th} day of life, that led to considerable weight loss. At the 20^{th} day of life, it was observed jaundice, hepatomegaly and slight crystalline opacification. Laboratory exams revealed increased conjugated and non-conjugated bilirubin, high blood level of reducing sugars and presence of galactose in urine (galactosuria). Diagnosis of galactosaemia was established and a diet based of soya was prescribed. Gradually, symptoms started to disappear with this diet and the child showed normal growth.
a) Galactosaemia can be caused by the absence or deficiency of which enzymes?
b) Which symptoms develop because of galactosaemia?
c) Which are the main food sources of galactose?
d) Explain the biochemical events responsible for the development of symptoms.
e) Galactose is a monosaccharide present in glycoproteins of plasma membranes. How can galactosaemic individuals synthetize galactose for their glycoproteins, if their diets should have low level of lactose?
f) Explain clinical and biochemical differences between cases of galactosaemia and secondary lactose intolerance.

Clinical case 1.1.10. *Effect of arsenate*

Arsenate is chemically and structurally like phosphate and certain enzymes that require phosphate can also use arsenate. However, compounds formed with arsenate are less stable than the corresponding compounds formed with phosphate.
a) What is the expected effect of replacing phosphate by arsenate in the reaction catalysed by gliceraldehyde-3-phosphate dehydrogenase?
b) Explain why arsenate is toxic for most organisms, specially under anaerobic conditions.

Clinical case 1.1.11. *Effect of fluoride*

Enolase is inhibited by fluoride ion.
a) Which metabolic intermediate is accumulated when this ion is added to a mixture of glucose, ATP, P_i, NAD^+ and all the enzymes required for glycolysis?
b) Explain how certain tissues continue to respirate (produce CO_2) in the presence of high concentrations of fluoride.
c) Which cells of our organism are affected by the inhibition of fluoride?

Clinical case 1.1.12. *Pyruvate carboxylase deficiency*

Explain the metabolic consequences observed if the enzyme pyruvate carboxylase in liver cells is mutated and is no longer regulated by acetyl-CoA. Justify considering the fasting and post-prandial state.

Clinical case 1.1.13. *Glucose-6-phosphatase deficiency (Von Gierke's disease)*

In glycogen metabolism, the Von Gierke's disease is caused by a liver deficiency of glucose-6-phosphatase. Explain symptoms developed by patients having this disorder.

Clinical case 1.1.14. *Glucose-6-phosphatase deficiency*

An 11-year-old boy was taken to the hospital due to severe hepatomegaly, weakness and pallor that were higher mainly in periods between meals. Clinical examination of the patient revealed a below minimal expectancy mental and physical development, detected by unsatisfactory school outcomes, height and weight below normal. Biochemical tests performed in a blood sample collected in fasting were the following:

Table 1.1.2. Results obtained in blood tests performed to the patient.

Parameter	Patient values	Reference range
Glucose	46.0 mg/dL	60.0-110.0 mg/dL
Pyruvate	0.40 mmol/L	0.05-0.10 mmol/L
Lactate	6.40 mmol/L	0.56-2.0 mmol/L
Free fatty acids	1.40 mmol/L	0.30-0.80 mmol/L
Triacylglycerols	3.05 g/L	1.50 g/L
Ketone bodies	40.0 mg/dL	3.0 mg/dL
Uric acid	9.50 mg/dL	6.0-7.0 mg/dL
pH	7.25	7.35-7.45
Total CO_2	14.0 mmol/L	24.0-30.0 mmol/L

It was also observed the presence of glycogen with normal structure (11 g/100 g of liver tissue; reference range = 8 g/100 g) and lipid content of 30 g/100 g of liver tissue (reference range = 5 g/100 g).
a) What is the enzyme deficiency observed?
b) In which tissues is this enzyme present?
c) Explain described symptoms.
d) Explain blood test results.
e) What is the adequate treatment for this condition?

Clinical case 1.1.15. *Biotin deficiency*

Dietary biotin is linked to the ε-amino groups of lysine. Biotinidase hydrolyses this bond and releases biotin that can be used as a cofactor of several enzymes. Biotinidase deficiency is rare but can happen.
a) What is the effect of this enzyme deficiency in gluconeogenesis?
b) What are the metabolic consequences that result from this condition?

Clinical case 1.1.16. *Hypoglycaemia*

A 6-year-old girl goes to the hospital due to constant hypoglycaemic episodes. The very severe fasting hypoglycaemia occurred frequently at night, and plasma glucose levels could even oscillate between 20 and 30 mg/dL. Glucagon (1 mg intramuscular), given in certain occasions, did not lead to increased glucose levels; however, when the same glucagon dosage was given one hour after a meal, plasma glucose levels raised to 60 mg/dL in 30 minutes. The hypothesis of this scenario being caused by a glycogen storage disease was discarded. Activities of glucose-6-phosphatase, glycogen phosphorylase, acidic phosphatase and phosphoglucomutase were normal, however fructose-1,6-biphosphatase activity was extremely low. The child had frequent hypoglycaemic episodes (fasting glycaemia around 30 mg/dL) and severe metabolic acidosis (arterial pH below 7.15), usually together with vomiting. The child was submitted to a test on the fasting effect to confirm that hypoglycaemia was caused by a deficiency in gluconeogenesis. After a period of 12, 18 and 21 hours of fasting, the results presented below were observed. The glucose tolerance test was normal, as well as plasma insulin levels. Both the fructose tolerance test and glycerol tolerance test led to hypoglycaemia.

Table 1.1.3. Observed values for plasma glucose levels, pH and total CO_2 after a fasting period of 12, 18 and 21 hours.

Fasting hours	D-glucose (mmol/L)	pH	Total CO_2 (mmol/L)
12	3.1	7.4	25.0
18	1.9	--	--
21	0.6	7.17	9.3

a) What is hypoglycaemia, and which are its main causes?
b) How the fasting hypoglycaemia in this patient can be explained?
c) Besides glucose, which sugars and sugar derived alcohols can improve the fasting hypoglycaemia of this patient? Explain.
d) Which are the causes of the metabolic acidosis observed in this patient?
e) Explain why premature children show higher sensitivity to hypoglycaemia than term children, having normal size for their age?
f) Post-prandial hypoglycaemia can be easily diagnosed through a glucose tolerance test. Explain.
g) Explain the most adequate food intake strategies for each case of hypoglycaemia.

Clinical case 1.1.17. *Hypoglycaemia and alcoholic intoxication*

A 45-year-old patient, with a background of chronic alcoholism, was found fainted on the street. Physical examination revealed a slight coma state, alcoholic breath, dehydration, physical weakness, lower limbs oedema and increased liver. Patient's blood tests revealed the following results:

Table 1.1.4. Observed results of patient blood tests.

Parameter	Patient values	Reference values
Alcohol	86 mmol/L	≤ 17.4 mmol/L
Glucose	3.1 mmol/L	3.3-8.4 mmol/L
Lactate	2.8 mmol/L	0.7-2.0 mmol/L
Urate	0.6 mmol/L	0.2-0.5 mmol/L
TGO	100 IU/mL	5.0-50.0 IU/mL
TGP	470 IU/mL	5.0-35.0 IU/mL
Amylase	800 IU/mL	50.0-150.0 IU/mL
Total bilirubin	4.8 mg/dL	0.2-1.0 mg/dL
Urea	10.0 mg/dL	14.0-18.0 mg/dL
Total proteins	4.5 mg/dL	6.0-8.0 mg/dL
Total CO_2	29 mmol/L	24-30 mmol/L

a) Liver commitment of this patient was quite advanced. Explain possible causes for the cirrhotic picture observed in chronic alcoholism.
b) Explain changes observed in biochemical tests of this patient.
c) Alcoholic individuals go from a phase of big alcohol tolerance to, in more advanced alcoholism stage, much lower tolerance. Explain.
e) Intravenous administration of glucose in this situation or in cases of alcohol intoxication is essential to re-establish patient glycaemia. It is also known that glucose increases liver alcohol metabolism. However, fructose is more effective. Explain why.

Clinical case 1.1.18. *Alcohol consumption*

Nowadays, excessive alcohol consumption is one of the severe health problems that affects our society. Among its more acute effects are hypoglycaemia, lactic acidosis and eventual coma.
a) Explain how these symptoms develop.
b) Why is gluconeogenesis decreased or even absent during acute alcohol consumption?
c) Simultaneous ingestion of ethanol and certain pharmaceutical products can be dangerous. Explain why.

Clinical case 1.1.19. *Glucose-6-phosphate dehydrogenase deficiency (Favism)*

A boy coming from an endemic region of malaria was admitted to the hospital with symptoms of that disease. Laboratorial diagnosis confirmed the suspicion and treatment was immediately started with antimalaria drugs. Two days later, the patient showed a worse scenario with dark urine and jaundice. Blood tests were performed again, and haemoglobin levels were drastically decreased together with the number of erythrocytes. Non-conjugated bilirubin levels were quite high. A deficiency of the enzyme glucose-6-phosphate dehydrogenase was suspected and to confirm this hypothesis, quantitative measurement of this enzyme in erythrocytes was performed, showing a very low activity.

a) To which metabolic pathway does this enzyme belong to and in which tissues does it occur?
b) Patients with this enzymatic deficiency can have serious consequences in certain situations. Explain.
c) Which pharmaceutical products can induce haemolytic anaemia in patients with this deficiency?
d) As a prophylactic measure, what can be done to protect these patients?
e) What would be the effect of vitamin E (antioxidant) in patients having this enzymatic deficiency?

1.1.4. **Further questions**

Question 1.1.1.
In a eukaryotic cell, where are the enzymes responsible for glycolysis located?

Question 1.1.2.
What is the purpose for the cell of the phosphorylation of glucose to glucose-6-phosphate?

Question 1.1.3.
What does the following reaction represent?
Glucose + ATP → Glucose-6-phosphate + ADP

Question 1.1.4.
In glycolysis, which hexose gives rise to trioses?

Question 1.1.5.
Indicate the oxidation-reduction reactions of glycolysis.

Question 1.1.6.
What is the effect of fructose-2,6-bisphosphate on the rate of glycolysis? Which step(s) is(are) affected?

Question 1.1.7.
In muscle what is the most likely use of glucose-6-phosphate?

Question 1.1.8.
How many moles of pyruvate are formed from one mole of glucose in glycolysis?

Question 1.1.9.
What is the production of NADH when 1 mole of glucose-6-phosphate is aerobically oxidized to pyruvate through glycolysis?

Question 1.1.10.
What is the production of NADH when 1 mole of glucose-6-phosphate is oxidized to lactate through glycolysis?

Question 1.1.11.
Considering the number of moles of ATP consumed and formed, establish the final ATP balance (energy balance) in the oxidation of one mole of glucose by the glycolytic pathway. Justify.

Question 1.1.12.
How is glucose transported to muscle cells? What kind of transporters are needed?

Question 1.1.13.
How is glucose transported across the basal membrane of intestinal cells? What kind of transporters are needed?

Question 1.1.14.
Phosphofructokinase is the main reaction controlling the flow of glycolysis. How is this enzyme regulated?

Question 1.1.15.
Pyruvate kinase is one of the flow regulation points of glycolysis. How is this enzyme regulated?

Question 1.1.16.
How is it possible to increase the rate of glycolysis?

Question 1.1.17.
Glucokinase and hexokinase can phosphorylate glucose to form glucose-6-phosphate. Explain why there are two enzymes to catalyse the same reaction and how they are regulated.

Question 1.1.18.
In the first step of glycolysis, hexokinase uses ATP to transfer a phosphate group to glucose and produce glucose-6-phosphate. Glucose-6-phosphate then continues to be oxidized by glycolysis to pyruvate which is a precursor of acetyl-CoA (which will then enter the Krebs cycle). Suppose that a cell has only glucose as an energy source and that suddenly hexokinase activity is inhibited in that cell. What will be the consequences?

Question 1.1.19.
Compare, in terms of substrate affinity, glucokinase and hexokinase. Explain how this is reflected in the enzyme kinetics parameters.

Question 1.1.20.
What is the isomerization of glucose-6-phosphate to fructose-6-phosphate for?

Question 1.1.21.
A patient has the following metabolic changes in erythrocytes.

Table 1.1.5. Metabolic changes observed in the patient.

High	Low
Fructose-1,6-biphosphate	ATP
2,3-Biphosphoglicerate	
Dihydroxyacetone phosphate	

What enzyme deficiency can explain these changes? Explain.

Question 1.1.22.
To match variations in cell requirements, enzymatic activities are controlled by a set of distinct mechanisms. In the process of glycolysis, give an example (explaining briefly) of:
a) Inhibition by products.
b) Allosteric control.

Question 1.1.23.
Enolase is inhibited by fluorine ion. What metabolic intermediate accumulates when that ion is added to a solution mixture of glucose, ATP, P_i, NAD^+ and all the enzymes necessary for glycolysis?

Question 1.1.24.
Define substrate-level phosphorylation. Which reactions of glycolysis fall into this category?

Question 1.1.25.
The glucose molecule must enter the cell to initiate the glycolytic pathway. Explain why glucose is phosphorylated immediately upon entering eukaryotic cells.

Question 1.1.26.
A student is producing glucose-6-phosphate from a very concentrated glucose solution, by the action of the enzyme glucokinase. As he cannot control the speed of the process, he decides to use one of the two hexokinase inhibitors he has available, one being a competitive inhibitor and the other a non-competitive one. Which would you choose? Justify.

Question 1.1.27.
Indicate and explain the effect on the intensity of glycolysis of an increase in the concentration of each of the following compounds:
a) Fructose-1,6-biphosphate.
b) Fructose-2,6-biphosphate.
c) Citrate.
d) NADH.
e) Glucose-6-phosphate.

Question 1.1.28.
In the first step of the glycolytic pathway, glucose is phosphorylated at carbon six (C6) to yield glucose-6-phosphate. This reaction is catalysed by hexokinase (ATP: D-hexose 6-phosphotransferase). Like other kinases, this enzyme needs Mg^{2+} for its activity. What role does this cofactor play?

Question 1.1.29.
One of the enzymes of glycolysis catalyses the physiologically irreversible reaction:
Phosphoenolpyruvate + A → Pyruvate + B
A and B correspond, respectively, to: ADP and ATP, ATP and ADP, H_2O and P_i or P_i and H_2O?

Question 1.1.30.
What is the molecular mechanism of activation of the enzyme pyruvate kinase by fructose-1,6-bisphosphate?

Question 1.1.31.
Describe the effect of glucagon on the activity of phosphofructokinase-2 and show the consequence of this effect on the activity of the glycolytic pathway.

Question 1.1.32.
Describe how fructose, galactose, and mannose enter the glycolytic pathway.

Question 1.1.33.
State, with justification, whether the following statements are true or false. Fructose…
a) Unlike glucose, cannot be metabolized by the glycolytic pathway.
b) In the liver, enters directly into glycolysis as fructose-6-phosphate.
c) Must be isomerized to glucose before it can be metabolized.
d) Is converted to the UDP-bound form and then epimerized to UDP-glucose.
e) Catabolism in the liver uses fructokinase and a specific aldolase that recognizes fructose-1-phosphate.

Question 1.1.34.
In the liver, does the major pathway for converting fructose to pyruvate include fructose-6-phosphate, fructose-1,6-bisphosphate, glucose-6-phosphate, or dihydroxyacetone phosphate? Justify.

Question 1.1.35.
State, with justification, whether the following statements are true or false. Galactosemia…
a) Is a genetic deficiency of the enzyme uridyltransferase that exchanges galactose-1-phosphate for glucose in UDP-glucose.
b) Results from a deficiency in an epimerase.
c) Does not appear at birth but symptoms develop later.
d) Is an inability to form galactose-1-phosphate.
e) Interferes with the use of fructose and galactose because the deficient enzyme is common to the metabolism of both sugars.

Question 1.1.36.
Explain, indicating the various catalytic reactions and enzymes, why fructose is metabolized in the liver more rapidly than glucose.

Question 1.1.37.
Compare, using the appropriate catalytic reactions and enzymes, the rate at which galactose is metabolized relative to that of glucose.

Question 1.1.38.
What compound accumulates in the liver after sucrose ingestion in an individual with hereditary fructose intolerance?

Question 1.1.39.
After ingestion of galactose, galactosemia remains high for about 30 minutes, but quickly drops to almost zero. Explain why.

Question 1.1.40.
What causes muscle fatigue during physical exercise? Explain which metabolic pathways are active during this activity.

Question 1.1.41.
In the absence of oxygen, what is the main function of homolactic fermentation?

Question 1.1.42.
Under anaerobic conditions, muscle performs glycolysis producing ATP and lactic acid. Why is lactate production necessary?

Question 1.1.43.
Why, in the cytoplasm of cells, is there much more lactate than pyruvate under anaerobic conditions, and more pyruvate than lactate under aerobic conditions?

Question 1.1.44.
Under anaerobic conditions, skeletal muscle can continue to produce ATP through glycolysis. Under these circumstances, what is glucose converted to?

Question 1.1.45.
What are the degradation products of one mole of glucose by glycolysis under aerobic conditions?

Question 1.1.46.
What are the degradation products of one mole of glucose by glycolysis in muscle under anaerobic conditions?

Question 1.1.47.
What metabolic pathway is common to the breakdown of glucose under aerobic and anaerobic conditions?

Question 1.1.48.
Louis Pasteur, the leading 19th century chemist and microbiologist, was the first scientist to make the following observation: Cells that completely oxidize glucose to CO_2 and H_2O use glucose more rapidly in the absence than in the presence of O_2. O_2 appears to inhibit glucose consumption. Explain in general terms the meaning of this observation, nowadays referred to as the Pasteur Effect.

Question 1.1.49.
What is the energy balance of glycolysis under anaerobic conditions?

Question 1.1.50.
Under anaerobic conditions, which molecules can be used by muscle cells to produce energy?

Question 1.1.51.
Explain what fermentations are, whether they allow the production of large amounts of energy, whether or not they require the presence of oxygen and in which organisms they occur.

Question 1.1.52.
Adding pure O_2 to a yeast culture growing in grape juice causes the yeast to multiply faster. What will be the effect of this addition on the wine?

Question 1.1.53.
What distinguishes fermentation from respiration?

Question 1.1.54.
What vitamins and minerals are needed for the conversion of glucose to lactate?

Question 1.1.55.
Sketch the alcoholic fermentation reactions that make possible to obtain NAD^+ in the oxidized form. Give examples of tissues or organisms where lactic and alcoholic fermentations occur. Under what conditions does oxidation of glucose to lactate occur in muscle?

Question 1.1.56.
Describe the three possible fates of pyruvate from glycolysis.

Question 1.1.57.
Compare the three irreversible reactions of glycolysis with the gluconeogenesis reactions that replace them in terms of reactants, products, enzymes, and coenzymes. What distinguishes glycolysis from gluconeogenesis?

Question 1.1.58.
Define gluconeogenesis and give examples of gluconeogenic compounds. Name the tissue responsible for gluconeogenesis.

Question 1.1.59.
Why is it important that gluconeogenesis is not the exact reverse of glycolysis?

Question 1.1.60.
What is the subcellular location of gluconeogenesis enzymes?

Question 1.1.61.
What is the function of gluconeogenesis? Describe the reactions of gluconeogenesis.

Question 1.1.62.
State under what circumstances gluconeogenesis occurs and which molecules activate it.

Question 1.1.63.
Describe the physiological conditions that activate gluconeogenesis.

Question 1.1.64.
Considering that gluconeogenesis is an energy-consuming process, what advantage does the organism have in carrying it out?

Question 1.1.65.
In gluconeogenesis, what enzymes are needed to convert phosphoenolpyruvate to pyruvate?

Question 1.1.66.
What is the energy balance of the synthesis of one mole of glucose from two moles of lactate during gluconeogenesis? How much NADH is needed?

Question 1.1.67.
What is the energy balance of synthesizing one mole of glucose from two moles of malate during gluconeogenesis? How much NADH is needed?

Question 1.1.68.
What is the energy balance of synthesizing one mole of glucose from two moles of oxaloacetate during gluconeogenesis? How much NADH is needed?

Question 1.1.69.
What is the energy balance of synthesizing one mole of glucose from two moles of pyruvate during gluconeogenesis? How much NADH is needed?

Question 1.1.70.
Explain how gluconeogenesis occurs using glycerol as a substrate.

Question 1.1.71.
Which metabolic pathway(s) have as their main function the maintenance of an adequate level of glucose in the blood?

Question 1.1.72.
What is the main tissue involved in gluconeogenesis to supply glucose for muscle glycogen synthesis after prolonged exercise?

Question 1.1.73.
What are the effects of increasing ATP or AMP concentration on the catalytic activities of phosphofructokinase-1 and fructose-1,6-bisphosphatase? What are the consequences of these effects on the relative flux of metabolites in gluconeogenesis and glycolysis?

Question 1.1.74.
What are the likely consequences of a genetic change that makes liver fructose-1,6-bisphosphatase less sensitive to regulation by fructose-2,6-bisphosphate?

Question 1.1.75.
Define:
a) Anaplerotic reactions.
b) Lactic aciduria.

Question 1.1.76.
The following two reactions constitute a futile cycle:
Glucose + ATP → Glucose-6-phosphate
Glucose-6-phosphate + H_2O → Glucose + P_i
Suggest how these cycles are controlled.

Question 1.1.77.
Explain why the high KM of glucokinase is important for the liver's role in blood glucose control.

Question 1.1.78.
Mature erythrocytes lack mitochondria but actively metabolize glucose. In response to a large supply of glucose to the red blood cell, lactate and CO_2 are formed.
a) Why is it necessary to form lactate to ensure glucose utilization?
b) In red blood cells, which metabolic pathway is used to form CO_2 from glucose?

Question 1.1.79.
In conditions of thiamine deficiency, plasma lactic acid elevation may occur. Explain why.

Question 1.1.80.
The enzyme lactate dehydrogenase constitutes a cellular mechanism that allows the continuation of the glycolytic process in aerobic or anaerobic conditions? Explain why.

Question 1.1.81.
The fungus *Saccharomyces cerevisiae* (baker's yeast) is a facultative anaerobe. When it grows in the absence of oxygen, it consumes much more glucose than when it grows in the presence of oxygen. Explain this difference in glucose consumption.

Question 1.1.82.
What symptoms a patient with glucose-6-phosphatase deficiency has? Explain. Give an example of another enzyme deficiency that leads to the same type of symptoms.

Question 1.1.83.
Ethanol ingestion leads to a redox imbalance, with a marked decrease in the NAD^+/NADH ratio. Explain how this imbalance is generated and indicate the main metabolic consequences.

Question 1.1.84.
The simultaneous ingestion of ethanol and some drugs can be dangerous. Explain.

Question 1.1.85.
What does the Cori cycle consist of and what is it for? What does the glucose-alanine cycle consist of and what is it for? Make a description of the cycles referring to the organs involved. Describe the conditions under which the Cori cycle and the glucose-alanine cycle operate.

Question 1.1.86.
Why is the malate-aspartate shuttle important for gluconeogenesis?

Question 1.1.87.
The enzyme glucose-6-phosphatase is present in the ER. Describe the symptoms of a defect in glucose-6-phosphate transport across the ER membrane.

Question 1.1.88.
Blood glucose quickly enters all tissues, but only specialized tissues such as the liver and kidney can release it into the bloodstream. Give two reasons why most tissues are unable to release glucose into the bloodstream.

Question 1.1.89.
Describe the function of fructose 2,6-biphosphate in the regulation of gluconeogenesis.

Question 1.1.90.
Why is gluconeogenesis reduced or not even occurring during acute ethanol intake?

Question 1.1.91.
Phosphofructokinase and pyruvate kinase are involved in the regulation of glycolysis/gluconeogenesis. Indicate which mechanism is involved referring to the changes that occur between the well-fed and fasting states.

Question 1.1.92.
The pentose-phosphate pathway and the glycolytic pathway are interdependent. Explain why.

Question 1.1.93.
In which cells does the pentose-phosphate pathway occur and what does it provide that could be useful for cellular metabolism?

Question 1.1.94.
What products are obtained from the oxidation of one mole of glucose-6-phosphate by the oxidative phase of the pentose-phosphate pathway?

Question 1.1.95.
State, justifying, whether the following sentences are true or false:
a) Lactate and acetyl-CoA, both products derived from pyruvate, are possible substrates for gluconeogenesis.
b) Mammals can carry out gluconeogenesis from odd-chain fatty acids.
c) The pentose-phosphate pathway is regulated to meet the cell needs for NADH and ribose-5-phosphate.
d) NADPH is necessary for reductive processes.

Question 1.1.96.
From six glucose molecules, what must be obtained via the pentose-phosphate pathway (possibly together with glycolysis or gluconeogenesis)?

Question 1.1.97.
Explain why an inherited deficiency of the enzyme glucose-6-phosphate dehydrogenase in erythrocytes is harmful.

Question 1.1.98.
In addition to the pentose-phosphate pathway, which metabolic reaction is an important source of the NADPH needed for cholesterol synthesis?

Question 1.1.99.
In what ways is the NADPH that the body needs for anabolic processes regenerated?

Question 1.1.100.
Which agent in human red blood cells plays an identical role to that played by ascorbic acid (vitamin C – antioxidant) in fibroblasts? Refer to the role played by this agent.

Question 1.1.101.
Avidin is a 70 kDa protein present in egg white with a high affinity for biotin. In fact, avidin is an inhibitor of biotin enzymes. Which of the following conversions would be inhibited by adding avidin to a cell homogenate? Justify.
a) Glucose to pyruvate.
b) Pyruvate to glucose.
c) Oxaloacetate to glucose.
d) Glucose to ribose-5-phosphate.
e) Pyruvate to oxaloacetate.
f) Ribose-5-phosphate to glucose.

Question 1.1.102.
Which enzyme, in purified form, is often used in the measurement of blood glucose?

1.2. **Krebs cycle, Electron transport chain and Oxidative phosphorylation**

1.2.1. **Short review**

Krebs cycle

The Krebs cycle (also called citric acid cycle) is a sequence of reactions where two carbon atoms of acetyl-coenzyme A (acetyl-CoA) are oxidised to CO_2. It is a central metabolic pathway for the release of energy from acetyl-CoA, that is produced in the catabolism of carbohydrates, fatty acids and amino acids. Acetyl-CoA and the enzymes that catalyse the Krebs cycle reactions are in the mitochondrial matrix, except one enzyme that is present in the inner membrane of mitochondria.

After entering the mitochondria, pyruvate is converted into acetyl-CoA by the enzymatic complex pyruvate dehydrogenase. This is a very complex reaction, and the catalysing enzyme requires the presence of 5 different coenzymes/cofactors: thiamine pyrophosphate (TPP), lipoic acid, coenzyme A (CoA), FAD and NAD^+. This reaction is the main regulatory point of mitochondrial metabolism, and the enzyme can be regulated in a direct and an indirect way. Direct regulation is performed by reaction products (NADH and acetyl-CoA) that inhibit pyruvate dehydrogenase and the reagents (NAD^+ and pyruvate) that are able to activate the same enzyme. In the indirect regulation, the target enzyme is a kinase that is responsible for the inactivation of the complex pyruvate dehydrogenase by phosphorylation. In this way, reaction products (acetyl-CoA and NADH) will activate the kinase and the reagents will inhibit this enzyme.

The eight reactions of the Krebs cycle and respective regulatory points are (Fig. 1.2.1):

1. Citrate synthase catalyses the condensation of acetyl-CoA with oxaloacetate to yield citrate. This reaction is reversible but is a main regulatory point. The activity of this enzyme is inhibited by succinyl-CoA and by low NAD^+/NADH ratio.

2. Aconitase catalyses reversibly the conversion of citrate into isocitrate.

3. Isocitrate dehydrogenase catalyses the oxidative decarboxylation of isocitrate to α-ketoglutarate. NAD^+ is reduced to NADH and CO_2 is released. The enzyme suffers allosteric inhibition by ATP and NADH and is activated by ADP and NAD^+.

4. The complex α-ketoglutarate dehydrogenase catalyses the conversion of α-ketoglutarate in the presence of coenzyme A, into succinyl-CoA. Again, NAD^+ is reduced to NADH and CO_2 is released. This enzymatic complex is inhibited by NADH and succinyl-CoA.

5. Succinate thiokinase catalyses the conversion of succinyl-CoA into succinate. In this reaction there is the phosphorylation of GDP, yielding GTP.

6. Succinate dehydrogenase catalyses the oxidation of succinate to fumarate. This is the only enzyme of the Krebs cycle that is attached to the inner membrane of mitochondria. Transfer of 2 H^+ to flavin adenine dinucleotide in the oxidised form (FAD) occurs, yielding $FADH_2$. This enzyme is inhibited by oxaloacetate.

7. Fumarase catalyses the reversible hydration of fumarate to malate.

8. Malate dehydrogenase catalyses the production of oxaloacetate and NADH from malate.

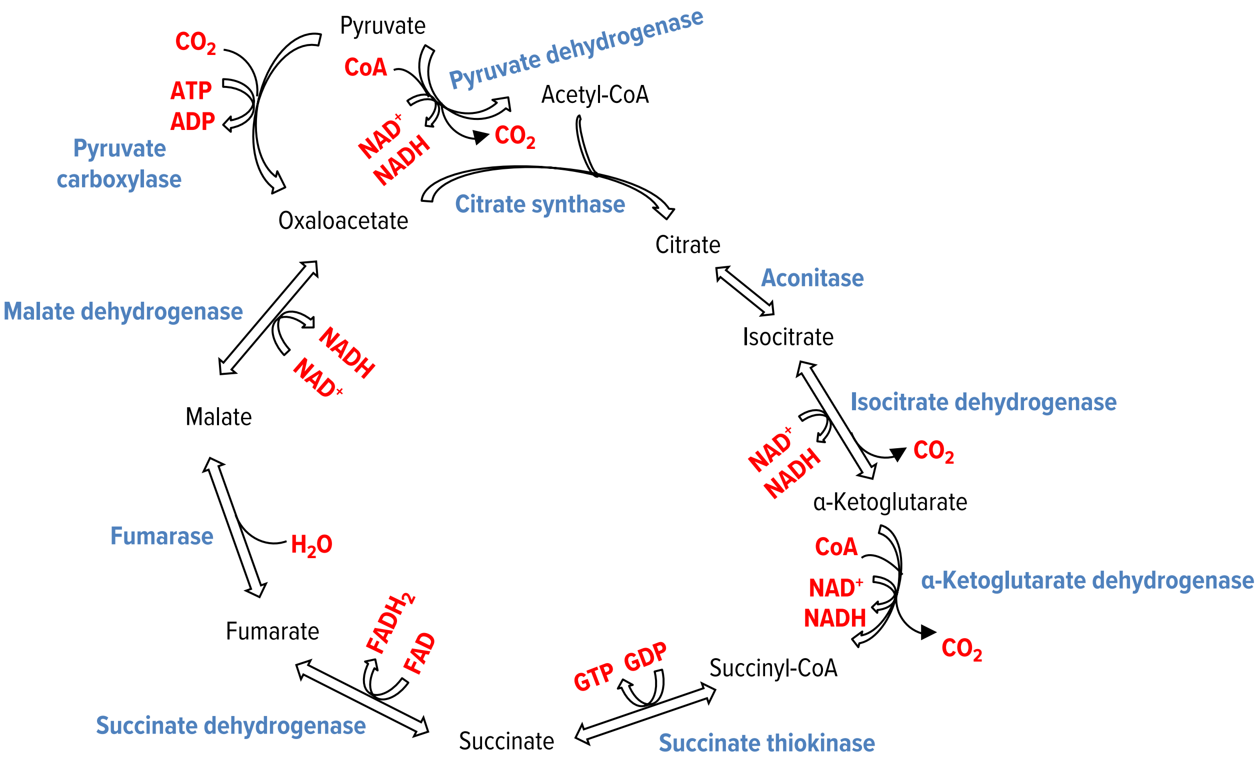

Fig. 1.2.1. The Krebs cycle.

The net reaction of the Krebs cycle is:

$$\text{Acetyl-CoA} + 3NAD^+ + FAD + GDP + P_i + 2H_2O \rightarrow 2CO_2 + 3NADH + FADH_2 + CoASH + GTP + 3\ H^+$$

The speed of consumption of acetyl-CoA in the Krebs cycle depends on the energetic state of mitochondria. In conditions of high energy, NADH and ATP levels are high and NAD^+ and AMP are low.

Many of the intermediaries of the Krebs cycle are used in the synthesis of other biomolecules, and several other biomolecules enter the Krebs cycle. For this reason, the Krebs cycle is considered amphibolic.

Electron Transport Chain and Oxidative Phosphorylation

In the electron transport chain (ETC), NADH and $FADH_2$, produced by carbohydrate and lipid oxidation, are oxidised by oxygen. ETC is composed of four enzymatic complexes located in the inner mitochondrial membrane:

1. Complex I or NADH dehydrogenase complex includes flavin mononucleotide in the oxidised form (FMN) and Fe-S groups. In this complex, electrons are transported from NADH to coenzyme Q_{10} (CoQ_{10}) or ubiquinone.

$$NADH + H^+ \rightarrow NAD^+ + 2H^+ + 2e^-$$

$$CoQ + 2\ H^+ + 2e^- \rightarrow CoQH_2$$

2. Complex II or succinate dehydrogenase complex includes FAD and Fe-S groups. In this complex, electrons are transported from $FADH_2$ to CoQ.

$$FADH_2 \rightarrow FAD + 2H^+ + 2e^-$$

$$CoQ + 2\ H^+ + 2e^- \rightarrow CoQH_2$$

3. Complex III or cytochrome bc_1 complex includes cytochromes *b* and c_1 and Fe-S groups. In this complex, electrons are transported from CoQ to cytochrome c.

4. Complex IV or cytochrome c oxidase complex includes cytochromes *a* and a_3. In this complex, electrons are transported from cytochrome c to oxygen, with the production of water.

$$\frac{1}{2}\ O_2 + 2\ H^+ + 2e^- \rightarrow H_2O$$

Oxidative phosphorylation consists of the synthesis of ATP catalysed by the complex ATP synthase, coupled with NADH and $FADH_2$ oxidation by oxygen in the ETC. The electron transfer through the components of the ETC leads to structural changes of their constituting proteins and to variation in pK_a values of their ionizable amino acid residues. The result is the transfer of protons from the mitochondrial matrix to the intermembrane space. The ATP synthase space contains a proton transport channel, that is the only route of re-entrance of protons into the mitochondrial matrix. The energy of the electrochemical potential gradient of protons is used in the synthesis of ATP from ADP and P_i. In this way, NADH oxidation leads to the production of around 3 molecules of ATP (2.5 ATPs) per oxygen atom reduced to water. On the other hand, $FADH_2$ oxidation only leads to the production of around 2 molecules of ATP (1.5 ATPs).

1.2.2. **Pathologies**

Pyruvate dehydrogenase deficiency

The pyruvate dehydrogenase complex is responsible for the conversion of pyruvate into acetyl-CoA, allowing entrance in the Krebs cycle (Fig. 1.2.1). The deficiency of this enzyme leads to the accumulation of the substrate pyruvate and considerable reduction on ATP synthesis in aerobic respiration. Cells must shift to anaerobic respiration to allow ATP production to proceed in glycolysis, being pyruvate converted into lactate. For this reason, this condition is characterised by the build-up of lactic acid in the blood leading to lactic acidosis. This can cause nausea and vomiting. To control blood pH, breathing problems and an abnormal heartbeat can develop. Since brain cells require high levels of energy, development of neurological problems can also occur, that can go from delayed development of mental abilities and motor skills to intellectual disabilities, seizures, hypotonia and low coordination.

This is a rare disease, with unknown prevalence, whose signs and symptoms usually develop after birth and differ widely between affected children.

Abnormal brain structures are present in some affected individuals and due to severe health problems, many patients do not survive after childhood.

Pyruvate dehydrogenase is a multimeric complex composed of three different polypeptide chains, E1 (alpha-ketoacid decarboxylase), E2 (dihydrolipoyl transacetylase) and E3 (dihydrolipoyl dehydrogenase or lipoamide dehydrogenase), each responsible for one of the steps in the conversion of pyruvate into acetyl-CoA.

Some other proteins are involved in the correct functioning of the complex, such as the E3 binding protein (that provides the correct structure to the complex ensuring its proper functioning), and the pyruvate dehydrogenase phosphatase and kinase that control the inactivation/activation of the complex by dephosphorylation/phosphorylation reactions.

The E1 polypeptide chain is formed by four subunits: two alpha subunits and two beta subunits. The *PDHA1* gene codes for the alpha subunit, and mutations in this gene lead to 80% of pyruvate dehydrogenase deficiencies. This gene is present in the X chromosome, leading to an X-linked pattern of heritage. Males are more affected by this condition since they only require one copy of the mutated gene to express the disorder. However, heterozygous females can also express the condition due to the inactivation of the normal X chromosome.

Pyruvate dehydrogenase deficiency can also result from mutations in other genes, being inherited with an autosomal recessive pattern. Mutations in other components of pyruvate dehydrogenase complex such as the *PDHB* gene coding for the beta subunit, the *DLAT* gene coding for the E2 chain, the *PDHX* gene coding for the E3 binding protein and the *PDP1* gene coding for pyruvate dehydrogenase phosphatase, have been identified in affected individuals. It is observed that reduction of function of one of the components of pyruvate dehydrogenase complex, affects the overall enzymatic activity. In the same way, mutations in other genes whose coded proteins regulate pyruvate dehydrogenase, also interfere with its catalytic activity.

Aconitase deficiency

Aconitase 2 is a mitochondrial enzyme that catalyses the conversion of citrate into isocitrate, via cis-aconitate, in the Krebs cycle (Fig. 1.2.1). This enzyme is encoded by the *ACO2* gene. This enzyme is related with several diseases since it is involved in ATP production, a central metabolic pathway.

Gastric cancer cells and prostate cancer cells having decrease expression of the *ACO2* gene, are associated with poor prognosis.

Aconitase deficiency is caused by mutations in the *ISCU* (iron-sulphur cluster scaffold) gene. The coded protein is involved in the build-up of the Fe-S cluster required for aconitase activity. Symptoms associated with this deficiency are myopathy and intolerance to physical activity. In some patients, exercise can be lethal due to circulatory shock.

Friedreich's ataxia, a neurodegenerative disease, is another disorder associated with aconitase. This disorder is caused by decreased activity of Fe-S proteins in aconitase and succinate dehydrogenase. This last enzyme also participates in the Krebs cycle, converting succinate into fumarate. The possible reason for this association is that the decreased enzymatic activities are correlated with high iron level in mitochondria and low in cytoplasm, leading to disruption of iron homeostasis.

α-Ketoglutarate dehydrogenase deficiency

α-Ketoglutarate dehydrogenase is an enzyme of the Krebs cycle responsible for the conversion of α-ketoglutarate into succinyl-CoA (Fig. 1.2.1). The mechanism of action of this enzyme is like pyruvate dehydrogenase. In this way, as discussed previously, it is an enzymatic complex constituted by three distinct subunits (E1, E2 and E3), each one responsible for one of the steps of the catalysed reaction.

α-Ketoglutarate dehydrogenase, fumarase and succinate dehydrogenase are the only enzymes of the Krebs cycle whose deficiency lead to human disorders.

Symptoms resulting from α-ketoglutarate dehydrogenase deficiency include hypotonia, hepatomegaly, lactic acidaemia, high creatine kinase and excretion of α-ketoglutarate in urine. These symptoms can develop immediately after birth and life expectancy is around 30 months of age. This deficiency can be associated with pyruvate dehydrogenase deficiency and in this case hypoglycaemia and neurological problems can develop.

Since it is a very rare condition, there is no data stablishing its incidence. The few reported cases are from the north of Africa. Nevertheless, it is believed that this medical condition is inherited as an autosomal recessive trait.

Fumarase deficiency

Fumarase (also called fumarate hydratase) is an enzyme of the Krebs cycle that catalyses the conversion of fumarate into malate (Fig. 1.2.1). This enzyme is coded by the *FH* (fumarate hydratase) gene.

Mutations in the *FH* gene lead to the loss of fumarase activity, resulting in disruption of ATP production in the Krebs cycle. This is particularly harmful for the nervous system, especially the developing brain. Children affected by this condition show microcephaly, altered brain structure, hypotonia, slow development and failure to thrive. Affected individuals may also have prominent forehead, micrognathia and ocular hypertelorism. Other possible symptoms are hepatosplenomegaly, and, during childhood excessive number of erythrocytes and deficient number of white blood cells.

Most affected individuals live only for a few months.

This is a very rare disorder, with only 100 affected individuals worldwide, being transmitted in an autosomal recessive pattern.

Complex I (NADH dehydrogenase) deficiency

Complex I is the first complex of the ETC occurring in mitochondria during cellular respiration. It is involved in the reception of electrons coming from NADH. These electrons are then transferred to complex III via CoQ and later to complex IV. This last complex gives electrons to the final acceptor, O_2, that is converted to H_2O. During electron transfer, there is also the passage of H^+ through complex

I, as well as complex III and IV. This generates a concentration gradient that forces the passage of hydrogen ions through ATP synthetase, leading to the production of ATP by oxidative phosphorylation. So, this complex is essential for the conversion of molecules into energy.

This disorder can result from mutations in several genes. Among these genes are genes coding for components of complex I, genes coding for proteins involved in the assembling of this complex or even genes coding for other functions that also interfere with the activity of this complex. Most of these genes are present in nuclear DNA but others are coded by mitochondrial DNA. The higher the number of mitochondria having the mutation, the more severe disease will develop.

Due to the diversity of possible involved genes, this disorder can have different types of heritage. If the mutated gene is present in the nucleus, the disease can be transmitted with an autosomal recessive or X-linked pattern, depending on the chromosome involved. However, if the mutated gene is in mitochondrial DNA, it will have a maternal inheritance, meaning that the disease is only transmitted by the mother. This happens because spermatozoid cells do not contribute with mitochondria to the developing embryo. All mitochondria are inherited from the oocyte. Mitochondrial disorders can show up in every generation and can affect males and females, being transmitted from one generation to the next by the mother.

Complex I deficiency results in the absence of this protein complex in ETC or in the loss of its function that leads to inability to produce ATP from NADH. Impairment of oxidative phosphorylation can lead to cell death due to the reduced availability of energy in cells. The most affected tissues are the ones that require high energy levels such as cardiac and skeletal muscles, brain and nervous system and kidneys.

Symptoms of this deficiency can appear since birth until adulthood and include several neurological changes such as encephalopathy, seizures, ataxia, dystonia and intellectual disability. Concerning muscular tissues, these patients can have hypotonia, myalgia and intolerance to exercise. Due to the ETC change, patients usually have high plasma levels of lactic acid that can lead to weakness, nausea and vomiting. The developed lactic acidosis can even be life-threatening.

Some patients with this condition develop a group of symptoms that are characteristic of a specific syndrome, like the Leigh syndrome characterized by developmental and psychomotor regression that leads to death 2-3 years after the onset of disease symptoms.

Another disorder that results from the deficiency of mitochondrial complex I is Leber hereditary optic neuropathy, manifested with vision problems due to degeneration of the optic nerve. These syndromes can also result from other causes.

1.2.3. **Clinical Cases**

Clinical case 1.2.1. *Pyruvate dehydrogenase deficiency*

A 3-year-old child shows symptoms of lactic acidosis and slow development of motor capacities, as well as delayed mental development. Analysis on enzymatic activity of the pyruvate dehydrogenase system revealed the presence of 15% of activity when compared with normal values.
a) How can the deficiency in pyruvate dehydrogenase explain these symptoms?
b) A therapy strategy applied in these cases is the administration of high dosage of thiamine, nicotinamide, lipoate and riboflavin. Explain this strategy.

Clinical case 1.2.2. *Fumarase deficiency*

A 1-month-old baby shows delayed development, hypotonia, lactic and pyruvic acidaemia and fumaric aciduria. The baby died at 8 months of age. Mitochondria isolated from skeletal muscle revealed selective defects on glutamate and succinate oxidation, although these compounds were normally oxidised in mitochondria isolated from liver cells. Fumarase activity was absent in mitochondria of both sources. Liver and muscular homogenates also showed very low activity of fumarase, indicating that the cytosolic form of the enzyme was also deficient. Differences in the intramitochondrial accumulation in the various organs led to the selective oxidative defects observed in mitochondria of skeletal muscle and not in liver. How can a deficiency of fumarase explain these symptoms?

Clinical case 1.2.3. *Aconitase deficiency*

Fluoroacetate is a compound used as rat poison. After entering cells, fluoroacetate is converted into fluoroacetyl-CoA in a reaction catalysed by the enzyme acetate thiokinase:

$$F\text{-}CH_2COO^- + CoA\text{-}SH + ATP \rightarrow F\text{-}CH_2(CO)\text{-}S\text{-}CoA + AMP + PP_i$$

The toxic effect of fluoroacetate was studied in experiments on heart metabolism from rats. After injection of a fluoroacetate solution (0.22 mmol/L), glucose absorption and glycolysis decreased; glucose-6-phosphate and fructose-6-phosphate accumulated. Analysis on the Krebs cycle intermediates showed levels below normal, except for citrate, whose levels were 10 times above normal.
a) Where is the blockage of the Krebs cycle? Why is citrate accumulated and all the other intermediates decreased? How can this inhibition be solved?
b) Why is glucose absorption and glycolysis decreased? Why are hexoses-monophosphate accumulated?
c) Why is fluoroacetate a lethal poison?

Clinical case 1.2.4. *α-Ketoglutarate dehydrogenase deficiency*

A newborn shows hypotonia, metabolic acidosis and hyperlactataemia. Neurologic deterioration led to child death at 30 months of age. Low levels of ketone bodies were observed in the plasma of the newborn. How can the deficiency in α-ketoglutarate dehydrogenase explain these symptoms?

Clinical case 1.2.5. *Complex I (NADH dehydrogenase) deficiency*

A newborn shows hypertrophic cardiomyopathy, delay in development and brain atrophy. After infection with varicella, the baby developed severe acidosis, convulsions and coma, and died of heart failure at 11 months of age. The diagnose was Leigh's syndrome. Enzymatic activities of complex I in muscles and fibroblasts were 20 and 36% of reference values, respectively. How can the deficiency in complex I (NADH dehydrogenase) explain these symptoms?

Clinical case 1.2.6. *Complex IV (cytochrome c oxidase) deficiency*

A boy shows delayed growth, hypotonia and episodes of metabolic acidosis. Lactate and pyruvate plasma levels were increased. The child died at age of 4. Biochemical tests revealed kinetic abnormal complex IV (cytochrome c oxidase). How can this result explain observed symptoms?

Clinical case 1.2.7. *Coenzyme Q_{10} deficiency*

A girl shows axial hypotonia, delayed growth during childhood, mental retardation, abnormal walk, muscular atrophy and high levels of lactic acid. She was diagnosed with Leigh's syndrome. Analysis revealed strong decrease in coenzyme Q_{10} (CoQ_{10}) levels in muscles and lymphoblasts. Supplementation of CoQ_{10} resulted in significant clinical improvement. Explain.

Clinical case 1.2.8. *Thiamine deficiency*

Individuals with thiamine deficiency show characteristic neurologic signs: loss of reflexes, anxiety and mental confusion. They can also have increased plasma levels of lactic acid. Suggest a reason why changes in brain function can result from thiamine deficiency.

Clinical case 1.2.9. *Lipoic acid deficiency*

A child that does not increase weight, shows frequent vomiting and metabolic acidosis. Levels of lactate, pyruvate and alanine were high. Treatment with thiamine, biotin and bicarbonate did not relief symptoms. However, administration of lipoic acid had a positive effect on lactic and pyruvic acidaemia.

a) What is the deficiency present in this patient?

b) Explain the therapeutic strategy with thiamine, biotin, bicarbonate and lipoic acid?

Clinical case 1.2.10. *Cyanide intoxication*

A 20-year-old man was hospitalized in a coma state, with suspicion of poisoning with some chemical substance. After clinical examination, it was confirmed potassium cyanide intoxication, a strong inhibitor of mitochondrial respiration.
a) Explain why cyanide has a high toxicity.
b) Explain the treatment strategy used in this type of intoxication.

Clinical case 1.2.11. *Carbon monoxide intoxication*

An anaemic individual having half of haemoglobin content in blood than normal, can show good health. However, an individual exposed to an amount of carbon monoxide able to fill half of available haem groups, becomes disabled. Explain why.

Clinical case 1.2.12. *Dinitrophenol intoxication*

A mitochondrial preparation was incubated in the presence of O_2 and oligomycin. Speed of O_2 consumption was continuously measured that, in these conditions, was almost null. If dinitrophenol is added to the preparation, what would be the effect on O_2 consumption? The use of dinitrophenol results in an increase in metabolic rate and consequent immediate increase in body temperature. Explain why.

Clinical case 1.2.13. *Nigericin and valinomycin intoxication*

Nigericin is an ionophore that allows the exchange of K^+ by H^+ through membranes. Valinomycin is an ionophore that transports K^+ but not H^+. What would be the effect of these transporters in terms of energy balance in oxidative phosphorylation?

1.2.4. **Further questions**

Question 1.2.1.
Metabolism consists of a tangle of many possible metabolic pathways. Glycolysis and the Krebs cycle are two of the main pathways.
a) Where, respectively, do glycolysis and the Krebs cycle take place in a eukaryotic cell?
b) What is the end product of glycolysis and what are the substances that start the Krebs cycle?

Question 1.2.2.
In a eukaryotic cell, where is the enzyme responsible for transforming pyruvate into acetyl-CoA?

Question 1.2.3.
What is the function of the Krebs cycle?

Question 1.2.4.
Indicate the steps in the Krebs cycle from succinate to oxaloacetate. For each step, refer the names of the intermediates and catalysing enzymes.

Question 1.2.5.
Indicate the steps in the Krebs cycle from citrate to succinyl-CoA. For each step, refer to the reactions and catalysing enzymes.

Question 1.2.6.
Write the reaction for the formation of acetyl-CoA from pyruvate and indicate:
a) The 5 necessary coenzymes.
b) The vitamins involved.

Question 1.2.7.
What are the products of the reaction catalysed by pyruvate dehydrogenase? How is the activity of this enzymatic complex regulated?

Question 1.2.8.
Which coenzymes participate in the mechanism of action of the pyruvate dehydrogenase complex? What role do they play?

Question 1.2.9.
Describe the role of Ca^{2+}, acetyl-CoA and NADH in the regulation of pyruvate dehydrogenase and the Krebs cycle.

Question 1.2.10.
Pyruvate dehydrogenase deficiency causes severe muscle pain. Explain why.

Question 1.2.11.
In the oxidation of acetyl-CoA in the Krebs cycle, indicate the enzyme that catalyses the reaction where there is production or consumption of:
a) CO_2.
b) GTP.
c) NADH.
d) $FADH_2$.
e) H_2O.

Question 1.2.12.
What is the production of NADH when one mole of acetyl-CoA is completely oxidized to CO_2 by the Krebs cycle?

Question 1.2.13.
What is the production of NADH when one mole of pyruvate is oxidized to acetyl-CoA?

Question 1.2.14.
How many moles of NADH and $FADH_2$ are produced by the complete oxidation of one mole of acetyl-CoA by the Krebs cycle? How many moles of carbon dioxide are released?

Question 1.2.15.
How many moles of ATP are produced when two moles of acetyl-CoA are converted to four moles of CO_2 through the citric acid cycle?

Question 1.2.16.
Which factor(s) slows down the Krebs cycle?

Question 1.2.17.
Consider the Krebs cycle and its control. Which steps are under control and which modulators are involved?

Question 1.2.18.
One of the regulatory points of the citric acid cycle is α-ketoglutarate dehydrogenase. What are the modulators of the activity of this enzyme?

Question 1.2.19.
Compare the skeletal muscle utilization of pyruvate of a sedentary worker and a runner during the marathon.

Question 1.2.20.
Malonate is a competitive inhibitor of succinate in the reaction catalysed by succinate dehydrogenase. Explain why an increase in oxaloacetate concentration can overcome malonate inhibition.

Question 1.2.21.
A runner needs a tremendous amount of energy during a race. Explain why using ATP for muscle contraction affects the Krebs cycle.

Question 1.2.22.
After several days of fasting, the ability of liver to metabolize acetyl-CoA through the Krebs cycle is dramatically diminished. Explain why.

Question 1.2.23.
Refer to the energy-producing capacity of the citric acid cycle.

Question 1.2.24.
Explain how the Krebs cycle can provide precursors for other metabolic pathways without depleting its own intermediates.

Question 1.2.25.
How many turns of the Krebs cycle does it take to convert the 6 carbons of glucose into CO_2?

Question 1.2.26.
If there is a deficit of oxaloacetate, how is it possible to replace this intermediate so that the citric acid cycle works?

Question 1.2.27.
The citric acid cycle transforms one mole of citrate into one mole of oxaloacetate, which is needed for the cycle to continue. If one of the cycle intermediates is used as a precursor for the biosynthesis of glucose or amino acids, can de novo synthesis of oxaloacetate from acetyl-CoA with citric acid cycle enzymes occur?

Question 1.2.28.
Although animals cannot synthesize glucose from acetyl-CoA, if a mouse is fed radiolabelled acetate, some radioactive label appears on the glycogen extracted from its muscles. Explain why.

Question 1.2.29.
Acetyl-CoA needed for fatty acid synthesis (in the cytosol) is produced in the mitochondria. Appropriately describe the mechanism by which acetyl-CoA crosses the mitochondrial membrane.

Question 1.2.30.
What is the meaning of the statement "The Krebs cycle is an amphibolic cycle"?

Question 1.2.31.
State, with justification, whether the following statements are true or false. The Krebs Cycle…
a) Is particularly active during physical exercise.
b) Is an amphibolic pathway.
c) Leads to complete oxidation of the coenzyme.
d) Occurs in all cells.
e) Is important to produce NAD^+.

Question 1.2.32.
In the first hours after a meal that contains carbohydrates (but not when they are absent or fasting), about 1/3 of the expired CO_2 results directly from the action of which enzyme?

Question 1.2.33.
Develop the following theme: "The Krebs cycle: its role in nutrient oxidation".

Question 1.2.34.
In a eukaryotic cell, where are the proteins responsible for the ETC located?

Question 1.2.35.
What are the main sources of electrons for the ETC?

Question 1.2.36.
Describe the process of electron transfer through the ETC.

Question 1.2.37.
Sketch the sequence of compounds in the ETC, indicating electron carriers and proton carriers.

Question 1.2.38.
In the ETC, what is the final electron acceptor?

Question 1.2.39.
Define oxidative phosphorylation. What is being phosphorylated? What is being oxidized?

Question 1.2.40.
How can the flow of H^+ from the intermembrane space to the mitochondrial matrix produce ATP?

Question 1.2.41.
Summarize the chemiosmotic theory. According to this theory, where does the energy for ATP synthesis come from? What ion is involved?

Question 1.2.42.
During the ETC, protons are transferred from one side of the inner membrane to the other. Which complexes allow the passage of protons, and which do not?

Question 1.2.43.
Explain how the ETC and oxidative phosphorylation are coupled. How can they be decoupled?

Question 1.2.44.
Describe the structure of ATP synthase (or F1F0-ATPase). Describe the role of each of the parts. Where is it located in the mitochondria?

Question 1.2.45.
What are the degradation products of one mole of glucose by glycolysis, followed by the Krebs cycle and oxidative phosphorylation under aerobic conditions?

Question 1.2.46.
In brown adipose tissue (which contains thermogenin), what are the degradation products of one mole of glucose by glycolysis, followed by the Krebs cycle and oxidative phosphorylation under aerobic conditions?

Question 1.2.47.
Why is complex II of the respiratory chain (succinate dehydrogenase) unable to pump protons across the inner mitochondrial membrane?

Question 1.2.48.
The inner membrane of mitochondria is impermeable to ATP and NADH. Explain:
a) How NADH produced in the glycolytic pathway can be oxidized in the respiratory chain.
b) How ATP produced in mitochondria can be used in the cytosol.

Question 1.2.49.
How many moles of CO_2 are produced per each mole of O_2 used in cellular respiration? Explain.

Question 1.2.50.
How many moles of CO_2 are produced per each mole of glucose used in cellular respiration? Explain.

Question 1.2.51.
How many moles of CO_2 are produced per each mole of pyruvate under aerobic conditions? Explain.

Question 1.2.52.
Is ATP hydrolysis a spontaneous or non-spontaneous process? Why? Which family of enzymes use ATP in these reactions?

Question 1.2.53.
Give examples of the importance of the ATP molecule to the cell.

Question 1.2.54.
Indicate the number of ATPs synthesized by oxidation of each NADH and $FADH_2$.

Question 1.2.55.
Develop the following theme: "The role of malate and glycerol-3-phosphate shuttles in aerobic glycolysis".

Question 1.2.56.
What does the shuttle of glycerol-3-phosphate consist of and what is it for?

Question 1.2.57.
How many moles of ATP are produced on the complete conversion of one mole of glucose to CO_2 and H_2O via glycolysis and the Krebs cycle (assume the malate-aspartate shuttle is being used)? Explain.

Question 1.2.58.
How many moles of ATP are produced on the complete conversion of one mole of glucose to CO_2 and H_2O via glycolysis and the Krebs cycle (assume the glycerol-3-phosphate shuttle is being used)? Explain.

Question 1.2.59.
Calculate ATP production, showing all the steps:
a) In the anaerobic oxidation of 1 mole of glucose.
b) In the aerobic oxidation of 1 mole of glucose.

Question 1.2.60.
How many moles of ATP can be formed by the complete oxidation of one mole of glycerol-3-phosphate to CO_2 under aerobic conditions?

Question 1.2.61.
Below are schematized, in simplified form, two biochemical processes:
A: Glucose → Pyruvic acid → Lactic acid
B: Glucose → Pyruvic acid → CO_2 and H_2O
a) What are processes A and B called?
b) Compare the two processes in terms of the energy yield each one provides.

Question 1.2.62.
Ethanol is oxidized in the liver to form acetate, which is converted to acetyl-CoA. Determine how many moles of ATP are produced from 1 mole of ethanol. Note that two moles of NADH are produced in the conversion of ethanol to acetate.

Question 1.2.63.
Explain how an ADP depletion affects NADH consumption and the rate of ATP synthesis in mitochondria.

Question 1.2.64.
What are the advantages and disadvantages of O_2-based metabolism?

Question 1.2.65.
As you are taking the Exam you are consuming oxygen. In which metabolic pathway does most oxygen consumption occur?

Question 1.2.66.
When O_2 is added to a cell suspension under anaerobic conditions and in a glucose-rich medium, the rate of glucose consumption decreases significantly once oxygen begins to be consumed. Additionally lactate accumulation ceases. This effect was first observed by Louis Pasteur in the 1860s and is an effect characteristic of most cells that can oxidize glucose under aerobic and anaerobic conditions.
a) What is the explanation for the cessation of lactate accumulation after the addition of O_2 to the medium?
b) Why does the addition of O_2 to the medium cause a decrease in glucose consumption?

Question 1.2.67.
Cyanide easily binds to the metallic portion of cytochromes. What are the consequences of cyanide poisoning? How does cyanide interfere with cellular respiration?

Question 1.2.68.
A mitochondrion was treated with a substance that causes the transport of H^+ ions across the membrane. What will be the effect of this treatment on breathing?

Question 1.2.69.
The reduction of oxygen to water that occurs in mitochondria can produce ROS that are highly reactive and have high destructive potential. What enzymes and compounds play an extremely important role in protecting cells against these species?

Question 1.2.70.
ATP synthesis by oxidative phosphorylation requires the presence of intact mitochondria. Explain why.

Question 1.2.71.
In porous membranes ATP synthesis does not occur. Explain why.

Question 1.2.72.
Name 3 inhibitors of the electron transport chain, indicating the transport complexes on which they act.

Question 1.2.73.
Define uncoupling agent and cite an example.

Question 1.2.74.
Arsenate is a phosphate-like ion. In the presence of arsenate, ATP production from ADP and free phosphate stops. What is the energy balance of glycolysis in the presence of arsenate?

Question 1.2.75.
Cyanide inhibits the terminal oxidase of the electron transport chain. What is the energy balance of glucose degradation in the presence of cyanide?

Question 1.2.76.
The consumption of dinitrophenol by animals results in an immediate increase in body temperature. Explain how compounds like dinitrophenol increase metabolic rates.

Question 1.2.77.
Check if malate and succinate oxidation is possible in the presence of rotenone.

1.3. **Glycogenolysis and Glycogenesis**

1.3.1. **Short review**

Glycogen stored in cells is the first energy reserve to be used, being a short-term store. It is produced after a meal and degraded in fasting periods. Glycogen stores last for 3-4 hours after a meal. In most cells capable of storing glycogen, this macromolecule is degraded to fulfil energetic requests. In liver cells, this store is used not only for ATP production but also for controlling plasma glucose levels. Whenever glucose plasma levels are low, glycogen is degraded and obtained glucose is transferred to the bloodstream. When glycogen stores are totally used up (during the night) liver will activate gluconeogenesis to control plasma glucose. The maintenance of constant plasma glucose levels is extremely important since some of our cells, such as brain cells and mature erythrocytes, cannot store glycogen depending entirely on blood glucose. Moreover, these cells use almost exclusively glucose as energy source.

Regulation of these two metabolic pathways is done by the action of two pancreatic hormones: insulin and glucagon.

Glycogen synthesis (glycogenesis) occurs after meals when plasma glucose levels are high. In these conditions, insulin is produced by the β-cells of pancreas. This hormone promotes entrance of glucose in cells and stimulates glycogen synthesis, activating the enzyme glycogen synthase, regulatory point of glycogenesis. At the same time, the key enzyme (glycogen phosphorylase) of glycogen degradation (glycogenolysis) is inhibited.

On the other hand, in fasting periods, when blood glucose is low, pancreatic α-cells produce the hormone glucagon. The target cells for this hormone are hepatocytes, activating metabolic pathways that allow the resetting of blood glucose: in the first place, glycogen degradation is activated and, when no more glycogen is available, gluconeogenesis is activated. Glucagon attaches to membrane receptors that interact with G protein. Activation of this protein will lead to the activation of the enzyme adenylcyclase, responsible for the synthesis of the secondary messenger cyclic adenosine monophosphate (cAMP), that will trigger cell response. This response involves the sequential activation of the following enzymes: cAMP dependent protein kinase, that phosphorylates phosphorylase kinase, that will phosphorylate glycogen phosphorylase. This last one, when phosphorylated, is converted in its active state, catalysing glycogen degradation. At the same time, cAMP dependent protein kinase will phosphorylate glycogen synthase, that in this condition, is inactive, preventing glycogen synthesis.

Another hormone that also interferes in glycogen metabolism is epinephrine. This hormone can act on two types of receptors: α-adrenergic and β-adrenergic. They both exist in liver cells making faster the response to this hormone. Independent on which receptor is being used, the cascades end up in the activation of glycogen degradation. The cascade of action on the β-adrenergic receptor is like the glucagon cascade. However, when it concerns the α-adrenergic receptors, the cascade involves the production of another secondary messenger, inositol-triphosphate (IP_3). For that, the attachment of epinephrine to the receptor, activates G protein, that will lead to the activation of phospholipase C. This one is responsible for the conversion of a membrane phospholipid in the secondary messengers, diacylglycerol (DAG) and IP_3. IP_3 acts on the ER membrane, leading to the exit of Ca^{2+} ions to the cytosol. The complex Ca^{2+}-calmodulin will now activate glycogen phosphorylase and inhibit glycogen synthase.

Glycogenolysis

Glycogen degradation involves:

1. The enzyme glycogen phosphorylase breaks down α-1,4 bonds of glycogen, adding P_i group to the released glucose unit. In this way, glucose-1-phosphate is produced. This enzyme is the regulatory point of glycogenolysis.

2. Glucose-1-phosphate must be converted into glucose-6-phosphate, to later be used in glycolysis or, in liver cells, be transformed into glucose and launched in the bloodstream. This position change in the phosphate group is catalysed by phosphoglucomutase.

3. Glycogen degradation is only complete with the breakdown of α-1,6 bonds present in branching points. For that, the debranching enzyme acts in two steps: in the first place, acts as an α-D-glucotransferase, transferring glucose units close to the glucose of the α-1,6 bond to a linear terminal; in the second place the enzyme will act as a glucosidase, cutting the α-1,6 bond, and releasing glucose.

Glycogen is totally degraded by the alternate action of the described enzymes.

Glycogenesis

In glycogen synthesis, the substrate use is the molecule of UDP-glucose produced from plasma glucose. Glucose enters cells according with its gradient, being immediately converted in glucose-6-phosphate. Phosphoglucomutase converts this last molecule into glucose-1-phosphate that will now be used to form UDP-glucose. These are the enzymes involved in glycogen synthesis:

1. Glucose-1-phosphate uridyltransferase converts glucose-1-phosphate into UDP-glucose, using uridine triphosphate (UTP). This reaction releases pyrophosphate (PP_i).

2. The enzyme glycogen synthase adds 1 unit of glucose to the glycogen molecule, using UDP-glucose as a substrate and establishing a α-1,4 bond. This enzyme is the regulatory point of glycogen synthesis.

3. To form branching points in the glycogen molecule, glucose units are added by the action of glycogen synthase, until a linear chain of around 12 units in length is obtained. Then, the branching enzyme transfers an oligosaccharide chain from the end of the growing chain to form the α-1,6 bond.

To act, glycogen synthase needs a starting molecule, a pre-existing glycogen molecule. However, in certain conditions (during the night) all stored glycogen was spent and it is necessary to perform *de novo* synthesis. The protein glycogenin acts as a primer molecule in the *de novo* glycogen synthesis. This is a self-glycosylating protein, able to add the first glucose unit to itself. Then, glycogen synthase is already able to act.

1.3.2. **Pathologies**

Liver glycogen phosphorylase deficiency

Glycogen phosphorylase is involved in glycogenolysis. This enzyme is able to cut α-1,4 bonds between glucose units of the macromolecule glycogen, releasing glucose-1-phosphate. Hepatic glycogen is used to control blood glucose levels, being synthetized when glucose blood levels are high and degraded when these levels decrease.

This enzyme is coded by the *PYGL* gene and mutations in this gene lead to glycogen storage disease VI (GSDVI). These mutations lead to deficiency or total absence of glycogen phosphorylase preventing liver cells from breaking down stored glycogen. This results in hypoglycaemia and in the increased use of lipids as energy source that can lead to ketoacidosis. Liver cells can still store glycogen that will not be degraded, becoming enlarged (hepatomegaly) and dysfunctional.

This disorder has an autosomal recessive pattern of transmission, and its prevalence is still not known. This condition can be underdiagnosed since some children may only have mild symptoms and some adults might show no symptoms at all.

Muscle glycogen phosphorylase deficiency (McArdle's disease)

McArdle's disease results from deficiency of muscular glycogen phosphorylase, leading to the inability to degrade glycogen and use the resultant glucose units for ATP production.

This condition results from mutations in the *PYGM* gene that codes for myophosphorylase, preventing muscular glycogen degradation. Consequently, muscles do not produce enough ATP, leading to intolerance to exercise.

This pathology is rare, transmitted as an autosomal recessive disorder and its prevalence is not known.

Symptoms associated with this deficiency include muscle pain, cramps and, in half of the patients, breakdown of muscular tissue can occur, evidenced by the release of myoglobin in urine. Myoglobin makes urine red or brown and can damage the kidneys. Half of the patients having myoglobinuria will probably face kidney failure that can cause death.

Debranching enzyme deficiency (Glycogenosis type III)

The debranching enzyme is responsible for cutting α-1,6 bonds present in the branching points of glycogen.

This enzyme is coded by the *AGL* gene and mutations in this gene lead to glycogen storage disease III (GSLIII). These mutations can lead to the production of a non-functional debranching enzyme leading to the development of GSDIIIa and GSDIIIb, or to an enzyme with reduced activity (GSDIIIc and GCDIIId). These four types of GSDIII can be distinguished by the pattern of symptoms. GSDIIIa is the most common form of this disorder, corresponding to 85% of all cases. GSDIIb is present in 15% of diagnosed cases. GSDIIIc and GSDIIId are very rare and have been identified in only a very small number of patients.

In all types of this enzyme deficiency, stored glycogen is partially broken down and its accumulation in cells can damage organs and tissues such as liver and muscles.

This disorder has an autosomal recessive transmission and has an incidence of 1 in 100,000 individuals in the United States.

Among the evidenced symptoms are hypoglycaemia, hyperlipidaemia and high levels of liver enzymes in blood that will appear in infancy. These children will develop hepatomegaly that can lead to cirrhosis and liver failure. Affected children usually have slow growth due to the liver problems that will result in short stature. In cases of GSDIIIa, patients may develop heart and skeletal muscle weakness, evidenced by cardiomyopathy and hypotonia.

Branching enzyme deficiency (Severe neonatal glycogenosis type IV)

The branching enzyme is responsible for the formation of α-1,6 bonds during glycogen synthesis. This enzyme is essential for the storage of glycogen in tissues. These stores will be used in fasting periods to produce ATP in muscular cells and to control blood glucose levels in liver.

Mutations in the gene coding for this enzyme, the *GBE1* gene, lead to the development of GSDIV characterized by the inability to produce normal glycogen molecules and to the accumulation of abnormal glycogen (very long unbranched chains) in cells, especially in the heart and muscles. Glycogen with the abnormal structure has low solubility and precipitates in liver.

The severity of this condition varies with the amount of enzyme being produced. In this way, one type of this condition is the fatal perinatal neuromuscular type where there is the excessive accumulation of fluid around the foetus and in the foetus body that results in decreased foetal movement, stiffness of joints after birth and low muscle tone. These children do not survive after birth due to weak heart and lungs.

Another form of this condition is the congenital muscular type that develops during infancy with cardiomyopathy. These children survive only for a few months after birth.

Other possible forms of this disorder are progressive hepatic type, non-progressive hepatic type and childhood neuromuscular type. In the progressive type, children develop hepatomegaly and cirrhosis and die from liver failure in early childhood. The non-progressive hepatic type is like the previous one, but liver involvement is not so severe. These children have muscle weakness and hypotonia and survive until they reach the adult state. Finally, the childhood neuromuscular type develops in late childhood and symptoms vary greatly among affected children.

It is transmitted with an autosomal recessive pattern and affects 1 in 800,000 individuals worldwide. Cases of GSDIV correspond to 3% of all glycogen storage diseases.

1.3.3. **Clinical Cases**

Clinical case 1.3.1 *Liver glycogen phosphorylase deficiency*

In the glycogen metabolism disease where liver phosphorylase is deficient hypoglycaemia is less severe than in the disease where glucose-6-phosphatase is deficient. Explain this fact.

Clinical case 1.3.2. *Muscle glycogen phosphorylase deficiency (McArdle's disease)*

A 65-year-old man shows weakness, mainly in arm muscles. He also shows difficulty in getting up from the chair and to raise his arms above the head, involuntary contractions and loss of muscular mass. Physical examination revealed atrophy and decreased strength in extremities of upper muscles (deltoid, biceps and triceps) and minimal bilateral weakness of the gluteus. Biopsy of quadricep muscles showed increased glycogen present in several fibres, absence of glycogen phosphorylase activity, decrease in oxidative enzymes and minimal neurogenic atrophy.
a) What is the reason for exercise intolerance?
b) What is the most likely enzymatic defect present in this patient?

Clinical case 1.3.3. *Debranching enzyme deficiency (Glycogenosis type III)*

A patient showed hypoglycaemia but less severe than the one observed in Von Gierke's disease, fasting tolerance and hepatomegaly. Other frequent associated signs are hypotonia and hypertrophic cardiomyopathy. Biochemical analysis included hypoglycaemia without acidosis, hypertriglyceridemia and hypertransaminasemia during childhood. This patient responded to glucagon.
a) What is the most likely enzymatic defect present in this patient?
b) Suggest the treatment for this patient.

Clinical case 1.3.4. *Debranching enzyme deficiency*

Disease in glycogen metabolism is caused by genetic defects on one or more enzymes involved in synthesis or degradation of glycogen. Patients with Cori's disease, caused by deficiency in the debranching enzyme, have swollen livers (hepatomegaly) and low plasma levels of glucose (hypoglycaemia). Suggest a possible cause for these symptoms.

Clinical case 1.3.5. *Branching enzyme deficiency*

A 62-year-old man showed progressive instability, proximal weakness, sensorial loss far from the origin point and slight cognitive loss during the last 6 years. Biochemical and genetic analyses revealed low levels of glycogen debranching enzyme. Explain these symptoms.

Clinical case 1.3.6. *Branching enzyme deficiency (Severe neonatal glycogenosis type IV)*

> Two newborns died with 4 and 10 weeks having clinical signs of a systemic storage disease. Prenatal history included reduced foetal movements. Caesarean was done at 36 weeks of gestation due to foetal risk. At birth, babies did not show severe hypotonia, they had hyporeflexia and did not show any activity of spontaneous respiration. Babies never showed active movements of suction and swallowing and were respirator-dependent till death. A muscle biopsy performed to both patients suggested that the stored material was abnormal glycogen.
> Activity of the branching enzyme in fibroblasts was low, confirming the diagnosis of glycogenosis type IV. Explain.

1.3.4. **Further questions**

Question 1.3.1.
What is obtained in the degradation of glycogen by the joint action of glycogen phosphorylase and debranching enzyme?

Question 1.3.2.
What is obtained in the degradation of glycogen by the joint action of glycogen phosphorylase, debranching enzyme and phosphoglucomutase?

Question 1.3.3.
A liver glycogen sample is incubated with Pi, glycogen phosphorylase and debranching enzyme. Which one is obtained, glucose-1-phosphate or glucose? Explain why.

Question 1.3.4.
A glycogen sample from a patient with liver disease was incubated with Pi, glycogen phosphorylase and debranching enzyme. The amount of glucose-1-phosphate produced was 100 times higher than glucose. What is the most likely enzyme deficiency in this patient? What is the likely structure of your glycogen?

Question 1.3.5.
The secretion of the hormone adrenaline activates glycogen metabolism, but its action is only temporary. What enzymes are needed to activate glycogen phosphorylase after adrenaline secretion? What enzymes are needed to reconvert phosphorylase a to phosphorylase b after adrenaline has disappeared?

Question 1.3.6.
State what consequences are expected from the following mutations:
a) Muscle phosphorylase loses cAMP binding capacity.
b) Mutation of Ser14 to Ala14 in liver phosphorylase.
c) Phosphorylase kinase from the liver has a higher-than-normal expression.
d) Loss of the protein phosphatase 1 subunit gene responsible for binding to glycogen.
e) Loss of the gene coding for glycogenin.

Question 1.3.7.

The V_{max} of the skeletal muscle glycogen phosphorylase enzyme is much higher than the V_{max} of the liver isoenzyme.

a) What is the physiological function of glycogen in muscles. And in the liver?

b) Why does the V_{max} of the muscle enzyme need to be higher than that of the liver?

Question 1.3.8.

During a fight-or-flight situation, the release of epinephrine promotes the breakdown of glycogen in the liver, heart and skeletal muscle. The end product of the degradation in the liver is glucose. On the other hand, the end product in skeletal muscle is pyruvate.

a) Why are different glycogen breakdown products observed in the two tissues?

b) What is the advantage for the organism, during the fight-or-flight situation, of having these specific glycogen degradation pathways?

Question 1.3.9.

Explain how *de novo* synthesis of glycogen occurs.

Question 1.3.10.

What substrate is used by the enzyme glycogen synthase in glycogen synthesis? How is this substrate produced?

Question 1.3.11.

What is the advantage of storing glucose in the form of glycogen instead of free glucose?

Question 1.3.12.

What is the energy balance of adding one mole of glucose to a chain of glycogen?

1.4. **Uronic acids and Proteoglycans**

1.4.1. **Short review**

Synthesis of glucuronic acid

Glucuronic acid is a uronic acid. Production of UDP-glucuronic acid occurs through the oxidation of UDP-glucose catalysed by UDP-glucose dehydrogenase (Fig. 1.4.1).

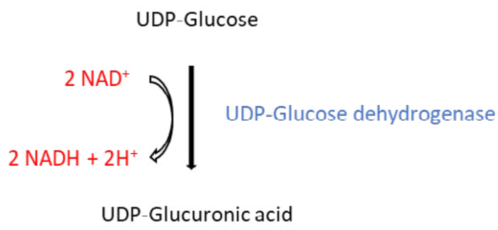

Fig. 1.4.1. Synthesis of UDP-glucuronic acid.

The biological significance of glucuronic acid extends to its ability to be conjugated with certain endogenous and exogenous substances, forming glucuronides. Conjugation produces a strongly acidic compound that is more water soluble at physiological pH than its precursor and therefore may alter the metabolism, transport, or excretion properties. Glucuronide formation is important in drug detoxification, steroid excretion, and bilirubin metabolism.

Oxidation pathway of glucuronic acid

The oxidation pathway of glucuronic acid (Fig. 1.4.2) represents another way of glucose oxidation. This pathway occurs in adipose tissue and involves several steps:

1. D-Glucuronic acid is metabolized to L-gulonic acid with NADPH reduction, catalysed by L-gulonic dehydrogenase.

2. L-Gulonic acid is oxidised to 3-ketogulonic acid with NAD^+, by β-L-hydroxy acid dehydrogenase.

3. 3-Ketogulonic acid is decarboxylated to L-xylulose by β-keto-L-gulonate decarboxylase.

4. L-Xylulose, a ketopentose excreted in pentosuria, is reduced to xylitol with NADPH, by L-xylulose reductase.

5. Xylitol is re-oxidised to D-xylulose with NAD^+, by xylitol dehydrogenase.

6. D-Xylulose is phosphorylated with ATP to xylulose-5-phosphate by D-xylulose kinase. Xylulose-5-phosphate can enter the pentoses-phosphate pathway.

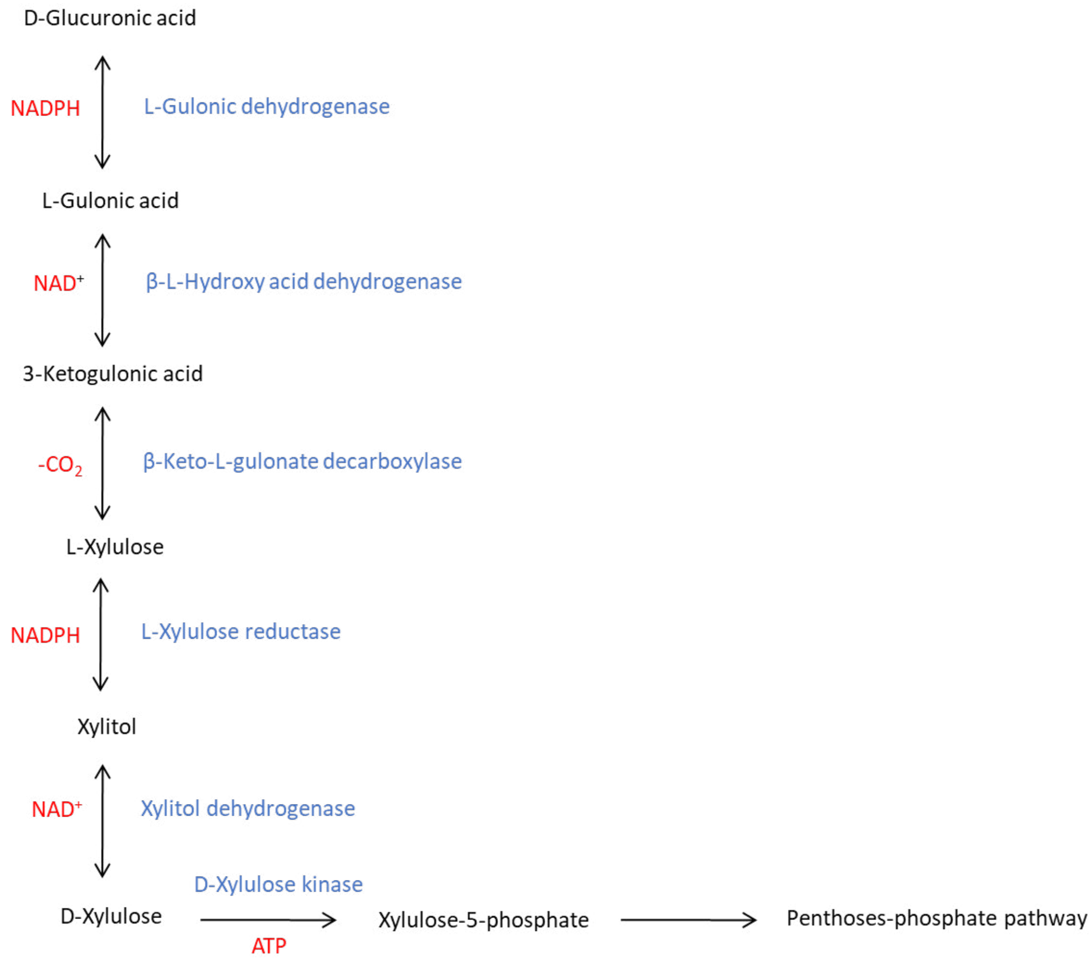

Fig. 1.4.2. Oxidation pathway of glucuronic acid.

Proteoglycans

Proteoglycans are poly-anionic molecules of high molecular mass that contain 95% or more of carbohydrates. Their heteropolysaccharide chains are glycosaminoglycans (GAGs), that are covalently attached to one protein. Most of these molecules are extracellular, and the body uses them to build bone, cartilage, tendons, corneas, skin and connective tissue. GAGs are also found in the fluids that lubricate joints.

Long chains of GAGs are repeated units of disaccharides, where one sugar is a hexosamine (N-acetylglucosamine, N-acetylgalactosamine or glucosamine) and the other is a uronic acid (D-glucuronic acid or L-iduronic acid). Other common constituents of GAGs are sulphate groups. The sulphate donor is 3'-phosphoadenosine-5'-phosphosulphate (PAPS) which is formed from ATP and sulphate in 2 steps.

Decarboxylation, oxidation-reduction, and transamidation of sugars may occur. An example of decarboxylation is the conversion of UDP-glucuronic acid to UDP-xylose (nucleotide sugar). Oxidation-reduction leads to the formation of deoxyhexoses and dideoxyhexoses. Transamidation leads to the formation of amino sugars, constituents of oligo- and polysaccharides and antibiotics.

Sialic acids, such as N-acetylneuraminic acid, belong to the family of sugars C9 and are derived from UDP-N-acetylglucosamine. Sialic acids are distributed in tissues, mostly in glycoproteins, glycolipids and proteoglycans.

Most important GAGs are hyaluronic acid, chondroitin-4-sulphate, heparan sulphate or heparin, keratan sulphate, and dermatan sulphate. An ancient name, mucopolysaccharides, is still in use, especially concerning a group of human genetic disorders, mucopolysaccharidoses.

1.4.2. **Pathologies**

Pentosuria

Pentosuria, an inborn metabolic disease inherited as an autosomal recessive, results from deficiency of NADP-linked L-xylulose reductase. This enzyme catalyses the reduction of L-xylulose to xylitol in the oxidation pathway of glucuronic acid (see Fig. 1.4.2) that is an alternate pathway of glucose-6-phosphate oxidation, which accounts for up to 5% of the total glucose catabolism in humans.

A person with clinically benign pentosuria excretes large amounts of pentose (2–4 g of L-xylulose daily) in the urine especially following intake of glucuronic acid. L-Xylulose is a reducing sugar, so it may give false diagnosis of diabetes. However, glucose metabolism is normal in people with pentosuria, and they are not diabetic. It is a harmless defect, and no cure is needed.

L-Xylulose reductase is a member of the short-chain dehydrogenase/reductase and aldo-ketoreductase super families. This enzyme is highly expressed in liver and kidneys of mammals, especially in epithelial cells of the proximal renal tubule.

L-Xylulose reductase also reduces highly reactive dicarbonyl compounds, thus performing a dual role in carbohydrate metabolism and detoxification. Dicarbonyl compounds are routinely generated during various normal metabolic reactions and tend to convert into advanced glycation end products that are frequently accumulated in the plasma proteins and tissues. A low L-xylulose reductase activity is implicated in age-related diseases including cancers, diabetes, and human male infertility.

Considering the multi-functional enzymatic activity of L-xylulose reductase, the healthy-in-overall persons with pentosuria may be differentially affected under some disease conditions such as diabetic nephropathy, cancers, and cataract.

Mucopolysaccharidoses

Mucopolysaccharidoses (MPS) are a group of human genetic disorders characterised by tissue accumulation and urine excretion of the GAGs of proteoglycans. This accumulation eventually causes progressive damage to cells, tissues, and various organ systems of the body.

MPS are classified as lysosomal storage diseases, a group of more than 40 genetic disorders that result from malfunctioning of the cells lysosomes. These disorders result from a deficiency of one or more lysosomal hydrolases responsible for the degradation of GAGs. Individuals with MPS either do not produce enough of one of the eleven enzymes required to breakdown these sugar chains into simpler molecules, or they produce enzymes that do not work properly. The enzyme deficiencies in the different types of MPS that have been identified are presented in Table 1.4.1.

Table 1.4.1. Enzyme deficiency in different types of mucopolysaccharidoses.

Syndrome	Enzyme deficiency	Accumulated GAGs
Hurler-Scheie (MPS type I)	α-L-Iduronidase	Heparan sulphate Dermatan sulphate
Hunter (MPS type II)	Iduronate-2-sulphatase	Heparan sulphate Dermatan sulphate
Sanfilippo (MPS type III) A B C D E	Heparan N-sulphamidase N-Acetyl-α-D-glucosaminidase Heparan acetyl-CoA: α-glucosaminide N-acetyltransferase N-Acetylglucosamine-6-sulphatase N-Glucosamine-3-O-sulphatase	Heparan sulphate
Morquio (MPS type IV) A B	Galactosamine-6-sulphatase β-Galactosidase	Keratan sulphate
Maroteaux-Lamy (MPS type VI)	N-Acetylgalactosamine-4-sulphatase (arylsulphataseB)	Dermatan sulphate
Sly (MPS type VII)	β-Glucuronidase	Heparan sulphate Dermatan sulphate
Natowicz (MPS type IX)	Hyaluronidase 1	Hyaluronic acid

These disorders are associated with a progressive accumulation of different types of GAGs in the lysosomes of various organs compromising their function. Overtime, these GAGs accumulate in the cells, blood and connective tissues. The result is permanent, progressive cellular damage which affects appearance, physical abilities, organ and system functioning, and, in most cases, mental development.

The clinical features of MPS differ depending on the specific enzyme deficiency, but major clinical features are mainly facial dysmorphism, hepatosplenomegaly, cardiac, respiratory, and skeletal involvement, and neurological, haematological, and ocular symptoms.

These disorders, with one exception (Hunter syndrome that exhibits X-linked recessive inheritance), are inherited in an autosomal recessive manner. Although the biochemical basis for this group of disorders is similar, their mode of inheritance as well as clinical manifestations may vary. For instance, both Hurler and Hunter syndromes are characterised by skeletal abnormalities and mental disability, which in severe cases may result in early death. In contrast, in Sanfilippo syndrome the physical defects are relatively mild, while the mental disability is severe.

Diagnosis can often be made through clinical examination and urine tests (excess GAGs are excreted in the urine). Enzyme assays (testing a variety of cells or body fluids in culture for enzyme deficiency) are also used to provide definitive diagnosis of one of the MPS. These disorders are amenable to prenatal diagnosis, using amniocentesis and chorionic villus sampling, since the pattern of metabolism of affected cells obtained from amniotic fluid is strikingly different from normal. Collectively, the incidence for all MPS is 1 per 30,000 births.

There is no cure for these disorders. Recently, many MPS have been recognized as diseases that could greatly benefit from an early diagnosis because the availability of treatments produces a better clinical outcome when started early in life. Usually, the treatment for MPS consists of prenatal identification and enzyme replacement therapy.

1.4.3. **Clinical cases**

Clinical case 1.4.1. *Deficiency of L-xylulose reductase (Pentosuria)*

Fasting urine of a 23-year-old male presented a positive result for the Benedict test and negative for a glucose-specific enzyme method. Urine sugar was identified as L-xylulose by chromatography and determined by an enzymatic method. A tolerance test with 50 g of glucose showed a normal glucose disappearance rate. The levels of L-xylulose in urine before and after oral administration of 10 g of D-glucuronolactone were 5.40 mmol/L (fasting) and 15.19 mmol/L (90 minutes after administration). Plasma levels of GOT, GPT, LDH, CK, urea, creatinine, uric acid, albumin and globulin were within the reference ranges. The activity of L-xylulose reductase in erythrocytes was measured fluorometrically, resulting in lower values than normal. Justify the results obtained within a clinical picture of pentosuria.

Clinical case 1.4.2. *Deficiency of α-L-iduronidase (MPS type I, Hurler-Scheie Syndrome)*

In a male newborn, MPS type I (Hurler-Scheie syndrome) was diagnosed by an enzymatic method. Up to 5 months, the patient showed no clinical findings beyond a high level of GAGs in the urine (651.7 g/mg creatinine, being 30-200 g/mg the reference value for his age), with an electrophoretic analysis revealing predominance of heparan sulphate and dermatan sulphate. Intravenous infusions of laronidase at a standard dose of 100 IU/kg body weight, were started at 5 months, and maintained regularly for 5 years, without significant adverse effects. Urinary GAGs excretion decreased to normal levels after 4 months of therapy, with complete disappearance of the heparan sulphate and dermatan sulphate bands on electrophoresis.
After 5 years of treatment with laronidase the patient had a normal height for his age, developed no coarse facial features, joint disease, or increased organ size, and showed no evidence of heart disease or abnormalities in the skeleton. The only sign of the presence of an MPS type I was a slightly hazy cornea at 12 months of age, which remained stable and did not compromise vision. Explain the clinical findings before and after treatment.

Clinical case 1.4.3. *Deficiency of iduronate-2-sulphatase (MPS type II, Hunter Syndrome)*

At 18 months of age, a child had macrocrania, macroglossia and small and poorly implemented teeth. He also presented short stature, hepatomegaly and skeletal disease, and progressively developed mental deficiency and cardio-respiratory disease. At age 3, a MPS type II (Hunter syndrome) was diagnosed when submitted to a surgery on an inguinal hernia. The diagnosis of MPS type II was established by an increase of heparan sulphate and dermatan sulphate in the urine and by a very low iduronate-2-sulphatase activity in leukocytes. Justify the established diagnosis.

1.4.4. **Further questions**

Question 1.4.1.

Of the following statements about glucuronic acid, state which ones are true, and which are false. Justify.

a) It increases the water solubility of compounds with which it is conjugated.
b) As a UDP derivative, it may be decarboxylated to a component used in the synthesis of proteoglycans.
c) It is a precursor of ascorbic acid in humans.
d) It can be converted to xylulose-5-phosphate and enter the pentose phosphate pathway.

Question 1.4.2.

Tell the purpose of the oxidation pathway of glucuronic acid, indicating the main reaction involved.

Question 1.4.3.

The active form of glucuronic acid in human metabolism is itself glucuronic acid, glucuronic acid-phosphate, UDP-glucuronic acid or glucuronic acid-CoA?

Question 1.4.4.

From the following statements about the conversion of fructose-6-phosphate to glucosamine-6-phosphate, please state which are true and which are false. Justify.

a) It is a transamination reaction with glutamate as a nitrogen donor.
b) It is stimulated by UDP-N-acetylglucosamine.
c) Requires that the fructose-6-phosphate is first bound to a nucleotide.
d) It is the first step in the formation of N-acetylated amino sugars.
e) It occurs only in the liver.

Question 1.4.5.

Which of the following interconversions of monosaccharides (or derivatives) require a sugar intermediate linked to a nucleotide? Justify.

a) Galactose-1-phosphate to glucose-1-phosphate.
b) Glucose-6-phosphate to mannose-6-phosphate.
c) Glucose to glucuronic acid.
d) Glucuronic acid to xylose.
e) Glucosamine-6-phosphate to N-acetylneuraminic acid (a sialic acid).

Question 1.4.6.

The carbohydrate moiety is attached to the dolichol phosphate prior to transfer to the protein for L-linked glycoproteins, O-linked glycoproteins, proteoglycans or GAGs?

Question 1.4.7.

Which of the following statements about fucose and sialic acid are true, and which are false? Justify.

a) They are most frequently found in N-linked glycoproteins.
b) They are those parts of the carbohydrate chains that are covalently bound to the protein.
c) They can be found in the structure of certain O-linked glycoproteins.
d) They are transferred to the carbohydrate chain when they are bound to the dolichol phosphate.
e) They are the repeating units of proteoglycans.

Question 1.4.8.

Of the following glycosaminoglycans, it is said that it is predominantly intracellular, the most abundant in the body and the only one not covalently bound to the protein:

a) Chondroitin sulphate.
b) Dermatan sulphate.
c) Heparin.
d) Hyaluronate.
e) Keratan sulphate.

Question 1.4.9.

Please state which of the following statements are true or false for proteoglycans. Justify.

a) Their specificity is determined, in part, by the action of glycosyltransferases.
b) Their synthesis is regulated, in part, by the inhibition of UDP-xylose in the conversion of UDP-glucose into UDP-glucuronic acid.
c) Their synthesis involves sulphating of carbohydrate residues by PAPS.
d) Their degradation is catalysed in the cytosol by non-specific glucosidases.

Question 1.4.10.

Develop the following theme: “Glycoproteins: their importance in health and disease”.

2. LIPID METABOLISM

CHAPTER CONTENTS

2.1. **β-Oxidation, Fatty acids synthesis and Ketone bodies**

2.1.1. **Short review**

β-Oxidation of fatty acids

In blood stream, short and medium chain fatty acids are transported associated with albumin. Long and very long chain fatty acids, since they are hydrophobic, must be incorporated in plasma lipoproteins to be able to reach cells, where they will be stored in the form of triacylglycerols or used as energy source.

Fatty acid degradation occurs in mitochondrial matrix in hepatic, adipose and muscular cells, in fasting periods, when glycogen stores have been depleted. This degradation is activated by the hormone glucagon in liver or by thyroid hormones or cortisol in other cells.

Once inside cells, fatty acids must enter the mitochondria, where their degradation will take place. For that, these molecules must cross two mitochondrial membranes, being required their association with different transporters. In cytosol, the fatty acid is attached to the first transporter, the coenzyme A, by the action of the enzyme acyl-CoA synthetase, present in the outer mitochondrial membrane. In this reaction, there is consumption of energy equivalent to 2 ATPs. Once linked to CoA, the fatty acid is able to cross the outer mitochondrial membrane. In the intermembrane space, the change in transporter will take place. The fatty acid is transferred from CoA to carnitine, by the action of carnitine acyltransferase I (enzyme present in the outer mitochondrial membrane that is a regulatory point), producing acyl-carnitine. This last molecule crosses the inner mitochondrial membrane. Once in the mitochondrial matrix, the fatty acid is again transferred to CoA, by carnitine acyltransferase II (enzyme present in the inner mitochondrial membrane). Now, in the form of acyl-CoA, the fatty acid is ready to be degraded.

Fatty acid degradation occurs in β-oxidation cycles (Fig. 2.1.1). In each cycle 2 carbons of the fatty acid are removed, and the number of cycles required for full degradation of the fatty acid depends on the length of the carbonated chain of the fatty acid. The β-oxidation cycle involves:

1. One oxidation catalysed by the enzyme acyl-CoA dehydrogenase, that leads to the formation of a double bond between the second and the third carbon of the fatty acid, producing trans-Δ^2-enoyl-CoA. In this reaction, $FADH_2$ is produced.

2. One hydration catalysed by enoyl-CoA hydratase, in which a H_2O molecule is added to the double bond, producing 3-L-hidroxyacyl-CoA.

4. One thiolysis catalysed by ketoacyl-CoA thiolase, using CoA as a coenzyme. This reaction involves the break of the bond between the second and third carbon of the fatty acid, releasing acetyl-CoA that can be used in the Krebs cycle. The fatty acid has now (n-2) carbons.

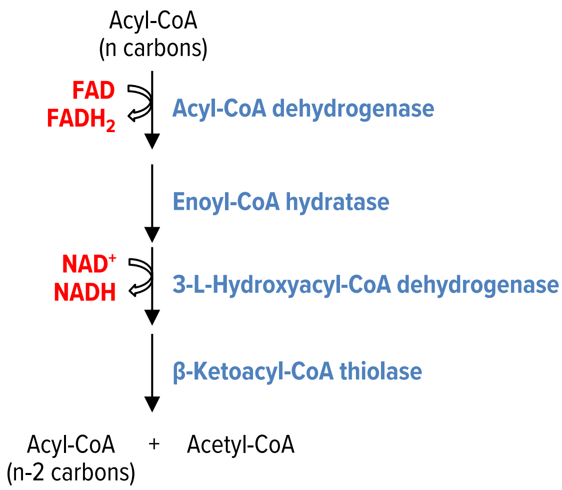

Fig. 2.1.1. Degradation of fatty acids by β-oxidation.

If the fatty acid has an even number of carbons, acetyl-CoA is the only degradation product, besides $FADH_2$ and NADH produced during the β-oxidation cycles. Degradation of fatty acids with odd number of carbons, besides the production of acetyl-CoA, there is the synthesis of propionyl-CoA in the last β-oxidation cycle. This one can be used in the Krebs cycle in the following way:

1. Propionyl-CoA is converted into methylmalonyl-CoA by propionyl-CoA carboxylase. As all carboxylation reactions, this one requires biotin as a cofactor.

2. Methylmalonyl-CoA is going to suffer the action of the enzyme methylmalonyl-CoA mutase (that uses cobalamin (vitamin B_{12}) as a cofactor), producing succinyl-CoA, that is a Krebs cycle intermediate.

Degradation of unsaturated fatty acids rises additional problems. Due to the presence of double bonds and their specific location, additional steps catalysed by enzymes are required for total degradation of these fatty acids, that include isomerizations and reductions. For this reason, the energy produced in the oxidation of an unsaturated fatty acid is always lower than the one produced from a saturated fatty acid with the same number of carbon atoms.

Fatty acid synthesis

After a carbohydrate meal, and stimulated by insulin secretion, fatty acid synthesis can occur, in the cytosol of hepatic and adipose cells. The substrate used is acetyl-CoA, that is produced in the mitochondrial matrix. Therefore, the first obstacle to overcome is the passage of acetyl-CoA to the cytosol since mitochondrial membranes are impermeable to the passage of this molecule. By the action of the enzyme citrate synthase, acetyl-CoA reacts with OAA, giving rise to citrate. This one crosses the mitochondrial membranes to the cytosol and by ATP-citrate lyase, the reverse reaction occurs, leading to acetyl-CoA and OAA, with consumption of 1 ATP. The shuttle is only finished with the return of OAA to the mitochondrial matrix. This is done by its conversion into malate by the enzyme malate dehydrogenase with consumption of 1 NADH and then malate into pyruvate by the malic enzyme, yielding 1 NADPH and releasing CO_2. Pyruvate enters the mitochondria and can again be converted in acetyl-CoA by pyruvate dehydrogenase.

Before starting the synthesis of the fatty acid, malonyl-CoA needs to be produced, to be used in each cycle of synthesis. Malonyl-CoA is produced by carboxylation of acetyl-CoA by acetyl-CoA carboxylase, consuming 1 ATP. Being a carboxylase, this enzyme requires biotin as a coenzyme. This reaction is the regulatory point in fatty acid synthesis, being activated by citrate and inhibited by long chain fatty acids. This enzyme is also stimulated by insulin.

Fatty acid synthesis (Fig. 2.1.2) occurs in cycles of addition of carbon atoms, and in each cycle, 2 carbons are added to the fatty acid. Each cycle involves:

1. Condensation of one acetyl unit or the product of the previous cycle with one malonyl unit. CO_2 is released in this reaction.

2. One reduction that uses NADPH as coenzyme.

3. One dehydration.

4. Another reduction that uses NADPH as coenzyme.

The end product of each cycle is the substrate of the following cycle, and the fatty acid grows 2 carbons in each cycle. The four reactions of each cycle are catalysed by a multimeric enzyme called fatty acid synthase. Palmitic acid (16:0) is the only fatty acid produced by this cytosolic enzyme.

Synthesis of fatty acids with more than 16 carbon atoms occurs in the mitochondrial matrix or smooth endoplasmic reticulum (SER), using palmitic acid as initial substrate. In the SER, synthesis occurs exactly in the same way as in cytosol, using malonyl-CoA in each cycle and NADPH as coenzyme in the reduction reactions. In the mitochondria, the process is slightly different: acetyl-CoA is used in each cycle and the first reduction uses NADH as coenzyme.

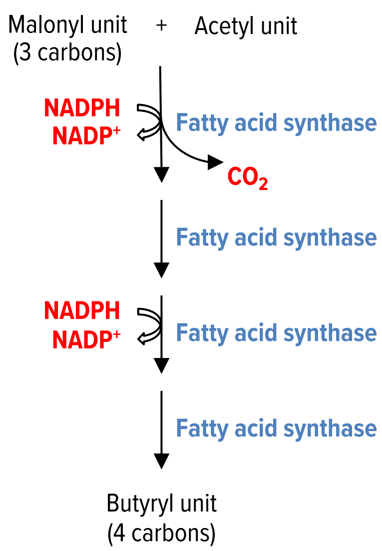

Fig. 2.1.2. First cycle in the synthesis of fatty acids. The butyryl unit is then used in the following cycle to react with another acetyl unit.

Synthesized fatty acids can suffer unsaturation by an electron transport system in the SER membrane that culminates in a specific desaturase. Humans only have desaturases for carbons 4, 5, 6 and 9, being these the only positions where double bonds can be added in fatty acids. For this reason, two fatty acids are considered essential, since they cannot be produced in the human being: ω_6 or linoleic acid ($18{:}2^{\Delta 9,12}$) and ω_3 or linolenic acid ($18{:}3^{\Delta 9,12,15}$). Several other unsaturated fatty acids can be produced in our organism from these two, by alternate addition of carbons and double bonds. One of these is arachidonic acid, precursor used in eicosanoid synthesis.

An interesting case occurs in cells of the mammary glands, during lactation period. These cells produce fatty acids with 8-12 carbons, due to the presence of an enzyme, thioesterase II, that interrupts the action of fatty acid synthase, when fatty acids reach that length.

Ketone bodies

Ketone bodies are produced from fatty acids and usually occur in long fasting periods. Synthesis of ketones is strongly regulated by insulin. This hormone is a potent inhibitor of lipid catabolism e consequent use of fatty acids.

Ketone bodies are produced in long starvation periods when glycogen stores have been depleted. Under these circumstances, lipidic stores will star to be degraded. Fatty acids are used in β-oxidation, yielding acetyl-CoA that can be used in the Krebs cycle. However, without glycogen reserves, there is no available glucose to produce pyruvate and this one be converted in OAA. If there is no way of producing OAA, the Krebs cycle cannot function.

To ensure that at least ATPs coming from $FADH_2$ and NADHs will still be produced in β-oxidation cycles, obtained acetyl-CoA molecules are converted in ketone bodies. Synthesis of these molecules follows the reactions:

1. Attachment of two acetyl-CoA molecules by acetoacetyl-CoA thiolase, producing acetoacetyl-CoA.

2. By the action of β-hydroxy-β-methylglutaryl-CoA (HMG-CoA) synthase, acetoacetyl-CoA is condensed with one more acetyl-CoA molecule, producing HMG-CoA.

3. HMG-CoA lyase converts HMG-CoA into acetoacetate and acetyl-CoA.

4. Acetoacetate can be converted into acetone with the release of CO_2, or into 3-hydroxybutirate producing NADH.

Once produced, ketone bodies are rapidly sent to the bloodstream, possibly causing ketoacidosis. Under these circumstances, and since the body is facing a hypoglycaemic state, brain cells can adapt to use ketone bodies as an energy source.

Use of ketone bodies as an energy source occurs in the mitochondrial matrix and involves:

1. Conversion of 3-hydroxybutirate into acetoacetate by 3-hydroxybutirate dehydrogenase with the production of NADH.

2. By the action of CoA transferase, acetoacetate is transformed in acetoacetyl-CoA. Used CoA results from a succinyl-CoA molecule.

3. Acetoacetyl-CoA thiolase breaks the bond between the second and third carbon of acetoacetyl-CoA, releasing two acetyl-CoA molecules that can be further used in the Krebs cycle.

2.1.2. **Pathologies**

Carnitine deficiency

Carnitine is essential for lipid catabolism, since, as described previously, it is required for the transport of fatty acids across the inner mitochondrial membrane, to reach the matrix where these lipids will be degraded by β-oxidation. Carnitine is acquired in the food diet, being required in fasting periods for production of ATP from fat degradation.

Primary carnitine deficiency results from mutations in the *SLC22A5* (Solute Carrier Family 22 Member 5) gene that codes for the OCTN2 protein, a transporter of carnitine into cells. These mutations can result in the absence or in the production of a non-functional protein, leading to a shortage of carnitine in cells and consequent inability to degrade fatty acids.

This condition has an autosomal recessive transmission and an incidence of 1 in 100,000 newborns. The incidence of this pathology is much higher in Japan, affecting 1 in 40,000 newborns.

This deficiency is particularly damaging in tissues that require high levels of ATP such as the heart and skeletal muscles, being muscle weakness one of the observed symptoms. Since fatty acids cannot be used as energy stores, the demand for glucose is much higher and, for this reason, patients develop hypoglycaemia. Moreover, tissues such as liver, heart and muscles will have an increase in fatty acid storage that can damage these cells.

Symptoms start to appear during infancy or early childhood and, besides the previously mentioned symptoms, include cardiomyopathy, vomiting and confusion. Severe brain dysfunction can also be present due to the lack of glucose, main substrate for ATP production in brain cells.

Patients with this disorder can have different degrees of severity depending on the amount of functional protein still present in cells. Some individuals may be asymptomatic and the ones developing symptoms have the risk of liver problems, heart failure and sudden death.

Carnitine palmitoyltransferase I deficiency

Carnitine palmitoyltransferase I is an enzyme present in the outer mitochondrial membrane, responsible for the transfer of the fatty acid from the transporter CoA to carnitine. This allows the fatty acid to cross the inner mitochondrial membrane, attached to carnitine, to be later degraded by β-oxidation and converted in ATP.

This enzyme is coded by the *CPT1A* gene and mutations in this gene lead to a severe reduction or elimination of carnitine palmitoyltransferase I activity, that will prevent energy production during periods of fasting, when glucose is no longer present.

This condition is a rare disorder transmitted with an autosomal recessive pattern, whose symptoms usually start to show up during early childhood.

Since fatty acids cannot be used as energy source but are still being produced and stored, liver, heart and brain cells can be severely damaged. Moreover, the requirement for glucose is higher in these patients and they usually develop hypoglycaemia. An increase in the use of proteins for energy production is observed, leading to increased plasma levels of ammonium. Due to the inability to use fatty acids, these patients also have low plasma levels of ketone bodies.

Carnitine acyltransferase II deficiency

Carnitine palmitoyltransferase II is an enzyme of the inner mitochondrial membrane that catalyses the transfer of fatty acids from carnitine to CoA, producing acyl-CoA that is then ready to be used in β-oxidation to produce ATP. This protein is encoded by the *CPT2* gene. Mutations in this gene lead to reduced or absence of enzyme activity and consequent inability to break the bond between carnitine and the fatty acid. In this way, fatty acids cannot be metabolized to produce energy. As discussed in the previous disease, patients will have a build-up of fatty acids and acyl-carnitine that will damage liver, heart and muscular cells. Symptoms developed in patients with this condition are like the one's characteristic of the deficiency in carnitine palmitoyltransferase I.

Since the severity of the disease is variable, this condition is defined in three types: lethal neonatal, infantile hepatocardiomuscular form and a myopathic form. The lethal neonatal type becomes evident at birth, by the development of respiratory and liver failure, seizures, cardiomyopathy and arrythmias. These newborns also have hypoketotic hypoglycaemia. The life expectancy of these children is a few days to a few months.

The infantile hepatocardiomuscular type is a less severe form, whose symptoms appear during the first year after birth. Problems associated with this condition are usually triggered by fasting periods or by viral infections and is characterised by recurrent episodes of hypoketotic hypoglycaemia. Children with this condition are at risk of liver failure, coma and sudden death.

The milder form, the myopathic type involves recurrent episodes of muscular pain and weakness resulting from the destruction of muscular tissues. This episodes of myalgia and rhabdomyolysis can be induced by stress, fasting and infections. The destruction of muscles leads to the release of myoglobin into the bloodstream and its consequent excretion through the kidneys, making urine red or brown. The excretion of this protein can damage kidneys and may lead to kidney failure. Symptoms are usually evident during childhood or adolescence.

Acyl-CoA dehydrogenase deficiency

Acyl-CoA dehydrogenase is coded by the *ACADM* gene and is involved in the degradation of fatty acids. This enzyme catalyses the first oxidation of the β-oxidation cycles, yielding $FADH_2$. Mutations in this gene lead to a shortage of the coded enzyme and inability to use fatty acids as an energy source. Signs and symptoms of this condition are like the ones described in the previous disorders.

The incidence of this disorder is 1 in 17,000 individuals, being more frequent in northern European individuals, with an autosomal recessive transmission.

β-Hydroxy-β-methylglutaryl-CoA lyase deficiency

HMG-CoA lyase is encoded by the *HMGCL* gene. This enzyme has an important role in the production of ketone bodies since it catalyses the conversion of HMG-CoA in acetoacetate. As explained before, this step is essential for the proper use of fatty acids as energy store in fasting periods. It is also an important step of leucine degradation.

Mutations in the *HMGCL* gene lead to reduced or absence of HMG-CoA lyase activity. Consequently, there will be a build-up of organic acids that result in metabolic acidosis. Moreover, the inability of producing ketone bodies, decreases fatty acid degradation and leads to an increase in the use of glucose as energy source that will culminate in the development of hypoglycaemia, especially in fasting periods.

Symptoms of this condition usually develop during the first year of life and include, beside the hypoglycaemia and acidosis, diarrhoea, vomiting, dehydration, lethargy and hypotonia. In untreated cases, patients can experience breathing problems, convulsions, coma and even death.

This is a rare disorder with less than 100 cases reported worldwide and is transmitted with an autosomal recessive pattern.

Ketoacidosis

Ketoacidosis results from an uncontrolled overproduction of ketone bodies, that leads to a metabolic acidosis state that can provoke changes in blood pH. Symptoms are diverse depending on the underlying cause, but the most frequent symptoms are nausea, vomiting, abdominal pain and weakness. To compensate the metabolic acidosis, patients usually tend to have a rapid deep breathing and their breath may also smell as acetone.

The most frequent cause of ketoacidosis is diabetes. Diabetic ketoacidosis can result from type 1 or late-stage type 2 diabetes, and main symptoms include hyperglycaemia, dehydration and ketogenesis due to the increase in degradation of fatty acids. Hyperkalaemia and hyponatremia may also develop.

Ketoacidosis may also result from prolonged and heavy alcohol consumption associated with poor nutrition. The need for ethanol catabolism interferes with gluconeogenesis since it consumes main substrates of this last pathway. In this way, glucose will not be produced from gluconeogenesis when hepatic glycogen is depleted. This leads to hypoglycaemia, accumulation of lactic acid and increase need for fatty acid catabolism for energy production which will increase the synthesis of ketone bodies. In this case, acidosis results from high plasma levels of lactic acid and ketone bodies.

A rare cause of ketoacidosis is starvation during pregnancy, lactation or acute disorder. Again, in long starvation periods glycogen stores are depleted and the organism relies on fatty acids for ATP production. As discussed above, this commonly leads to excessive ketone bodies production. Moreover, the high levels of hormones, including glucagon, present in pregnant women lead to increased plasma levels of fatty acids and consequent rise in ketone bodies. In the same way, children usually have lower levels of glycogen stores and in acute infection circumstances (particularly gastrointestinal illness), may produce high levels of glucagon and other catabolic hormones that will activate fatty acid degradation and may lead to ketoacidosis.

Ketoacidosis can also be developed from the use of certain medications (salicylates or isoniazid) or from the ingestion of toxic products such as methanol and acetone.

2.1.3. **Clinical cases**

Clinical case 2.1.1. *Carnitine deficiency*

A baby was taken to the hospital showing heart failure, central nervous system dysfunction, hypoglycaemia, weakness, reduced muscular tonus, high levels of circulating fatty acids, almost null levels of ketone bodies and high plasma levels of ammonium. Liver and muscle biopsy revealed accumulation of triacylglycerols and very low levels of carnitine. Treatment by administration of carnitine led to disappearance of symptoms. Explain why.

Clinical case 2.1.2. *Carnitine deficiency*

A child did not tolerate physical activity, getting tired very fast. A muscular biopsy revealed that muscles had very high levels of triacylglycerols, but only 1/6 of carnitine levels when compared with a person without any muscular problem.
a) Explain the intolerance to exercise observed in this child.
b) Explain the accumulation of triacylglycerols in muscles.

Clinical case 2.1.3. *Carnitine palmitoyltransferase deficiency*

Deficiency in carnitine palmitoyltransferase in muscles causes cellular lesion and recurrent muscular weakness, especially during fasting and/or physical activity. The deficiency of this enzyme in liver cells leads to an increased liver volume, fatty liver, hypoglycaemia and decreased plasma levels of ketone bodies. Explain the cause of these symptoms.

Clinical case 2.1.4. *Carnitine acyltransferase II deficiency*

Explain why individuals with hereditary deficiency of carnitine acyltransferase II have muscular weakness. Why are these symptoms more severe in fasting periods?

Clinical case 2.1.5. *Acyl-CoA dehydrogenase deficiency*

Deficiency in acyl-CoA dehydrogenase leads to inability to degrade fatty acids. Symptoms of this genetic disease are for instance nauseas and vomiting. These symptoms can be relieved by regular ingestion of food and avoiding fasting periods above 12 hours. Explain the biochemical base of this strategy for symptoms relief.

Clinical case 2.1.6. *Acyl-CoA dehydrogenase deficiency*

In Jamaica, there are a few cases of poisoning due to the ingestion of a certain fruit. Poisoned individuals show violent vomiting, severe hypoglycaemia followed by convulsions, coma and death. The fruit contains hypoglycine A, an unusual amino acid that is metabolized by acyl-CoA dehydrogenase (enzyme that catalyses the first reaction of fatty acids β-oxidation) producing a reactive intermediate that inactivates the enzyme. Why are these patients completely depleted of glycogen stores?

Clinical case 2.1.7. *β-Hydroxy-β-methylglutaryl-CoA lyase deficiency*

If deficiency in HMG-CoA lyase is not treated, can lead to death during childhood. Symptoms can include metabolic acidosis, hypoglycaemia, sensitivity to leucine in diet, carnitine deficiency, hepatomegaly, fever, sleepiness and coma. Treatment involves leucine restriction, supplementation of glucose to prevent hypoglycaemia and supplements of carnitine. Explain this strategy.

Clinical case 2.1.8. *Ketoacidosis*

A diabetic 22-year-old woman went to the hospital. This woman has a history of 2 days of vomiting and abdominal pain. She was confused and her respiration was deep and fast. Her breath had a characteristic smell.

a) What is the most likely diagnosis?

b) Which simple tests could be done to confirm diagnosis?

c) Which laboratory tests should be done?

Clinical case 2.1.9. *Ketoacidosis*

Ketone bodies are associated to a severe metabolic disturbance that can cause death in patients with diabetes mellitus type I. However, the capacity to produce ketone bodies was positively selected during evolution. Explain the importance of the capacity to produce ketone bodies in survival of a mammal.

2.1.4. **Further questions**

Question 2.1.1.
How are free fatty acids transported in the blood?

Question 2.1.2.
How and where does fatty acid activation take place?

Question 2.1.3.
In a eukaryotic cell, is the enzyme responsible for the activation of fatty acids found in the inner mitochondrial membrane, in the cytosol, in the mitochondrial matrix or in the intermembrane space?

Question 2.1.4.
Describe the transport pathway of fatty acids to the mitochondria.

Question 2.1.5.
What is the origin and metabolic and physiological importance of carnitine?

Question 2.1.6.
In a eukaryotic cell, is carnitine acyl transferase I found in the outer mitochondrial membrane, in the cytosol, in the mitochondrial matrix or in the intermembrane space?

Question 2.1.7.
In a eukaryotic cell, is carnitine acyl transferase II found in the inner mitochondrial membrane, in the cytosol, in the mitochondrial matrix or in the intermembrane space?

Question 2.1.8.
Is the passage of fatty acids into the mitochondrial matrix coupled with the production of CO_2, the consumption of NADH, the synthesis of ATP, the loss of electrons or the hydrolysis of ATP?

Question 2.1.9.
In a eukaryotic cell, are the enzymes responsible for the degradation of fatty acids found in the inner mitochondrial membrane, in the cytosol, in the mitochondrial matrix or in the intermembrane space?

Question 2.1.10.
Comment on the following statement: "Palmitoyl-CoA-carnitine acyltransferase controls substrate availability for β-oxidation".

Question 2.1.11.
Comment on the following statement: "The formation of a carnitine fatty acid ester is necessary for recognition by a membrane carrier protein."

Question 2.1.12.
Carnitine is a special amino acid derived from lysine. What kind of athletes would suffer the most from a low-lysine diet, and why?

Question 2.1.13.
Explain why individuals with an inherited deficiency of carnitine palmitoyl transferase II have muscle weakness. Why are these symptoms more severe in times of fasting?

Question 2.1.14.
In congenital defects of β-oxidation of fatty acids, a low-fat diet and carnitine supplement are used.
a) Briefly describe the process of β-oxidation of fatty acids.
b) What is the biochemical basis for that therapeutic action?

Question 2.1.15.
What is β-oxidation? Considering a β-oxidation cycle, what product is formed?

Question 2.1.16.
Say, justifying, if the β-oxidation of fatty acids removes 1-carbon units, removes 2-carbon units, has acetyl-CoA as the only end product or can occur in the cytosol.

Question 2.1.17.
Summarize the chemical transformations that occur during fatty acid activation and degradation to acetyl-CoA.

Question 2.1.18.
What are the similarities between the β-oxidation of fatty acids and the citric acid cycle?

Question 2.1.19.
Explain whether the products of β-oxidation include $FADH_2$, NADH, ATP, or CO_2.

Question 2.1.20.
Fatty acids are metabolically degraded to acetyl-CoA. This compound is also an activator of the enzyme pyruvate carboxylase, an enzyme that catalyses the conversion of pyruvate to oxaloacetate. How does the activation of the pyruvate carboxylase enzyme contribute to energy production from fatty acids?

Question 2.1.21.
Why can't fatty acids be used by neurons as a source of energy under anaerobic conditions?

Question 2.1.22.
Why are mammals unable to carry out gluconeogenesis from even-chain fatty acids?

Question 2.1.23.
Why are unsaturated fatty acids preferable to saturated fatty acids for individuals whose caloric intake must be limited?

Question 2.1.24.
Why are unsaturated fatty acids preferable to saturated fatty acids for good health?

Question 2.1.25.
Why are odd-chain fatty acids preferred over even-chain fatty acids, especially if the diet consists solely of fats?

Question 2.1.26.
Suppose you must survive on a diet of seal fat, with few or no carbohydrates.
a) Can you survive? How?
b) If in fact you didn't have any carbohydrates in your diet, would you prefer to consume fatty acids with even or odd number of carbon atoms?

Question 2.1.27.
How many ATPs are produced in the complete oxidation to CO_2 and H_2O of a 12-carbon saturated fatty acid? Justify by presenting all calculations.

Question 2.1.28.
How many moles of ATP are produced in the total oxidation of 1 mole of:
a) Myristic acid (14:0).
b) Palmitic acid (16:0).
c) Arachidic acid (20:0).

Question 2.1.29.
Compare energy balances, in ATPs, on:
a) Degradation of stearic acid (18:0) to CO_2.
b) Degradation of fatty acid with 19 carbons to CO_2.

Question 2.1.30.
What additional steps are needed to oxidize unsaturated and odd-chain fatty acids?

Question 2.1.31.
The oxidation of linoleic acid (18:2($\Delta^{9,12}$)) requires two enzymes that are not involved in the oxidation of stearic acid (18:0).
a) Indicate in which steps these enzymes work and what are their functions.
b) How many moles of ATP are produced in the total oxidation of linoleic acid?

Question 2.1.32.
Compare, in terms of energy yield, the total degradation of 1 mole of stearic acid (18:0) and 3 moles of glucose.

Question 2.1.33.
How is the degradation of fatty acids regulated?

Question 2.1.34.
Compare the following aspects of fatty acid degradation and synthesis: location where they occur; transporter of acyl groups; reducers and oxidants; direction in which they proceed; organization of the enzymatic system.

Question 2.1.35.

Compare β-oxidation with biosynthesis of decanoic acid (10:0) in the following aspects: (Answer in the form of a table)

a) Starting compound.

b) Intracellular localization of the enzymes that catalyse the reactions of both processes.

c) Oxidation-reduction coenzymes used.

d) Carrier of the acyl group.

e) Way in which carbon units are removed or added.

f) ATP balance (consider oxidation to CO_2 and H_2O).

g) High ATP/ADP ratio as a determinant of the occurrence of one or the other.

h) Low ATP/ADP ratio.

Question 2.1.36.

In a eukaryotic cell, is the enzyme responsible for the synthesis of malonyl-CoA found in the inner mitochondrial membrane, in the cytosol, in the mitochondrial matrix or in the intermembrane space?

Question 2.1.37.

The enzyme acetyl-CoA carboxylase is the main point of regulation in the biosynthesis of fatty acids. Some of the enzyme properties are described below:

- Addition of citrate or isocitrate increases the enzyme V_{max} by a factor of 10.
- The enzyme exists in two interconvertible forms that differ markedly in their activities:

protomer (inactive) ⇆ filamentous polymer (active)

Citrate and isocitrate bind preferentially to the filamentous form, and palmitoyl-CoA binds preferentially to the protomer. Explain how these properties are consistent with the regulatory role of acetyl-CoA carboxylase in fatty acid biosynthesis.

Question 2.1.38.

Does the enzyme acetyl-CoA carboxylase require biotin, N-acetylneuraminic acid, lipoic acid, N-acetylglutamate or pyridoxal phosphate as a cofactor?

Question 2.1.39.

Which of the following is not required for fatty acid synthesis: acetyl-CoA, ATP, NADPH, or NADH?

Question 2.1.40.

Can the fatty acid shown below be produced in animals?

$CH_3\text{-}(CH_2\text{-}CH{=}CH)_3\text{-}(CH_2)_7\text{-}COO^-$

Question 2.1.41.

How many moles of acetyl-CoA, malonyl-CoA, ATP, and NADPH are consumed for each mole of palmitic acid (16:0) synthesized?

Question 2.1.42.

What is the consumption of ATP and NADPH (or equivalents) in the synthesis of stearic acid (18:0), knowing that the elongation of palmitic acid occurs in the mitochondria. Explain.

Question 2.1.43.
Explain the consumption in ATP and NADPH (or equivalents) of stearic acid synthesis (18:0), knowing that elongation of palmitic acid occurs in the ER.

Question 2.1.44.
How many moles of NADPH are needed for the synthesis of 1 mole of oleic acid (18:1(Δ^9))? And how many moles of ATP?

Question 2.1.45.
Comment on the following statement: "Acetyl-CoA carboxylase is the regulatory point of fatty acid synthesis".

Question 2.1.46.
Comment on the following statement: "Acetyl-CoA carboxylase is activated by citrate".

Question 2.1.47.
Acetyl-CoA needed for fatty acid synthesis (in the cytosol) is produced in the mitochondria. Appropriately describe the mechanism by which acetyl-CoA crosses the mitochondrial membrane.

Question 2.1.48.
The acetyl group of acetyl-CoA, produced during oxidative decarboxylation of pyruvate in mitochondria, is transferred to the cytosol via the malate shuttle.
a) Write the overall reaction for the transfer of an acetyl group from the mitochondria to the cytosol.
b) What is the cost of this process in ATPs per acetyl group?

Question 2.1.49.
Explain why linoleic acid (18:2($\Delta^{9,12}$)) is an essential fatty acid.

Question 2.1.50.
Describe the main regulatory mechanisms of fatty acid metabolism in humans.

Question 2.1.51.
Briefly explain the role of the liver in fatty acid metabolism.

Question 2.1.52.
In a patient with type I diabetes mellitus (insulin dependent) there is an increase in plasma fatty acids. Explain why.

Question 2.1.53.
In the synthesis of triacylglycerols, there are enzymes that catalyse the transfer of acyl residues. Is the donor substrate always a lecithin, 2-mono-acyl-glycerol, an acyl-CoA or acetyl-CoA?

Question 2.1.54.
Consider an individual who has deficiencies in the carnitine synthesis pathway and who does not ingest adequate amounts of carnitine in his diet. Will the concentration of ketone bodies in the blood of this individual after an overnight fast be higher or lower than in a normal individual?

Question 2.1.55.
Do ketone bodies circulate in the bloodstream as water-soluble molecules, associated with the surface of plasma lipoproteins, non-covalently bound to albumin, esterified to carnitine, or esterified to coenzyme A?

Question 2.1.56.
In people who are on hunger strike, ketone bodies are the main source of energy of brain cells. How many moles of ATP are produced per each mole of β-hydroxybutyrate?

Question 2.1.57.
During a prolonged fasting period, brain cells spare glucose utilization in favour of ketone bodies. What are the metabolic advantages of this alternative? Explain how ketone bodies are synthesized and how they are used.

Question 2.1.58.
Why does the breath of an untreated diabetic smell like acetone?

Question 2.1.59.
In prolonged fasting, increased blood levels of fatty acids are found, and ketone bodies are formed. Comment on this statement and refer to the main energy sources of the brain, muscle and liver in this situation.

2.2. Cholesterol and Plasma lipoproteins

2.2.1. Short review

Cholesterol synthesis

Steroids are complex derivatives of triterpenes, having a cyclopentanoperhydrophenanthrene nucleus composed of three hexagonal rings and one pentagonal. Terpenes are composed of repetitive structural units of 5 carbons (isoprenoids). Each steroid is distinguished by the position of double bonds and substituents (hydroxyl, carbonyl and alkyl groups). Cholesterol has 1 hydroxyl (linked to C-3), being classified as sterol.

Cholesterol is derived from two sources, from food diet and from endogenous synthesis. When the diet provides enough cholesterol, the endogenous synthesis decreases. Biosynthesis is stimulated when food is low in cholesterol. Although all tissues can produce cholesterol, most is synthesized in the liver. Cholesterol biosynthesis begins with acetyl-CoA and is a very complex process involving 32 different enzymes, some of which are soluble in the cytosol and others are bound to the membrane of the endoplasmic reticulum. Biosynthesis of cholesterol can be divided into 3 phases:

1. Formation of HMG-CoA from acetyl-CoA.

2. Conversion of HMG-CoA into squalene. The regulatory enzyme is HMG-CoA reductase that catalyses one step in this conversion, the transformation of HMG-CoA into mevalonate.

3. Conversion of squalene to cholesterol.

Cholesterol as a precursor of steroid hormones and bile salts

Cholesterol is an essential component of cell membranes, being also precursor of steroid hormones, vitamin D and bile salts.

The initial reaction in the synthesis of steroid hormones (conversion of cholesterol to pregnenolone) is catalysed by desmolase, a mitochondrial enzyme. After its synthesis, pregnenolone is transported to the ER, where it is converted into progesterone. Pregnenolone and progesterone are precursors of all other steroid hormones.

Cholesterol and other steroids cannot be degraded. In liver cells, they are converted into derivatives (bile acids), which because of their solubility properties allow their excretion. Conversion of cholesterol to bile acids begins when hydroxyl groups are introduced into the phenanthrene ring of cholesterol. Conversion of cholesterol to 7-α-hydroxycholesterol, catalysed by cholesterol-7-α-hydroxylase (microsomal enzyme), is the rate-limiting step of this transformation. Products (colic and deoxycholic acid) are converted into bile salts by microsomal enzymes that catalyse conjugation reactions (solubility increases by conversion to a derivative containing a water-soluble group, amides and esters).

Bile salts are components of bile, yellow-green liquid produced by hepatocytes that helps in lipid digestion. In addition to bile salts, bile contains cholesterol, phospholipids, and bile pigments (products of haem group degradation).

Plasma lipoproteins

Plasma lipoproteins are proteins covalently bound to lipid groups. They also contain various types of lipid-soluble antioxidant molecules (α-tocopherol and several carotenoids). Their role is to carry lipid molecules (triacylglycerols, phospholipids and cholesterol) through the bloodstream from one organ to another. Apolipoproteins or apoproteins are the protein components of lipoproteins and have several possible roles such as coenzymes, enzymatic activity or signal for activation of endocytosis.

Each type of lipoprotein contains a neutral lipid core, composed of cholesterol esters and/or triacylglycerols. This core is surrounded by a phospholipid layer, cholesterol and proteins. The charged and polar residues on the lipoprotein surface allow interaction with the blood.

Chylomicrons are the largest type of lipoprotein having extremely low density. Ingested lipids are incorporated into chylomicrons in the cells of the intestinal wall, being released into the lymphatic circulation. This is the exogenous route of lipid transport. During chylomicrons transport to the liver, triacylglycerols are degraded by an enzyme present in the blood capillaries, lipoprotein lipase (LPL). As a result of triacylglycerols degradation, resulting fatty acids start to be captured by cells, leaving only reminiscent chylomicrons which, due to the presence of apolipoprotein E (apo E), are endocytosed by hepatocytes (which have apo E receptor).

In the liver, endogenous lipid transport is now initiated by the synthesis of very low-density lipoproteins (VLDL). These mainly carry triacylglycerols to tissues. Again, during VLDL transport through blood flow, triacylglycerols degradation occurs by LPL, leading to the maturation of these lipoproteins in low-density lipoproteins (LDL). LDL is responsible for the distribution of lipids to all tissues. These lipoproteins have apo B,E which activates endocytosis by cells having apo B,E receptor (LDL receptor).

High plasma LDL levels are directly related to high risk of coronary artery disease since they have high amounts of cholesterol and cholesterol esters. Mutations in the gene encoding the LDL receptor, that inhibit the production of this receptor, prevent the internalization of this type of lipoproteins. This is the leading cause of the metabolic disease familial hypercholesterolemia. Individuals who are homozygous for this mutation have 10 times higher than normal plasma levels of cholesterol and may suffer from cardiovascular disease in young ages (between 0 and 30 years).

Finally, high-density lipoproteins (HDL) are produced in the liver but go to tissues to collect the excess of cholesterol and carry it back to the liver. HDLs circulate continuously and contain an enzyme that converts free cholesterol into cholesterol esters, the lecithin cholesterol acyltransferase (LCAT). To perform this double path, HDL suffers a change in the composition of apolipoproteins during their maturation. Initially, HDLs are rich in apo A, which allows them to collect cholesterol from tissues. However, during their maturation, they lose apo A and acquire apo E, which allows the return of HDL to the liver.

The liver, the only organ that can eliminate cholesterol excess, converts most of it to bile acids, which are released into the intestine to allow emulsification of ingested lipids. As a result of its role in eliminating excess cholesterol, high levels of plasma HDL are associated with low risk of coronary artery disease.

2.2.2. **Pathologies**

Lipoprotein lipase deficiency (Hyperlipidaemia type I)

LPL, present in capillaries, is responsible for the degradation of triglycerides transported in lipoproteins such as chylomicrons and VLDL.

LPL deficiency has an autosomal recessive transmission and results from mutations in the *LPL* gene, located in chromosome 8p21.3. The reduction of LPL content and consequently of its activity leads to the build-up of triglycerides in blood and tissues.

Patients with LPL deficiency usually develop symptoms before 10 years of age and can even show first symptoms by age 1. The first evidenced symptom is abdominal pain with diverse degrees of intensity that result from acute pancreatitis. If not treated, pancreatitis can become chronic, and can lead to severe pancreatic damage that can be life-threatening. The increased level of fat in the body may lead to hepatosplenomegaly. This results from the action of macrophages that take up lipids from the bloodstream, to reduce lipid blood levels, and deliver them to the liver and spleen where they are accumulated. Patients can also develop yellow deposits of fat under the skin, known as eruptive xanthomas. If blood fat levels are too high, they can accumulate in blood vessels and in the retina leading to lipemia retinalis. Finally, increased lipid content may also provoke neurological changes such as memory loss, depression and dementia.

Familial hypercholesterolemia (Hyperlipidaemia type IIA)

Familial hypercholesterolaemia is a genetic disorder with autosomal dominant transmission, having as main characteristic the presence of high levels of plasma cholesterol, mainly in LDL. This leads to an early onset of cardiovascular disease. Another physical sign normally present in these patients is the presence of yellow deposits of cholesterol-rich fat that can develop in various places of the body, like tendons, iris and around the eyelids.

The most common form of this condition results from mutations in the *LDLR* gene, that codes for the LDL receptor. This protein is responsible for removing LDL from the circulation, which are later used to produce energy in cells. It is estimated that 1 in 300-500 individuals in the whole world carry one mutation in this gene. Patients can be heterozygous or homozygous for these mutations. Heterozygous individuals have 2 times higher levels of plasmatic LDL and a consequent onset of coronary artery disease between 40 and 60 years of age. On the other hand, homozygous patients show 5-7 times higher blood LDL levels and for this reason have a very early onset of coronary artery disease (0-30 years of age), even during childhood. The frequency of homozygous in the population is much lower, 1 in 1,000,000 individuals since both parents must be heterozygous for these mutations.

More than 1,000 different mutations in the *LDLR* gene have already been described and different mutations lead to five major classes of familial hypercholesterolaemia: in class I, no LDL receptor is produced; in class II, this receptor protein is not correctly transported from the rough endoplasmic reticulum (RER) to the Golgi apparatus and consequently is not inserted in the cell membrane; in class III, no proper binding of LDL to the receptor takes place, due to a receptor defect; in class IV, after binding of LDL to the receptor, endocytosis does not occur; and in class V there is no recycling of the receptor to the cell membrane.

Other possible causes of this condition are mutations in the gene coding for Apo B. These mutations have a prevalence of 1 in 1,000 individuals. Apo B is the main apolipoprotein present in LDL and is the signal recognized by the LDL receptor, inducing endocytosis. Mutations in this gene lead to a defective protein that has a reduced ability to bind the receptor. Consequently, hypercholesterolaemia develops. The severity of this condition is determined by the number of mutant copies present in the individual.

Other genes have also been associated with the development of this disease, such as the *PCSK9* and *LDLRAP1* genes, but their pathophysiological mechanisms are still not known.

Apolipoprotein C-II deficiency (Hyperlipoproteinaemia type IB)

Apolipoprotein C-II (Apo C-II) is a constituent of chylomicrons and VLDL and works as an activator of LPL, present in capillaries. LPL is involved in the degradation of triglycerides transported in the mentioned lipoproteins, providing free fatty acids to cells.

This protein is encoded by the *APOC2* gene and mutations in this gene lead to a pathology that is clinically like LPL deficiency, and for that reason is called hyperlipoproteinemia type IB. It is characterised by pancreatitis, hepatosplenomegaly and the development of xanthomas. Patients have high plasma levels of triglycerides, cholesterol and chylomicrons.

Apo C-II deficiency is significantly less frequent than LPL deficiency. It is transmitted with an autosomal recessive pattern, since the *APOC2* gene is present in chromosome 19q13.32.

High density lipoproteins deficiency (Tangier's disease)

Tangier disease results from mutations in the *ABCA1* gene, belonging to the ATP-binding cassette family of genes, and codes for the ATP-binding cassette transporter ABCA1. This protein, also known as cholesterol efflux regulatory protein (CERP), is an important regulator of cholesterol and phospholipids in cells. It is produced in many tissues being present in higher levels in the liver macrophages.

The role of the ABCA1 protein is to move cholesterol and phospholipids across the membrane to the outside of the cell that are then trapped by Apo A-I to be incorporated by HDL and transported to the liver.

Mutations in the *ABCA1* gene prevent the removal of excess cholesterol and phospholipids from cells, leading to their accumulation in tissues. The build-up of these lipids can lead to changes in cell function or even cell death.

Patients have low levels of HDL that is associated with increased risk of cardiovascular disease. Other possible symptoms developed by these individuals are neuropathy, splenomegaly, hepatomegaly, corneal clouding, and type 2 diabetes.

This condition has an autosomal recessive transmission, and is very rare, with only 100 reported cases worldwide.

Apolipoprotein E (Apo E) receptor deficiency

Apo E is an essential component of lipoprotein particles in the brain and periphery. Human apo E exists in three isoforms: apo E2, apo E3 and apo E4. Apo E mediates cholesterol metabolism by binding to various receptors. The LDLR, a transmembrane receptor expressed in the liver, brain, and other tissues, has a high affinity for apo E and is the only member of its receptor family to demonstrate apo E isoform-specific binding affinity (E4 > E3 >> E2).

The apo E-LDLR interaction appears critical in both cardiovascular disease and Alzheimer's disease. For example, disparities in cardiovascular disease risk generally associated with the *APOE* genotype disappear in patients who lack functional LDLR, suggesting that the disease-modifying effects of apo E isoforms depend on the presence of a fully functional LDLR protein. In mice, the introduction of human LDLR results in higher plasma cholesterol and increased cardiovascular complications in the presence of E4. This effect does not occur in mice that express E3 and, in the presence of E2 lowers cholesterol and improves cardiovascular outcomes.

In the context of Alzheimer, disease risk is modified by several LDLR polymorphisms. Furthermore, the risk associated with a certain LDLR polymorphism differs dramatically depending on the *APOE* genotype of patients, implying that a functional interaction between apo E and LDLR determines the risk of Alzheimer's disease. The apo E-LDLR interaction affects regional brain apo E levels, brain cholesterol, and cognitive function in an apo E isoform-dependent manner.

2.2.3. **Clinical cases**

Clinical case 2.2.1. *Hyperlipidaemia*

A 53-year-old man showed the following clinical results in a blood sample collected in fasting:

Table 2.2.1. Results obtained in patient blood tests.

Parameter	Patient values	Reference values
Total cholesterol	8.4 mmol/L	< 6.5 mmol/L
Triacylglycerols	6.8 mmol/L	< 2.5 mmol/L
Glucose	9.8 mmol/L	4-5.5 mmol/L
g-GT	138 U/L	< 36 U/L

This man is non-smoker, has blood pressure of 145/95 mmHg and is obese with fat distribution in the central region of the body.

a) What other information or tests would help in the following of this patient?

b) Which treatment options should be considered in this case?

Clinical case 2.2.2. *Hyperlipidaemia*

A 36-year-old man went to the ophthalmologist for prescription of reading glasses. During the exam, the doctor observed that the patient had bilateral cornea *arcus senilis*. Further exams showed he had tendons xanthomas, mainly in Achilles tendons. Blood pressure was normal, and the patient did not smoke and did not have excess weight. His father died of heart attack at age of 40. The patient performed a resting electrocardiogram that was normal, showing only ischemic changes during exercise. Fasting blood tests showed the following results:

Table 2.2.2. Results obtained in patient blood tests.

Parameter	Patient values (mmol/L)	Reference values (mmol/L)
Total cholesterol	13.2	< 6.5
Triacylglycerols	1.3	< 2.5
LDL	11.4	< 3.2
HDL	1.2	> 1.2

a) What is the most likely diagnosis for this clinical condition?
b) Based on cholesterol metabolism, explain the observed changes in performed biochemical analyses.
c) Which strategy concerning food diet and pharmacological treatment should be used? Explain.

Clinical case 2.2.3. *Lipoprotein lipase deficiency (Hyperlipidaemia type I)*

A 44-year-old woman showed weight loss and tiredness episodes. She stared to have pain in shoulders and thighs. An X-ray revealed lesions in humerus and femur bones. This patient had familial background of hyperlipidaemia. Biochemical blood tests revealed the following results, indicating hyperglycaemia and hyperlipidaemia.

Table 2.2.3. Results obtained in patient blood tests.

Parameter	Patient values (mg/dL)	Reference values (mg/dL)
Total cholesterol	890	< 219
Triacylglycerols	12349	< 149
Glucose	690	< 109

Further analysis revealed increased levels of VLDL and chylomicrons. Plasma LPL decreased (≤ 9 ng/mL; reference value = 40-60 ng/mL) after intravenous injection of heparin.
a) The stablished diagnose was hyperlipidaemia caused by LPL deficiency. Explain.
b) It was concluded that diabetes mellitus developed secondarily due to the lipidic metabolic disturbance. Explain.

Clinical case 2.2.4. *Familial hypercholesterolemia (Hyperlipidaemia type IIA)*

A 32-year-old woman went to the hospital due to an acute myocardial infarction. Blood tests revealed that plasma cholesterol levels were 420 mg/mL, but triacylglycerols were normal. LDL levels were high. Coronary angiography showed the presence of severe arteriosclerosis in 3 coronary arteries. Her father and two of her five brothers also had hypercholesterolemia and tendon xanthomas.
a) What is the most likely diagnosis for this clinical picture?
b) How can be explained observed changes in performed biochemical tests?

Clinical case 2.2.5. *Familial hypercholesterolaemia (Hyperlipidaemia type IIA)*

A 50-year-old man shows strong thoracic pain, related to physical activity. Two measurements of fasting plasma cholesterol were 10 and 11 mmol/L (reference value = 4.5-7.0 mmol/L). Blood tests also showed increased LDL levels. Angiography showed narrowing and irregularities in two of the three coronary branches. The patient was submitted to cardiac surgery and recommended to poor cholesterol diet. During ambulatory treatment it was observed an increased plasma cholesterol and treatment with oral intake of resins that attach to biliary salts was introduced. The patient also had a family history of hypercholesterolaemia.
a) What is the biochemical defect responsible for the increase in plasma cholesterol in familial hypercholesterolemia?
b) Which other diseases can be associated with atherosclerosis?
d) Describe the mechanism of cholesterol biosynthesis regulation.
e) Explain the base of hypercholesterolaemia treatment.

Clinical case 2.2.6. *Hypercholesterolaemia*

An asymptomatic 38-year-old woman performed blood tests and found out a fasting plasma cholesterol of 8.7 mmol/L (reference value < 6.5 mmol/L).
a) What other information and analyses would be required for the follow-up of this woman?
b) What can be concluded from the performed tests?

Clinical case 2.2.7. *Hypercholesterolaemia*

One of the most effective therapeutic measures in hypercholesterolaemia is the administration of pharmaceutical products such as lovastatin, known inhibitors of liver 3-hydroxy-3-methyl-glutaryl CoA reductase.
a) What is the origin of plasma cholesterol?
b) Why does lovastatin lead to reduction in cholesterol levels?

Clinical case 2.2.8. *Hypercholesterolaemia*

A patient with hypercholesterolaemia (330 mg/dL) was treated with a diet without cholesterol for 3 months, but cholesterol levels only decreased to 300 mg/dL. Subsequently, the patient was treated with a resin that attaches to biliary acids, leading to their excretion in big amounts in faeces. This treatment decreased cholesterol levels to 220-250 mg/dL, a value that is considered acceptable for this patient.

a) Explain the high levels of cholesterol in this patient after 3 months of cholesterol-free diet.

b) How did the resin decrease plasma concentration of cholesterol?

Clinical case 2.2.9. *Apolipoprotein C-II deficiency (Hyperlipoproteinemia type IB)*

A 41-year-old man shows high plasma triacylglycerols and chylomicrons, but low LDL and HDL. The patient suffers from abdominal pain, eruptive xanthomas and hepatomegaly. An apo C-II variant with lower molecular mass and more acidic isoelectric point was found in the patient. The hypertriglyceridemia was corrected by infusion of normal plasma or by injection of a peptide with 44-79 amino acids biologically active, fragment of apo C-II. The effect persisted for 13 to 20 days after the injection of the synthetic peptide. Explain the diagnosis of apo C-II deficiency.

Clinical case 2.2.10. *High density lipoproteins deficiency (Tangier's disease)*

A 49-year-old man had during the last 15 years episodes of appendicular pain. Physical examination revealed absence of tendon reflexes, loss of pain and temperature feeling, changes in nails and skin, loss of facial hair, and moderate splenomegaly. Laboratory tests showed undetectable plasma HDL and decreased total cholesterol and apo A-I. Explain the diagnosis of Tangier's disease.

Clinical case 2.2.11. *Apolipoprotein E receptor deficiency*

Which symptoms are associated with the deficiency in the production of apo E receptor? Explain.

Clinical case 2.2.12. *Hypertriglyceridemia and alcohol intoxication*

Excessive alcohol consumption is, nowadays, one of the severe health problems that affects our society. Among its most acute metabolic effects, is hypoglycaemia, lactic acidosis and eventually coma. Another problem also related with alcohol ingestion, but related with excessive chronic consumers, is the development of "fatty liver" and hepatic cirrhosis. "Fatty liver" results from an excessive accumulation of triacylglycerols in liver. Knowing that ethanol is metabolized by the enzyme alcohol dehydrogenase, being converted to acetaldehyde that is then converted to acetate and this one to acetyl-CoA, explain why chronic alcoholism leads to the development of the referred clinical condition.

2.2.4. **Further questions**

Question 2.2.1.
Summarize the chemical events of cholesterol biosynthesis.

Question 2.2.2.
Which one is not required in cholesterol biosynthesis: acetyl-CoA, NADPH, ATP or NADH?

Question 2.2.3.
Does the HMG-CoA reductase enzyme participate in the synthesis of fatty acids, keto acids, cholesterol, urea or ammonia? Explain the role of this enzyme.

Question 2.2.4.
Comment on the following statement: "In the biosynthesis of cholesterol, HMG-CoA reductase catalyses the limiting step".

Question 2.2.5.
A class of drugs called statins inhibit HMG-CoA reductase. What is the effect of this drug on patients?

Question 2.2.6.
Comment on the following statement: "3-hydroxy-3-methylglutaryl-CoA reductase is a target of pharmacological actions to decrease circulating cholesterol levels".

Question 2.2.7.
Comment on the following statement: "Lipoprotein lipase uses a source of lipids present in plasma as a substrate".

Question 2.2.8.
State whether the following statement is true or false and justify: "To avoid the risk of severe atherosclerosis, one should ideally eliminate all cholesterol from the diet".

Question 2.2.9.
Explain the role of mevinolin in the treatment of hypercholesterolemia.

Question 2.2.10.
Briefly describe the catabolism of LDL in mammalian cells, focusing on the regulatory effects of cholesterol entry into cells.

Question 2.2.11.
Why are plasma cholesterol levels dependent on LDL receptor activity? What is the metabolic consequence for a person with LDL receptor deficiency?

Question 2.2.12.
What is the role of HDL? What about LDL? Point out two known functions for apolipoproteins.

Question 2.2.13.

Apo A-I, the main protein constituent of HDL, is an activator of LCAT. Based on your knowledge of the metabolism of that type of plasma lipoprotein, state the importance of this fact.

2.3. **Membrane lipids**

2.3.1. **Short review**

Phospholipid metabolism

Phospholipids are structural components of cell membranes, emulsifiers and surfactants and are amphipathic molecules (have hydrophobic and hydrophilic domains).

There are 2 types of phospholipids, phosphoglycerides and sphingomyelins. Phosphoglycerides contain glycerol, fatty acids, a phosphate group and an alcohol (e.g., choline). Phospholipid molecules are the most numerous in cell membranes. The simplest phosphoglyceride, phosphatidic acid, is the precursor of all other phosphoglyceride molecules. They are classified according to the alcohol esterified with the phosphate group (e.g., phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, diphosphatidylglycerol, and phosphatidylinositol). Sphingomyelins have sphingosine instead of glycerol.

The phospholipid biosynthesis reactions occur in the SER. Synthesis of phosphatidylethanolamine and phosphatidylcholine are similar. Synthesis of phosphatidylethanolamine (Fig. 2.3.1) begins in the cytosol when ethanolamine enters the cell and is immediately phosphorylated. Phosphoethanolamine reacts with cytidine triphosphate (CTP) to form the activated CDP-ethanolamine intermediate. CDP-ethanolamine is converted to phosphatidylethanolamine when reacted with DAG (catalysed by a SER enzyme).

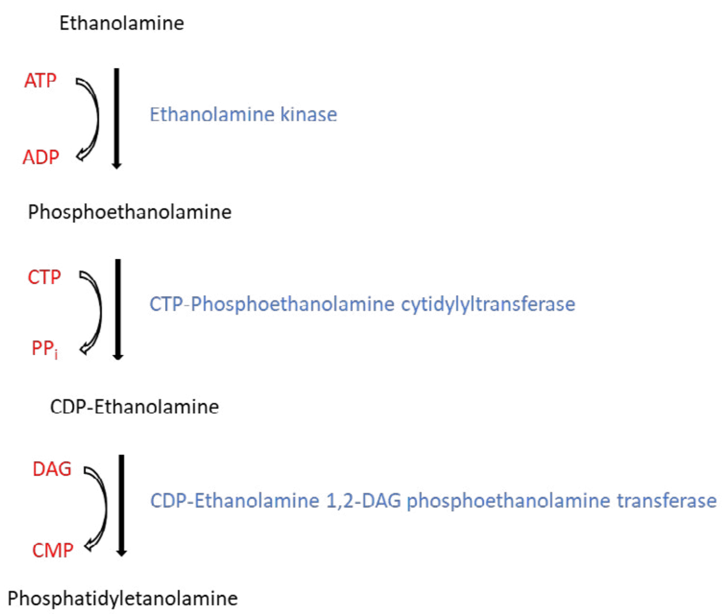

Fig. 2.3.1. Synthesis of phosphatidylethanolamine.

Phosphatidylcholine is also synthetized in the liver, using phosphatidylethanolamine as a precursor (Fig. 2.3.2). Phosphatidylethanolamine is methylated in 3 steps by the enzyme phosphatidylethanolamine-N-methyltransferase to form the trimethylated product phosphatidylcholine. S-adenosylmethionine (SAM) is the methyl group donor in this set of reactions.

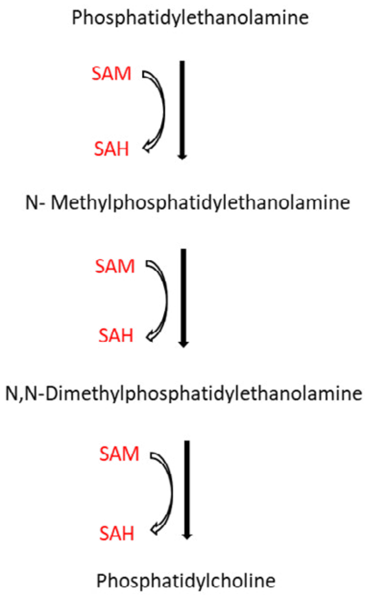

Fig. 2.3.2. Synthesis of phosphatidylcholine.

Phosphatidylserine is synthetized (Fig. 2.3.3) in a reaction in which the ethanolamine residue of phosphatidylethanolamine is exchanged by serine. This reaction, catalysed by a SER enzyme, is reversible. In mitochondria, phosphatidylserine is converted to phosphatidylethanolamine in a decarboxylation reaction.

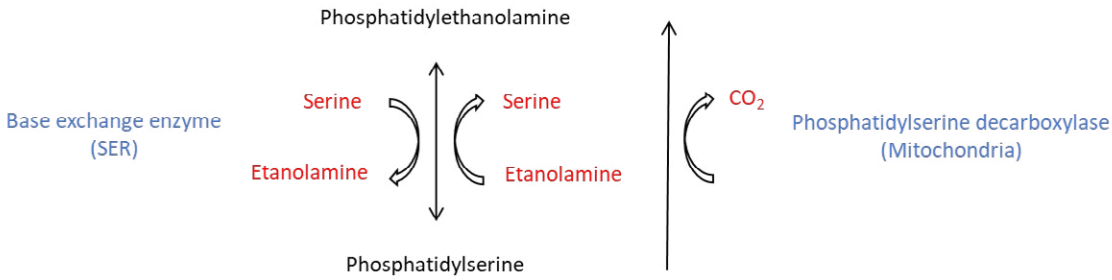

Fig. 2.3.3. Synthesis of phosphatidylserine.

Sphingolipid metabolism

Sphingolipids are important components of cell membranes. They contain long chains of amino alcohols (sphingosine in animals). The nucleus is ceramide, a fatty acid derived amide from sphingosine.

Glycosphingolipids are a complex group of sphingolipids, in which the ceramide backbone is modified by the addition of a carbohydrate head group via an O-glycosidic bond. The simplest glycosphingolipids are termed cerebrosides and have a single monohexose (glucose or galactose) moiety linked to ceramide. During evolution, the carbohydrate head group has increased in complexity, being the most common the ones derived from the core structure glucosylceramide. Galactosylceramide and its sulphated derivative have a much more restricted distribution and are generally confined to myelin and the kidney. When the oligosaccharide head group of glucosylceramide-derived glycosphingolipids contains a sialic acid, they are charged and termed gangliosides and are the major glycoconjugates found in the nervous system.

The synthesis of ceramide (Fig. 2.3.4) begins with the condensation of palmitoyl-CoA with serine to form 3-ketoesphinganine, catalysed by 3-ketoesphinganine synthase (pyridoxal-5'-phosphate-dependent enzyme). The 3-ketoesphinganine is then reduced by NADPH to form sphinganine. The synthesis of sphinganine occurs in the SER. Then, in a 2-step process involving acyl-CoA and $FADH_2$, the sphinganine is converted to ceramide.

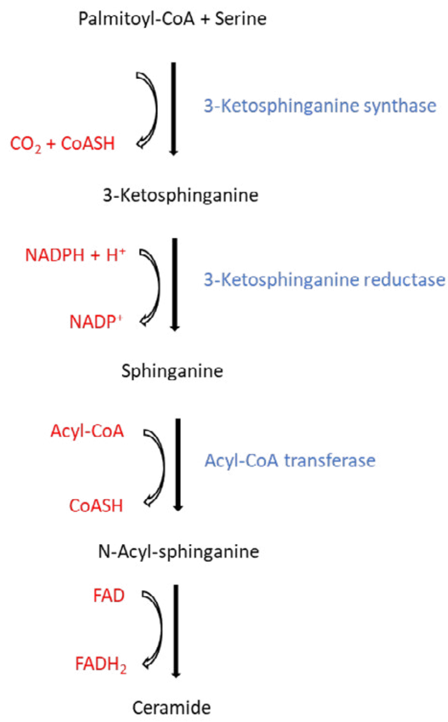

Fig. 2.3.4. Synthesis of ceramide.

Sphingomyelin and sphingolipids are synthetized (Fig. 2.3.5) on the luminal side of the Golgi complex membrane. Sphingomyelin is synthetized when ceramide reacts with phosphatidylcholine. In an alternative reaction, CDP-choline is used instead of phosphatidylcholine. When ceramide reacts with UDP-glucose, glucosylceramide (a common cerebroside, glucosylcerebroside) is formed. Galactocerebroside (precursor of other glycolipids) is synthesized when ceramide reacts with UDP-galactose. Sulphatides are synthesized when galactocerebrosides react with a sulphate donor molecule, 3'-phosphoadenosine-5'-phosphosulfphate (PAPS). Transfer of sulphate groups is catalysed by the enzyme microsomal sulphotransferase.

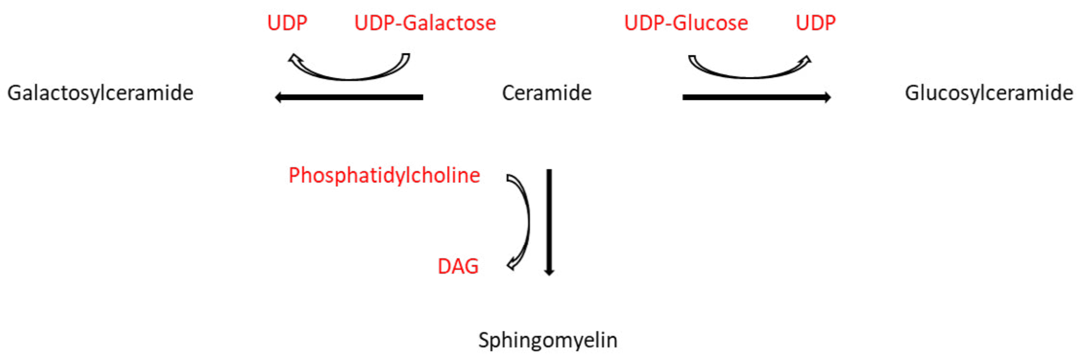

Fig. 2.3.5. Synthesis of sphingomyelin and sphingolipids.

2.3.2. **Pathologies**

Sphingolipidoses

Sphingolipids are normally degraded in lysosomes of phagocytic cells, particularly histiocytes or macrophages of the reticuloendothelial system, located mainly in the liver, spleen and bone marrow. The global pathway of sphingolipid catabolism is composed of a series of enzymes that cleave specific bonds in these compounds (Fig. 2.3.6).

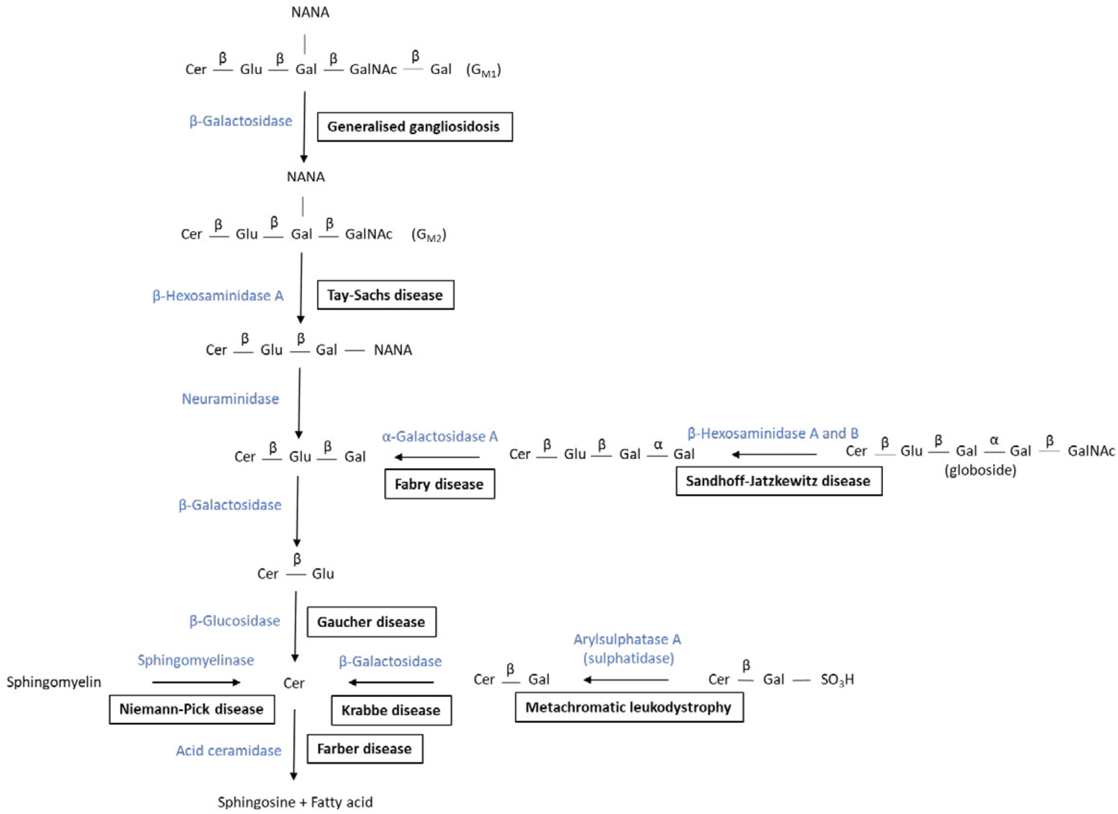

Fig. 2.3.6. Sphingolipids catabolic pathway catalysed by lysosomal enzymes. Some sphingolipidoses are shown. NANA: N-acetylneuraminic acid (sialic acid); Cer: ceramide; Glu: glucose; Gal: galactose; GalNAc: N-acetylgalactosamine.

Specific diseases called sphingolipidoses, occur when the activity of one of the hydrolytic enzymes is markedly reduced due to a genetic error. Due to the presence of a defective or absent enzyme, its substrate will accumulate and deposit within the lysosomes of the tissue responsible for the catabolism of that sphingolipid (Table 2.3.1 and Fig. 2.3.6). Therefore, sphingolipidoses are a group of inherited lysosomal storage diseases.

Table 2.3.1. Enzyme deficiency in different sphingolipidoses.

Disorder	Enzyme deficiency	Principal storage substance
Tay-Sachs disease	Hexosaminidase A	Ganglioside G_{M2}
Gaucher disease	Glucocerebrosidase	Glucocerebroside
Fabry disease	α-Galactosidase A	Ceramide trihexoside
Niemann-Pick disease	Sphingomyelinase	Sphingomyelin
Globoid leukodystrophy (Krabbe disease)	Galactocerebrosidase	Galactocerebroside
Metachromatic leukodystrophy	Arylsulphatase A (sulphatidase)	Sulphatide
Generalised gangliosidosis	G_{M1} ganglioside: β-galactosidase	G_{M1} ganglioside
Sandhoff-Jatzkewitz disease	Hexosaminidase A and B	G_{M2} ganglioside (globoside)
Farber disease	Acid ceramidase	Ceramide
Fucosidosis	α-L-Fucosidase	Pentahexosylfucoglycolipid

Individually, sphingolipidoses are rare and occur with incidences below 1:100,000. However, in some ethnic groups the incidence is higher.

In sphingolipidoses, symptoms are permanent and progressive, the clinical picture is independent of intercurrences, there is no relation to food intake, and clinical manifestations occur in all ages. Patients may have, for example, hepatosplenomegaly, hypotonia, developmental delay and cardiomyopathy.

2.3.3. **Clinical cases**

Clinical case 2.3.1. *Deficiency of hexosaminidase A (Tay-Sachs disease)*

A 30-year-old man has an 8-year history of progressive weakening of his right body. Symptoms progressed to the left arm and leg. He developed dysarthria and dysphagia. He also noted difficulty in recalling recent events. Cerebrospinal fluid examination was normal, as were haematological and biochemical tests: complete blood count, erythrocyte sedimentation rate, urea and creatinine, electrolytes, liver function, thyroid function, and vitamin B12. Serologic tests for HIV, HTLV-1, syphilis (VDRL test), hepatitis B and C were negative. Patient serum was also analysed by a fluorometric method for hexosaminidase A: 280 nmol/h/mL (reference value: 550-1675 nmol/h/mL) and 32% total hexosaminidase (reference value: 45-70%). According to these results, a diagnosis of hexosaminidase A deficiency (Tay-Sachs disease) was established. Justify the established diagnosis.

Clinical case 2.3.2. *Deficiency of glucocerebrosidase (Gaucher disease)*

A 47-year-old female patient presented for 3 months with asthenia, anorexia, postprandial infarction, unquantified weight loss, and lower limb edema, especially in the left. She currently had hepato-splenomegaly (22 cm spleen and solid nodules) and macrocytic anemia.
Bone biopsy showed multiple spinal space occupation by histocyte cells with very wide cytoplasm containing a slightly irregular oval nucleus, whose histomorphological characteristics indicated storage disease, probably Gaucher disease. This diagnosis was further corroborated by enzymatic analysis that showed glucocerebrosidase deficiency in both leukocytes and fibroblasts (Table 2.3.2).

Table 2.3.2. Results obtained in the patient's blood tests.

Parameter	Patient values (nmol/h/mg)	Reference values (nmol/h/mg)
Leukocytes galactosidase	103	73-585
Leukocytes glucocerebrosidase	0.8	2.8-19
Plasma acid phosphatase	4234	10-150
Plasma D-chitotriosidase	29375	10-85
Fibroblasts galactosidase	212	166-2037
Fibroblasts glucocerebrosidase	35	103-552

a) Justify the established diagnosis.
b) How can changes in the performed biochemical analysis be explained?

Clinical case 2.3.3. *Deficiency of α-galactosidase A (Fabry disease)*

A 70-year-old woman had long-standing chronic renal failure, lower limb paralysis due to a stroke 30 years ago, pacemaker implantation 15 years ago due to bradycardia, and a diagnosis of Alzheimer's disease 4 years ago. Plasma assay for α-galactosidase A enzyme activity was 0.54 μmol/L/h (reference value > 2.7 μmol/L/ h) and in leukocytes was 18.4 μmol/mg protein/h (reference value > 1.4 μmol/mg protein/h). Ophthalmologic examination was hampered by the advanced state of Alzheimer's disease. At biomicroscopic examination, she had pseudophaly and cornea verticilata. A diagnosis of α-galactosidase A deficiency (Fabry's disease) was established. Justify the established diagnosis.

2.3.4. **Further questions**

Question 2.3.1.
Briefly describe the process of phosphatidylethanolamine and phosphatidylcholine biosynthesis.

Question 2.3.2.
Does CDP-X (where X is the appropriate alcohol) react with 1,2-diacylglycerol in the primary biosynthetic pathway of phosphatidylcholine, phosphatidylinositol or phosphatidylserine?

Question 2.3.3.
Which of the following is not a precursor or intermediate in sphingomyelin biosynthesis: palmitoyl-CoA, lysophosphatidate, phosphatidylcholine or acyl-CoA?

Question 2.3.4.
Comment on the following statement: "Newborns, especially when premature, may suffer from breathing difficulties due to insufficient synthesis of glycerophospholipids used as surfactants".

3. AMINO ACID AND PROTEIN METABOLISM

CHAPTER CONTENTS

3.1. **Amino acid degradation and Urea cycle**

3.1.1. **Short review**

Amino acid degradation

Muscle proteins constitute the second largest energy reserve in the human body. However, it is the last reserve to be used for ATP production and is only consumed in a long fasting period.

Degradation of dietary or endogenous proteins originates amino acids that can be used in cells for the synthesis of new proteins, nitrogen bases, neurotransmitters, phospholipids, coenzymes, porphyrins, as well as other nitrogenic compounds, besides being degraded for ATP production.

The first step in amino acid degradation is the removal of the nitrogen containing moiety, the amine group, the most toxic component of the molecule. In humans, 86% of the excretion of this nitrogen occurs in the form of urea. Nitrogen may also be removed from amino acids in the form of ammonium ion (2-8%).

The carbon skeleton of amino acids can be used for ATP production, entering directly or indirectly into the Krebs cycle. The carbon skeleton of certain amino acids can also be used to form lipid stores (fatty acids and sterols). These amino acids are called ketogenic. On the other hand, if the carbon skeleton is used for restoring glycogen stores, the amino acids are named glycogenic. Moreover, some amino acids are both glucogenic and ketogenic, as is the case with isoleucine.

The liver is the main organ involved in the metabolism of nitrogen and carbon skeletons, being the most active organ in both the synthesis and degradation of amino acids. Amino acids can enter the cell by several mechanisms, one of which is the γ-glutamyl pathway, which involves the formation of γ-glutamyl conjugates of amino acids that can enter the cell. Once inside the cell, most of the nitrogen is removed from amino acids by hepatic enzymes that transaminate them or oxidatively remove nitrogen, producing NH_4^+.

Transaminations are catalysed by aminotransferases that use pyridoxal-phosphate (PLP) as a coenzyme. In these reactions, transfer of the amino group of an amino acid to a ketoacid occurs, leading to the production of a new amino acid and a ketoacid. One of the amino acid/ketoacid pairs of these reactions is fixed, being always the glutamate/α-ketoglutarate pair.

Since there is no elimination but only transfer of the amino group, transaminations are coupled with the oxidative deamination catalysed by glutamate dehydrogenase. In this reaction, glutamate is converted into its corresponding ketoacid, α-ketoglutarate, with production of NADH and elimination of the amino group in the form of ammonium ion. Another oxidative deamination that may occur, catalysed by the L-amino acid oxidase, involves the conversion of the amino acid into its corresponding ketoacid, with formation of $FMNH_2$ and release of ammonium ion.

In addition to the action of the above enzymes, serine and threonine may undergo non-oxidative deamination through the action of serine-threonine dehydratase. The products of this reaction include pyruvate or α-ketobutyrate, with the elimination of ammonium ion.

The ammonium released in the described reactions can be used to form certain amino acids such as glutamate, by glutamate dehydrogenase, or glutamine from glutamate by glutamine synthetase. In this way, it is possible to reduce plasma ammonium levels since this ion is highly toxic. However, most ammonium released in amino acids catabolism is converted into urea by the urea cycle, which is easily excreted in the urine.

Urea cycle

The purpose of the urea cycle (Fig. 3.1.1) is the conversion of the ammonium ion released during amino acid catabolism, into a less toxic form of nitrogen elimination, to urea. The synthesis of urea begins with:

1. Synthesis of carbamoyl phosphate. This reaction is catalysed by carbamoyl phosphate synthetase, which needs N-acetylglutamate as a cofactor. Synthesis of a carbamoyl phosphate molecule uses ammonium ion and carbon dioxide and consumes 2 ATPs. This enzyme is found in the mitochondria and together with citrulline synthetase constitute the two steps of this metabolic pathway that occur in mitochondria. All other steps occur in the cytoplasm.

The following steps in urea production are:

2. Citrulline synthase catalyses the second step of the synthesis of urea in which citrulline is produced from ornithine and carbamoyl phosphate.

3. In the cytosol, citrulline is condensed with aspartate to form argininosuccinate by argininosuccinate synthetase.

4. Argininosuccinate is then broken down into arginine and fumarate. Fumarate can be used in the Krebs cycle, resulting in OAA. This molecule, by transamination, gives rise to aspartate that will be used in the urea cycle.

5. Arginine will serve as a substrate for arginase, producing urea and ornithine thus completing the urea cycle.

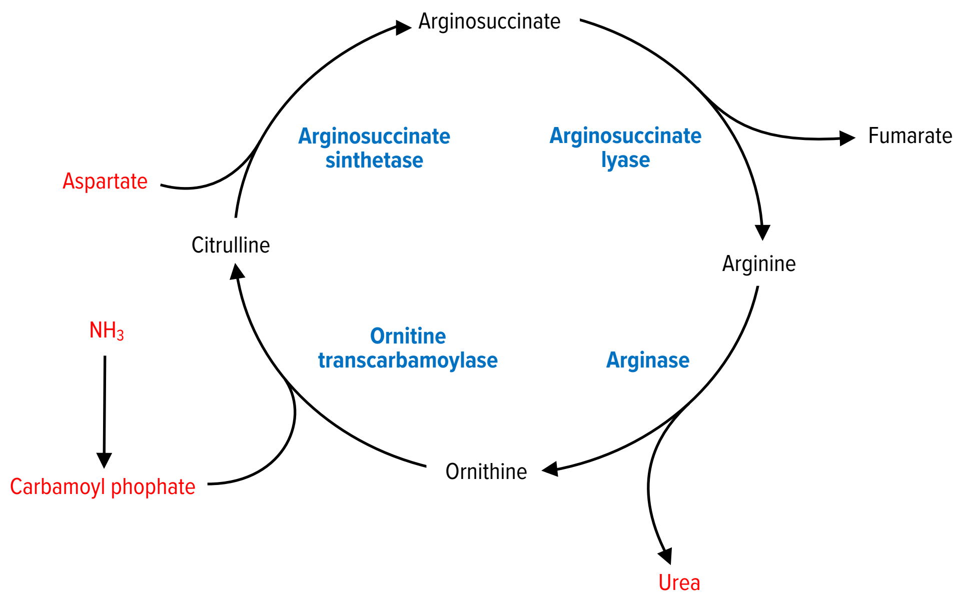

Fig. 3.1.1. Scheme of the Urea cycle.

The synthesis of one urea molecule consumes the equivalent of 4 ATPs.

Regulation of the urea cycle can be done in a short term by allosteric activation of the enzyme carbamoyl phosphate synthetase by N-acetylglutamate. High concentrations of arginine stimulate the synthesis of the cofactor N-acetylglutamate, from glutamate and acetyl-CoA. It can still be regulated in the long term by ingested food diet. A high protein intake induces high levels of aminotransferases and urea cycle enzymes, while a low protein diet has the opposite effect.

3.1.2. **Pathologies**

Pyridoxine deficiency

As pyridoxine (vitamin B6) is present in most foods, dietary deficiency is rare. The richest sources of vitamin B6 include fish, beef liver and other organ meats, potatoes and other starchy vegetables, and non-citrus fruits. Notably, the vitamin B6 found in meat is more easily absorbed and used by the body than the vitamin B6 from plants and vegetables. This can be important for those who exclusively follow a vegetarian or vegan diet. Inadequate vitamin B6 status is often associated with low concentrations of other B vitamins such as vitamin B12 and folic acid (vitamin B9).

Secondary disability can result from several conditions. Individuals with compromised kidney function who are receiving dialysis or who have had a transplant are more likely to be deficient in vitamin B6. This is due to the increased metabolic clearance of PLP, which is the biologically active form of vitamin B6. Additionally, those with autoimmune disorders such as celiac disease and inflammatory bowel disease (e.g., Crohn's disease and ulcerative colitis) are more likely to have deficiencies because of decreased absorption from the gut and increased inflammation caused by the underlying disease. Lastly, those with alcohol dependence are also at a higher risk. The alcohol is broken down into acetaldehyde, which lowers the total PLP. Another well-known cause of pyridoxine deficiency is the use of isoniazid, an antibiotic used to prevent and treat tuberculosis. Isoniazid causes a depletion of vitamin B6 by inhibiting an enzyme (pyridoxine phosphokinase) necessary for the synthesis of pyridoxine.

Individuals with borderline concentrations of vitamin B6 or mild deficiency may have no signs or symptoms for months or even years. Individuals with pyridoxine deficiency can often experience skin problems such as seborrheic dermatitis, characterized by a red, itchy rash on the scalp, face, neck, and upper chest. It usually has an oily, scaly appearance and can cause swelling or white patches. One of the functions of vitamin B6 is to produce collagen, a skin protein that provides strength and elasticity. Without adequate levels of vitamin B6, the skin barrier can breakdown, leading to dermatological conditions. Cheilosis (i.e., peeling of the lips and cracks at the corners of the mouth) and glossitis (i.e., swollen tongue) are also characteristic signs. In the case of glossitis, the tongue becomes enlarged, smooth, and red as it loses the papillae, or raised bumps, on the tongue.

Importantly, vitamin B6 deficiency can cause nerve damage, leading to burning pain, twinges, and tingling in the arms, legs, hands, and feet. It can feel like “pins and needles” and can also result in clumsiness, balance problems, and difficulty walking. Individuals may also experience confusion, depression, electroencephalogram abnormalities, seizures, and a weakened immune system.

Vitamin B6 is needed to produce antibodies and white blood cells, such as T cells, needed to fight infection. Without these cells that regulate immune function, the organism is unable to respond properly. In addition, microcytic anaemia, characterized by the presence of small, pale red blood cells, can also occur as the body needs vitamin B6 to produce haemoglobin and increase the amount of oxygen carried by haemoglobin.

Those with prolonged or severe pyridoxine deficiency may experience peripheral neuropathy and a pellagra-like syndrome. Pellagra is a disease that results from deficiency of vitamin B3 (niacin), and is characterized by dermatitis, dementia and diarrhoea.

In babies, vitamin B6 deficiency causes irritability, abnormal hearing and seizures.

Argininosuccinic aciduria

The degradation of amino acids leads to the production of ammonium, a very toxic form of nitrogen elimination. This ammonium is converted into urea, a less toxic compound, through the urea cycle. Argininosuccinic aciduria is a genetic disorder with autosomal recessive transmission that results from a defect in one enzyme of the urea cycle. This is a monogenic disorder, with a prevalence of 1 in 70,000 live births, that manifests as a multifactorial disease at the phenotypic level.

Mutations in the gene encoding argininosuccinate lyase (*ASL* gene), present on chromosome 7q11, are responsible for the development of this condition. This enzyme catalyses the step of the urea cycle where argininosuccinate is converted into arginine and fumarate (Fig. 3.1.1). If this transformation does not occur, hyperammonaemia will develop, leading to encephalopathy and respiratory alkalosis. The signs develop in the first weeks of life and include mental and physical retardation, convulsions, skin lesions, increase in liver size and sudden unconsciousness.

Two different forms of this disease have been identified differing in the age of symptom’s development: an early-onset and a late-onset type. The early-onset or severe form results from the complete or nearly complete absence of the ASL enzyme and develops in the neonatal period, within 24-72 hours after the birth, usually after protein ingestion. The main characteristics of this form are refusal to eat, lethargy, lack of appetite, vomiting and irritability. Affected children may also show cerebral oedema and hepatomegaly, together with breathing problems. The condition might progress to the development of chronic liver disease and, due to the high level of ammonium in blood, can lead to coma.

The late-onset or milder form results from a partial loss of the ASL enzyme and develops later during infancy or childhood or even in adulthood. Affected children can have failure to thrive, ataxia and lethargy. Some individuals with this type of the disease may be asymptomatic. Both forms can be associated with long-term health problems such as liver dysfunction and neurocognitive disabilities.

Arginase deficiency

Arginase is an enzyme of the urea cycle, responsible for the conversion of arginine into ornithine with the release of urea. As the deficiency of other urea cycle enzymes, the deficiency of arginase leads to the slow down or blockage of this metabolic pathway with consequent increase in plasma levels of ammonium. However, unlike other urea cycle disorders that manifest with acute hyperammonemic crisis, arginase deficiency is a chronic condition that develops in late childhood by developmental delay and spastic disparesis. Patients may also experience lethargy, irritability, vomiting, ataxia, hepatomegaly and seizures.

This condition is transmitted with an autosomal recessive pattern, being caused by mutations in the *ARG1* gene, coding for arginase. This is the least common of all urea cycle disorders with an incidence of 1 in 300,000-1,000,000 births.

N-acetyl glutamate synthetase deficiency

This is another condition that results in the inability to convert ammonium in urea. The N-acetyl glutamate synthetase enzyme is responsible for the production of N-acetyl glutamate, essential activator of the enzyme carbamoyl-phosphate synthetase. This one catalyses the conversion of ammonium in carbamoyl-phosphate that then proceeds to the urea cycle.

This is an autosomal recessive disorder that results from mutations in the *NAGS* gene that codes for N-acetyl glutamate synthetase. The deficiency of this enzyme will lead to the accumulation of the neurotoxic compound ammonium. This enzyme deficiency can be partial or total leading to milder or severe forms of the disease, respectively. In the severe form, symptoms develop in the neonatal period while the milder form is only evident during infancy, childhood or even adulthood. Symptoms can vary between affected individuals and include diarrhoea, vomiting, progressive lethargy, hepatomegaly and in more severe cases seizures, confusion and cerebral oedema. These symptoms may evolve to coma due to the hyperammonaemia and to neurological alterations such as learning disabilities and developmental delay. If not treated it can become life-threatening.

3.1.3. **Clinical cases**

Clinical case 3.1.1. *Pyridoxine deficiency*

Which metabolic pathway(s) can be affected by a poor pyridoxine diet? Justify.

Clinical case 3.1.2. *Hyperammonaemia*

Consider a clinical condition of hyperammonaemia:
a) What can be the cause for this clinical condition?
b) Which are the observed symptoms?
c) What type of treatment should be used?
d) Can this condition result from hormonal changes?

Clinical case 3.1.3. *Hyperammonaemia: arginase deficiency*

During a week the development of a newborn seemed normal. Since then, he developed progressive lethargy and survival problems with recurrent episodes of vomiting and irritability. He was admitted to the hospital in metabolic disequilibrium, and a blood sample was tested revealing the results presented below. Later, the baby fell into a coma. However, treatment was started immediately, allowing to revert the phenotype and his life was no longer in danger.

Table 3.1.1. Results observed in patient blood tests.

	pH	Urea (mmol/L)	NH_3 (μmol/L)	Glutamine (μmol/L)	Citrulline (μmol/L)	Arginino succinic acid
Patient values	7.55	0.7	420	1.5	1200	Undetectable
Reference values	7.35-7.45	2.5-7.0	12-60	0.45-0.75	10-30	Undetectable

a) What is the most likely diagnosis in this individual. Explain.
b) What could be responsible for the episodes of lethargy, vomiting and irritability that led to hospital admission. Explain why.

Clinical case 3.1.4. *Hyperammonaemia*

A patient having severe liver dysfunction, with digestive haemorrhage and in coma state, was submitted to blood tests:

Table 3.1.2. Results of patient blood tests.

Parameter	Patient values	Reference values
Total cholesterol	90 mg/dL	130-230 mg/dL
Total proteins	4.5 mg/dL	5-8 mg/dL
Albumin	2 mg/dL	3-5 mg/dL
Globulins	4 mg/dL	2-3 mg/dL
Ammonium	40 IU	18 IU
Prothrombin	70%	100%

a) Based on the metabolic role of liver, discuss in detail the observed changes.
b) Administration of arginine could lower plasma ammonium levels. Explain why.

Clinical case 3.1.5. *Hyperammonaemia*

A 29-year-old boy, with a 6-year history of recurrent episodes of nausea, vomiting and strange behaviour, goes to the doctor. He observes behaviour changes such as loss of sense of reality, glazed eyes, constantly crying and babbling, and with agitated behaviour. Observed clinical data were the following:

Table 3.1.3. Results observed in patient blood tests.

Parameter	Patient values (μmol/L)	Reference values (μmol/L)
Carbamoyl phosphate synthetase	1.96	1.63
Ornithine transcarbamoylase	8.86	34.60
Argininosuccinate synthetase	Not determined	0.33
Argininosuccinate lyase	1.64	1.00
Arginase	434	274
Plasma alanine	357-783	230-510
Plasma glutamine	1223-1400	390-650
NH_4^+	70	45

What is the most likely diagnosis? Explain observed blood tests.

Clinical case 3.1.6. *N-acetyl glutamate synthetase deficiency*

Consider a newborn with a diagnosed hereditary hyperammonaemia whose citrulline plasma levels were low and hepatic biopsy detected absence of N-acetylglutamate synthetase activity.
a) Which enzyme(s) of the urea cycle are deficient?
b) What is the relationship between the enzyme N-acetylglutamate synthetase and the urea cycle?

3.1.4. **Further questions**

Question 3.1.1.

For cells to catalyse transamination reactions between the 20 different amino acids and corresponding α-keto acids,

amino acid 1 + α-ketoacid 2 $\leftrightarrows$ α-ketoacid 1 + amino acid 2

about 202 = 400 enzymes would be needed. However, only about 20 transaminases are found. Explain why using the mechanism of the following reaction:

oxaloacetate + alanine $\leftrightarrows$ aspartate + pyruvate

Question 3.1.2.

Describe what happens in reactions catalysed by aminotransferases.

Question 3.1.3.

What is the essential cofactor for transaminases (aminotransferases)?

Question 3.1.4.

What products can be produced from an amino acid by the action of an aminotransferase?

Question 3.1.5.

What are transamination reactions? Give an example. What vitamin is involved? Which amino acids do not undergo transamination?

Question 3.1.6.

What are reductive amination/oxidative deamination reactions? Give an example.

Question 3.1.7.

Explain how PLP facilitates amino acid catabolism.

Question 3.1.8.

Which of the following intermediates can be produced from an amino acid by the action of a transaminase: α-ketoglutarate, oxaloacetate, pyruvate and/or succinate?

Question 3.1.9.

What is the meaning of essential amino acid. Which amino acids belong to this group?

Question 3.1.10.

Can a diet rich in alanine but deficient in aspartate lead to serious complications? Explain.

Question 3.1.11.

Write a balanced equation for the synthesis of glucose from aspartate via oxaloacetate. Indicate the coenzymes involved in the process.

Question 3.1.12.

Tyrosine is not an essential amino acid, but individuals with a genetic deficiency in phenylalanine hydroxylase require tyrosine in their diet for normal growth. Explain why.

Question 3.1.13.
Can amino acids be used as an energy source under anaerobic conditions? Explain why.

Question 3.1.14.
Glutamine is degraded to NH_4^+, CO_2 and H_2O. How many moles of ATP can be generated from one mole of this amino acid?

Question 3.1.15.
Describe the degradation of glutamate to CO_2.

Question 3.1.16.
Explain the role of alanine in a situation of physical exertion.

Question 3.1.17.
Amino acids are commonly classified as being glycogenic or ketogenic. What is the meaning of these classifications?

Question 3.1.18.
In general, what is the fate of the carbon and nitrogen in amino acids?

Question 3.1.19.
Are non-essential amino acids for humans those not incorporated into protein, not needed in the diet if enough precursors are present, the same for adults and children, or not generally supplied by the diet?

Question 3.1.20.
Does ammonia production in the reaction catalysed by the enzyme glutamate dehydrogenase require the participation of NADH or NADPH? Can that reaction be reversed to consume ammonia if it is present in excess? Is this reaction favoured by high levels of ATP or GTP? Is it inhibited when gluconeogenesis is active?

Question 3.1.21.
What are the metabolic products obtained in the degradation of amino acids?

Question 3.1.22.
Describe how each of the following amino acids is degraded: lysine, glutamate, glycine, aspartate, tyrosine, and alanine.

Question 3.1.23.
In individuals with phenylketonuria, is tyrosine an essential amino acid? Explain.

Question 3.1.24.
The urine of an individual who is deficient in phenylalanine hydroxylase has high levels of phenyllactate. Explain why.

Question 3.1.25.
Propionyl-CoA and L-methylmalonyl-CoA are intermediates in the conversion of which of the following to succinyl-CoA: histidine, isoleucine, and/or valine?

Question 3.1.26.
Amino acids whose degradation produces α-ketoglutarate include: arginine, glutamine, leucine and/or histidine?

Question 3.1.27.
Amino acids whose degradation gives rise to acetyl-CoA include: arginine, glutamine, leucine and/or histidine?

Question 3.1.28.
Amino acids whose degradation yields oxaloacetate include: arginine, glutamine, leucine and/or histidine?

Question 3.1.29.
Amino acids whose degradation gives rise to acetyl-CoA include: cysteine, glycine, serine and/or histidine?

Question 3.1.30.
Illustrate the pathway of alanine degradation to a Krebs cycle intermediate. Indicate required enzymes and cofactors.

Question 3.1.31.
Concerning the degradation of amino acids, classify as true or false the following statements:
a) Urea is formed in the liver being later converted to ammonia and thus excreted.
b) Alanine and glutamine are vehicles for transporting ammonia to the liver.
c) The carbon skeleton is always converted to glucose or acetyl- CoA and 3-phosphoglycerate.

Question 3.1.32.
Almost all organisms produce ammonia. Where does it come from and how is it eliminated from the body?

Question 3.1.33.
What organ is involved in the synthesis of urea? Where are located the enzymes responsible for the urea cycle?

Question 3.1.34.
What is the function of the urea cycle? Indicate the origin of each of the atoms of the urea molecule that is released in the cycle (except for the hydrogens). Although most steps in the cycle take place in the cytoplasm, some steps take place in the mitochondrial matrix. Is there any reason for this?

Question 3.1.35.
The urea cycle occurs partially in the cytosol and partially in the mitochondria. Indicate the reactions that take place in the cytosol and those that take place in the mitochondria.

Question 3.1.36.
Symptoms of a partial deficiency of a urea cycle enzyme can be alleviated with a low-protein diet. Explain why.

Question 3.1.37.
Individuals with very high protein diets are advised to drink plenty of water. Explain why.

Question 3.1.38.
What is the essential cofactor to the mitochondrial carbamoyl phosphate synthetase enzyme that catalyses the first step of urea synthesis?

Question 3.1.39.
A group of cats were submitted to fasting and then divided into three sub-groups: one placed on an arginine-free diet; another on an arginine diet; and the later on a diet with ornithine instead of arginine. The first group developed ammonia toxicity overtime.
a) Explain why the cats were fasted.
b) Why did cats on a diet without arginine show ammonia toxicity?
c) Why cats on a diet without arginine but with ornithine did not show ammonia toxicity?

Question 3.1.40.
Explain why a high concentration of ammonia slows down the Krebs cycle.

Question 3.1.41.
Comment on the following statement: "The urea cycle is an important metabolic detoxification cycle".

Question 3.1.42.
Explain the importance of aspartate in the excretion of ammonia in ureotelic organisms.

Question 3.1.43.
Can a clinical condition of hyperammonaemia result from a failure of an enzyme in the urea cycle? Is it treated with a high-protein diet? Can it result from a failure of the β-cells of the pancreas? Is it devoid of effects on the central nervous system?

Question 3.1.44.
Why is it that urea excretion greatly increases in the first few days of a prolonged fasting period and decreases dramatically if it goes on for a longer time?

Question 3.1.45.
Hyperammonaemia and hypoalbuminemia are characteristic of patients with liver failure. Explain why.

3.2. **Amino acid synthesis**

3.2.1. **Short review**

The human being can synthesize some, but not all, of the amino acids he needs. Non-essential amino acids may be obtained from intermediates of the central metabolism or, in some cases, from dietary amino acids.

3-Phosphoglycerate is precursor of the synthesis of serine and glycine. Cysteine can be produced from serine or using methionine (an essential amino acid) from the diet. The amino acids alanine, glutamate and aspartate can be obtained by transamination of pyruvate, α-ketoglutarate and OAA, respectively. Asparagine can be obtained from aspartate and glutamine from glutamate. Glutamate can still be used for the formation of proline and arginine.

Finally, tyrosine is synthesized in the body from an amino acid in the diet, phenylalanine, by the action of the enzyme phenylalanine hydroxylase. Deficiency of this enzyme leads to the pathology phenylketonuria, which is manifested by excretion of phenylketonic compounds in urine, albinism and deep mental disabilities. Nowadays, the early detection of this disease is performed, which allows the prevention of the development of symptoms, through changes in the food diet.

There is still a group of amino acids that cannot be produced in humans and are therefore considered essential: phenylalanine, isoleucine, leucine, lysine, methionine, threonine, tryptophan and valine. The amino acids histidine and arginine are also considered essential during growth.

3.2.2. **Pathologies**

Methylmalonic acidaemia

Methylmalonic acidaemia was first reported in 1967. It is a genetically heterogeneous disorder of methylmalonate and cobalamin (vitamin B12) metabolism. According to the phenotype, two main forms of the disease have been identified, including isolated methylmalonic aciduria and combined methylmalonic aciduria and homocystinuria. Isolated methylmalonic aciduria is due to defects of methylmalonyl-CoA mutase or the synthesis of its coenzyme adenosylcobalamin. On the other hand, combined methylmalonic aciduria and homocystinuria is characterized by high plasma homocysteine and decreased levels of adenosylcobalamin and methylcobalamin coenzymes. In addition, some subtypes of benign methylmalonic acidaemia have been described.

Patients with this disorder have significant morbidity and mortality, and a poor long-term survival prognosis. Elevated urea and urinary methylmalonic acid are predictors of adverse patient outcomes. The onset of manifestations of methylmalonic acidaemia varies from the neonatal period to adulthood. Affected children often have anorexia, failure to thrive, hypotonia, developmental delay, progressive renal failure, functional immunological impairment, optic nerve atrophy, and hematologic abnormalities. Atypical and "benign"/adult methylmalonic acidaemia is associated with increased, albeit mild, urinary excretion of methylmalonate. However, it is uncertain whether individuals with these conditions will develop symptoms.

Phenylalanine hydroxylase deficiency (Phenylketonuria)

Phenylketonuria is a disorder usually caused by mutations in the gene coding for phenylalanine hydroxylase (*PAH* gene). Phenylalanine is an essential amino acid, meaning that it cannot be produced in the human body (must be obtained from the food diet), being converted to tyrosine by the action of PAH in the organism (Fig. 3.2.1). The deficiency of this enzyme leads to the accumulation of phenylalanine. Brain cells are very sensitive to increased levels of phenylalanine and brain damage may develop. The excess of phenylalanine is converted into three phenylketonic compounds, phenyllactate, phenylpyruvate and phenylacetate, that start to be excreted in the urine. The name of this disorder comes from the excretion of these compounds in urine.

Moreover, the described reaction is the only source of tyrosine in the human body, so this amino acid becomes essential for these patients. Tyrosine is a precursor of the synthesis of melanin and neurotransmitters. Consequently, the lack of tyrosine leads to the development of albinism and intellectual disability.

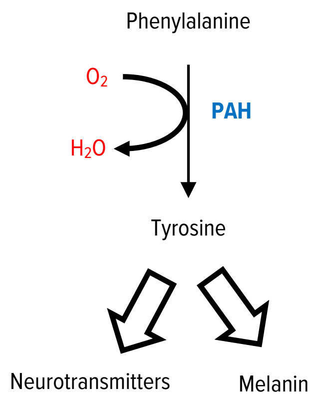

Fig. 3.2.1. Reaction catalysed by phenylalanine hydroxylase (PAH).

Three hundred different alleles of the *PAH* gene are responsible for the development of phenylketonuria. The combination of alleles present in each patient leads to different degrees of symptoms, that can vary from mild to severe. In the most severe form, classical phenylketonuria, the newborn looks normal until a few months old but then starts to develop seizures, delayed development, behaviour problems and psychiatric disorders. The mild forms show lower risk of brain damage and in some cases the only developed signal is the excretion of phenylketonic compounds in the urine.

There is a high variability in the prevalence of this disease among different geographic regions. In the USA it is 1 in 10,000-15,000 newborns.

The children of mothers having phenylketonuria with uncontrolled phenylalanine levels have a high risk of intellectual problems, since they were exposed to high levels of this amino acid before birth.

Kwashiorkor´s disease

Kwashiorkor´s disease is characterized by peripheral oedema in a person suffering from starvation. Oedema results from a loss of fluid balance between hydrostatic and oncotic pressures across the walls of capillary blood vessels. Albumin concentration contributes to oncotic pressure, allowing the body to maintain fluids within the vasculature. Children with Kwashiorkor´s disease have profoundly low levels of albumin and, as a result, are intravascularly depleted. Subsequently, antidiuretic hormone (ADH) increases in response to hypovolemia, resulting in oedema. Plasma renin also responds aggressively, causing sodium retention. These factors contribute to oedema.

Kwashiorkor´s disease is also marked by low levels of glutathione (antioxidant). This is thought to reflect high levels of oxidative stress in the malnourished child. High levels of oxidants are commonly seen during starvation and are even seen in cases of chronic inflammation. A reversal measure would be to improve nutritional status and sulphur-containing antioxidants.

The aetiology of Kwashiorkor´s disease is unknown, but diets based primarily on maize, cassava or rice are often associated with the disease. It was previously believed to be due to protein deficiency and low levels of antioxidants and aflatoxins. There is evidence for these associations, however, treatment using diets rich in protein and antioxidants has not been successful. Aflatoxin, formerly considered the aetiology of Kwashiorkor´s disease, is not always associated with the disease in certain populations. Some factors that are consistently associated with this illness include recent weaning, recent infection (particularly measles), and childhood disruptions (death of parents, temporary home environment, poverty).

The clinical manifestations of Kwashiorkor´s disease include: peripheral pitting oedema that begins in dependent regions and proceeds cranially; marked muscle atrophy; abdominal distension (with or without dilated bowel loops and hepatomegaly); round face (prominence of the cheeks, or "moon facies"); thin, dry, peeling skin with confluent areas of scaling and hyperpigmentation; dry, full, hypopigmented hair that falls out or is easily plucked; hepatomegaly (from fatty liver infiltrates); growth retardation; psychic changes (anorexia, apathy); skin lesions/dermatitis (perineum, groin, limbs, ears, armpits); subcutaneous fat retention with loose inner inguinal skin folds.

Some complications of Kwashiorkor´s disease include: hepatomegaly (resulting from the fatty liver); cardiovascular system collapse/hypovolemic shock; urinary tract infections; abnormalities of the gastrointestinal tract including atrophy of the pancreas with subsequent glucose intolerance, atrophy of the mucosa of the small intestine; lactase deficiency; ileus; bacterial overgrowth, which can lead to bacterial septicaemia and death; loss of immune function, antioxidant function, subsequent infections, septic shock, and death; endocrinopathies where insulin levels are decreased, growth hormone is increased, but insulin-like growth factor levels are reduced, leading to insulin intolerance; metabolic disturbances and hypothermia; impaired cellular functions, including endothelial dysfunction; electrolyte abnormalities.

In Kwashiorkor´s disease, mortality decreases as the age of disease onset increases. Children may not grow or develop abnormally and may remain stunted. There can be serious complications when treatment is not started earlier in the course of the illness, including shock, coma, and permanent physical and mental disabilities. Kwashiorkor´s disease can be fatal if left untreated.

Tetrahydrobiopterin (BH4) deficiency

Tetrahydrobiopterin (BH4), synthesized from GTP through several enzymatic reactions, is a cofactor for PAH. BH4 deficiency causes the body to accumulate an abnormally high level of phenylalanine. In addition, BH4 is also a cofactor for tyrosine hydroxylase and tryptophan hydroxylase, which are involved in the biosynthesis of dopamine and serotonin, respectively. Therefore, patients with hyperphenylalaninemia resulting from BH4 deficiency also manifest neurological findings related to deficiencies of these neurotransmitters.

BH4 deficiency is caused by pathogenic variants in any of several genes, including the *GCH1*, *PCBD1*, *PTS*, and *QDPR* genes, being inherited in an autosomal recessive pattern. Four enzyme deficiencies that lead to defective BH4 formation cause hyperphenylalaninemia with concomitant dopamine and serotonin deficiencies: autosomal recessive GTP cyclohydrolase I deficiency, 6-pyruvoyl-tetrahydropterin synthase deficiency, dihydropteridine reductase deficiency, and pterin-4-α-carbinolamine dehydratase deficiency. More than half of the reported patients had 6-pyruvoyl-tetrahydropterine synthase deficiency. Autosomal dominant forms of GTP cyclohydrolase I deficiency and sepiapterin reductase deficiency result in neurotransmitter deficiencies without hyperphenylalaninemia.

Symptoms can range from very mild to severe. Infants with BH4 deficiency appear normal at birth but may develop neurological symptoms. In 1-3% of infants with hyperphenylalaninemia, the defect resides in one of the enzymes required for production or recycling of the cofactor BH4. If these infants are misdiagnosed as having phenylketonuria, they may deteriorate neurologically despite adequate control of plasma phenylalanine. Plasma levels of phenylalanine may be as high as classic phenylketonuria or may be in the milder range. However, the clinical manifestations of neurotransmitter disorders differ greatly from those of phenylketonuria. Neurological symptoms of neurotransmitter disorders usually manifest in the first few months of life and include extrapyramidal signs (choreoathetotic or dystonic limb movements, axial and truncal hypotonia, hypokinesia), feeding difficulties, and autonomic abnormalities. Intellectual disability, convulsions, hypersalivation and swallowing difficulties are also observed. Symptoms are usually progressive and often have a marked diurnal fluctuation.

Prognosis and outcome depend heavily on the age of diagnosis and introduction of treatment, but also on the specific nature of the pathogenic variant and the resulting enzyme defect. Diagnosis is based on symptoms, clinical examination, and blood and urine tests. Despite the low incidence of defects in BH4 synthesis, all new-borns with hyperphenylalaninemia detected by new-born screening should be screened for defects in BH4 synthesis. BH4 deficiency and the responsible enzyme defect can be diagnosed by several studies.

BH4 may be a scavenger of ROS. It can react with various reactive species such as hydroxyl and thiyl radicals and render them harmless. Decreased levels of BH4 in the cerebrospinal fluid have also been observed in diseases such as Parkinson's, autism, depression and Alzheimer's.

3.2.3. **Clinical cases**

Clinical case 3.2.1. *Methylmalonic aciduria*

A patient suffering from methylmalonic aciduria (high levels of methylmalonic acid) has high levels of homocysteine and low levels of methionine in blood and tissues. Folic acid levels are normal.
a) Which vitamin is deficient?
b) How can this deficiency produce the described symptoms?
c) Why is this vitamin deficiency more likely to occur in a person having a strict vegetarian diet?

Clinical case 3.2.2. *Phenylalanine hydroxylase deficiency (Phenylketonuria)*

A 10-year-old child was hospitalized due to a series of symptoms. Among them are an agitated and aggressive behaviour, a delay in psychomotor development, convulsions, microcephaly, and reduced growth. This child also showed yellowish hair, light and extremely dry skin. Neurological tests demonstrated mental disability. Serum level of phenylalanine was 20 mg/dL (reference value: 1.2-4.0 mg/dL).
a) What is the diagnosis of this clinical picture?
b) Which treatment strategies should be used?

Clinical case 3.2.3. *Phenylalanine hydroxylase deficiency (Phenylketonuria)*

The urine of an individual with phenylalanine hydroxylase deficiency had high levels of phenyl-lactate.
a) Explain why.
b) For this patient tyrosine is an essential amino acid. Explain why.

Clinical case 3.2.4. *Kwashiorkor's disease*

One of the symptoms present in Kwashiorkor's disease, the disease of protein deficiency in children's diet, is skin and hair depigmentation. Explain the biochemical base of this symptom.

Clinical case 3.2.5. *Tetrahydrobiopterin deficiency*

Individuals that cannot produce tetrahydrobiopterin must take supplements of 3,4-dihydroxyphenylalanine (L-DOPA) and 5-hydroxytryptophan. Explain why.

3.2.4. **Further questions**

Question 3.2.1.
Write a balanced equation for the synthesis of alanine from glucose.

Question 3.2.2.
Describe the biosynthesis of glutamate from pyruvate.

Question 3.2.3.
What are the metabolic precursors of non-essential amino acids?

Question 3.2.4.
Many of the most used herbicides inhibit the synthesis of aromatic amino acids. Explain why these compounds can be used around animals.

Question 3.2.5.
Why is the enzyme glutamine synthetase so important in mammals?

Question 3.2.6.
Alanine is being synthesized from its metabolic precursors. With the compounds below make a table with two columns. The first column with the compounds that are precursors, and the second with the compounds that are not precursors of alanine:
- phosphoenolpyruvate – glycogen
- acetyl-CoA – carbamoylphosphate
- glucose - glyceraldehyde-3-phosphate
- malate – malonyl-CoA
- palmitic acid – acetoacetate

Question 3.2.7.
Using reactions, illustrate how α-ketoglutarate can be converted to glutamate through two pathways. Indicate required enzymes and cofactors.

Question 3.2.8.
Illustrate the pathways of synthesis of the following amino acids: glutamine, methionine, threonine, glycine, and cysteine.

Question 3.2.9.
The amino acids glutamine and glutamate are central to amino acid metabolism. Explain why.

Question 3.2.10.
Identify the compounds designated by the letters A, B, C and D and indicate which reaction occurs in the conversion of A to aspartate. Indicate another metabolic pathway or reaction in which the compound designated by the letter C plays an identical role.
A + Glutamate → B + Aspartate
C + Aspartate + ATP → D + Asparagine + AMP + PP_i

Question 3.2.11.
Illustrate the synthesis pathway of asparagine and glutamine from a Krebs cycle intermediate. Indicate required enzymes and cofactors.

Question 3.2.12.
Give two examples of amino acid metabolic disorders citing which process is affected, the deficient enzyme and the developed symptoms.

Question 3.2.13.
Individuals who cannot produce tetrahydrobiopterin must take L-DOPA and 5-hydroxytryptophan supplements. Explain why.

3.3. **Haemoproteins**

3.3.1. **Short review**

Haemoglobin and myoglobin

Haemoglobin and myoglobin are haemoproteins, proteins with the haem prosthetic group. They are oxygen-binding proteins, that carry (haemoglobin) or store (myoglobin) oxygen.

The haem group consists of an iron in the centre of a tetrapyrrolic ring, protoporphyrin IX. In the haem group, iron is bound to four nitrogen atoms in the centre of the protoporphyrin ring. However, iron forms six bonds. In haemoglobin and myoglobin, the 5^{th} bond is for the nitrogen of histidine, and the 6^{th} bond for oxygen.

Haemoglobin is a spherical molecule present in red blood cells, with the function of transporting oxygen from the lungs to all tissues. Haemoglobin contains four subunits (2 α subunits and 2 β subunits). Each subunit contains a haem group that reversibly binds oxygen.

Myoglobin is a reservoir for oxygen in skeletal and cardiac muscle cells, giving these tissues their characteristic red colour. Myoglobin is a simple polypeptide chain that contains 8 α-helix sections, with a haem group that reversibly binds oxygen.

Although haemoglobin and myoglobin bind oxygen reversibly, haemoglobin has a complex structure and more complicated binding properties. Interactions between subunits are affected by ligand binding.

When haemoglobin is oxygenated, the saline bridges and certain hydrogen bonds break, while the $\alpha_1\beta_1$ and $\alpha_2\beta_2$ dimers slide over each other and rotate 15°. The deoxygenated conformation (deoxyHb) is the tense (T) state and the oxygenated (oxyHb) is the relaxed (R) state. Due to the interaction between the subunits, the haemoglobin oxygen saturation curve has a sigmoidal shape.

On the other hand, the myoglobin oxygen saturation curve has a hyperbolic shape. This simple pattern is a consequence of the simple structure of myoglobin and reflects the role of this protein in oxygen storage. The myoglobin oxygen saturation curve is to the left of the haemoglobin oxygen saturation curve, so myoglobin releases oxygen only when the oxygen concentration in muscle cells is very low (during intense exercise). Myoglobin has a higher affinity for oxygen than haemoglobin, so oxygen moves from the blood to the muscles.

The binding of ligands other than oxygen affects the binding properties of oxygen to haemoglobin. The saturation with oxygen decreases when the pH decreases. The binding of H^+ to ionizable groups in haemoglobin decreases saturation with oxygen by converting the haemoglobin to its T-state. H^+ binds preferentially to deoxyHb.

Active cells (requiring large amounts of oxygen for energy production) produce a large amount of H^+ and CO_2. As CO_2 diffuses into the blood, it reacts with water to form HCO_3^- and H^+ (bicarbonate buffer). Small amounts of CO_2 bind to haemoglobin amine groups (forming carbamate or $NHCOO^-$) and the deoxy form of the protein is stabilized.

2,3-Bisphosphoglycerate (BPG) is also an important regulator of haemoglobin function. Although most cells have trace amounts of BPG, red blood cells have considerable amounts. In the absence of BPG, haemoglobin has a high affinity for oxygen. As with H^+ and CO_2, binding to BPG stabilizes deoxyHb. A negatively charged BPG molecule binds to a central haemoglobin cavity that has positively charged amino acids. BPG decreases the affinity between oxygen and haemoglobin.

Haem biosynthesis

Haem synthesis (Fig. 3.3.1), also known as porphyrin synthesis, is an important process that normally occurs in the liver and bone marrow. The first and last three steps are catalysed by mitochondrial enzymes. Intermediate stages occur in the cytosol. The organic portion of the haem is derived entirely from glycine and succinyl-CoA. Reactions occurring in groups attached to the tetrapyrrolic ring involve stained porphyrogen intermediates.

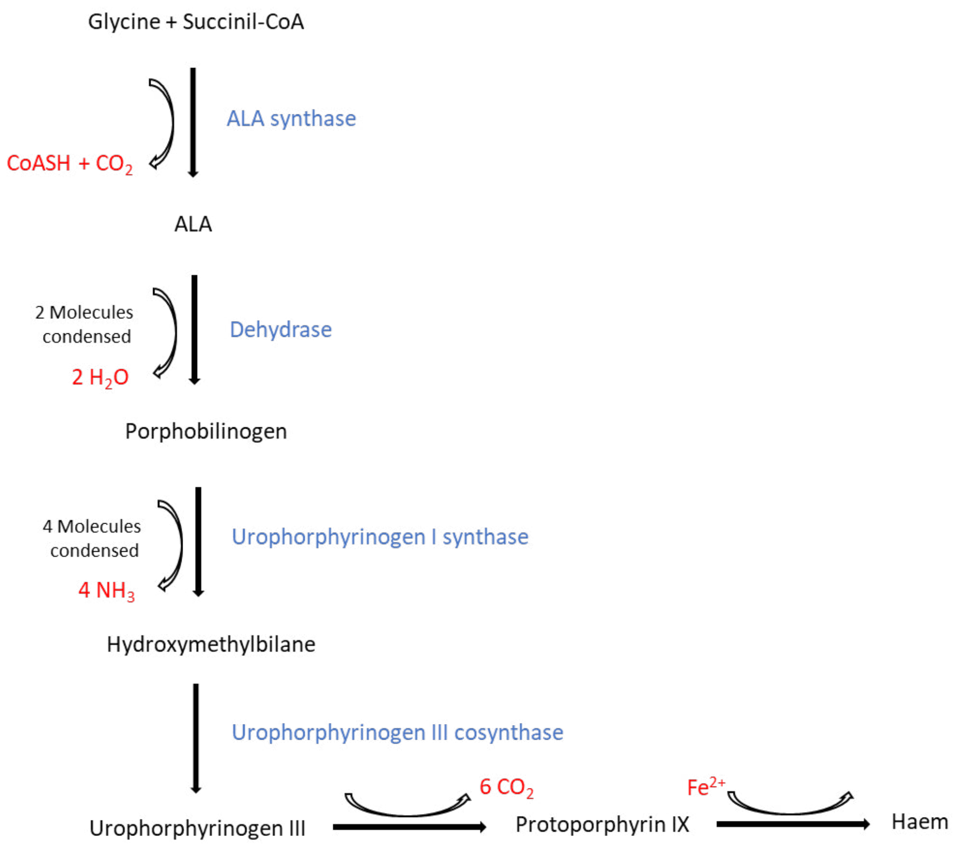

Fig. 3.3.1. Haem biosynthesis. ALA: Aminolevulinate.

Haem catabolism

Erythrocytes have a medium half-life of 120 days. After their destruction, their components are reused: the protein fraction (globin) is reused as such or in the form of their constituent amino acids, as well as the iron.

Bilirubin is the product of a series of reactions that degrade the haem group of several haemoproteins in the cells of the reticuloendothelial system - liver, spleen and bone marrow.

The first step of haem degradation (Fig. 3.3.2) involves a microsomal enzyme system (haem oxygenase) that requires oxygen and NADPH. In the second stage biliverdin is converted to bilirubin in a reaction catalysed by a cytoplasmic enzyme (biliverdin reductase). It is estimated that one gram of haemoglobin generates 35 mg of bilirubin and that the daily production of bilirubin in a healthy individual is around 250-350 mg.

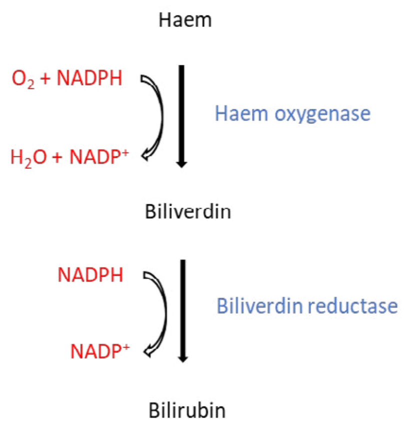

Fig. 3.3.2. Haem catabolism.

Bilirubin is a very toxic compound. It inhibits the synthesis of DNA and proteins and the metabolism of carbohydrates in the brain. Bilirubin, shortly after being produced, is poorly soluble in aqueous media such as plasma, and therefore circulates bound to albumin (up to 25 mg per 100 mL of plasma). The association of bilirubin with plasma albumin protects cells from the toxic effects of this molecule. Excess bilirubin may also bind more loosely to albumin and as such, it readily detaches and diffuses into tissues. Some drugs, such as certain antibiotics, compete with bilirubin for binding to albumin and thereby promote their diffusion into tissues. This bilirubin is called unconjugated or indirect.

In the liver, bilirubin is released from the albumin and captured by a carrier mediated system. Subsequently, bilirubin is conjugated (Fig. 3.3.3) with polar molecules (glucuronic acid), forming bilirubin-monoglucuronate and bilirubin-diglucuronate, which makes it water soluble and thus the possibility of being excreted in the bile. This step occurs in SER, under the action of the enzyme bilirubin UDP-glucuronosyltransferase. The activity of this enzyme can be induced by drugs such as phenobarbital. Most bilirubin excreted in bile is in the form of diglucuronide. After conjugation, bilirubin is excreted in the bile by an active transport system, against a concentration gradient. This bilirubin is called conjugated or direct.

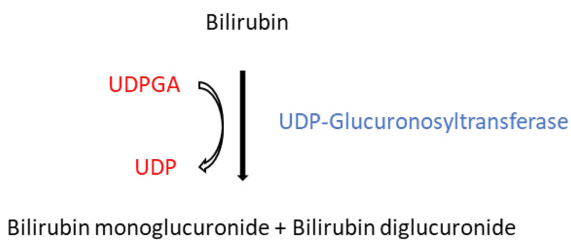

Fig. 3.3.3. Conjugation of bilirubin with glucuronic acid. UDPGA: UDP-glucuronic acid.

Once in the intestine, the glucuronides are separated by the action of specific bacterial enzymes, giving rise to the urobilinogen which is colourless. In the terminal ileum and colon, part of this urobilinogen is absorbed by the liver and then re-excreted, constituting the enterohepatic cycle. In the colon, urobilinogen is oxidized, forming urobilin. This molecule has colour, being responsible for the coloration of the faeces.

3.3.2. **Pathologies**

Iron-deficiency anaemia

Anaemia is the decrease in blood's ability to carry oxygen due to a decrease in the total number of erythrocytes, a decreased haemoglobin concentration per erythrocyte or a combination of both factors.

Iron-deficiency anaemia is the most common cause of anaemia in the world. It is characterized by a reduction or absence of iron stores, low serum iron concentration, low haemoglobin levels, reduced haematocrit, and increased platelet count.

Microscopic examination of a blood smear in patients with iron-deficiency anaemia usually reveals the characteristic findings of microcytic (small in size) and hypochromic (underpigmented) red blood cells. These changes in the red cells result from a decrease in the rate of globin synthesis when haem is not available. A bone marrow aspiration will not reveal the presence of storage iron and the serum ferritin values will be practically zero. The serum transferrin value (expressed as the total iron-binding capacity) will be high (upper normal limits: 410 μg/dL) with a serum iron saturation of less than 16%.

The causes of an iron-deficiency anaemia are an insufficient supply of iron through food, a creation of iron reserves that are too low during foetal life, an abnormally weak digestive iron absorption, major iron losses through chronic bleeding or iron reserves below requirements. It usually affects pregnant women, children of preschool and school age, babies with low birth weight and women of childbearing age.

The management of these patients should include careful examination of the cause and source of bleeding and iron supplementation. The latter is usually supplied in the form of oral ferrous sulphate tablets. Occasionally, intravenous iron therapy may be required. When iron deficiency is severe, transfusion with packed red blood cells may also be indicated.

Haemochromatosis

Haemochromatosis is an inherited recessive disorder associated with a marked inadequate increase in iron absorption. In such cases, serum transferrin can be almost completely saturated with iron. Iron overload may occur in patients, so that the body's iron content can be increased to values of up to 100 g.

When the binding capacity of iron transporters reaches saturation, highly reactive free iron radicals are generated, that can lead to generalized cellular dysfunction. Thus, the combined effects of systemic iron overload and associated oxidative stress in patients with untreated hemochromatosis result in tissue damage that precipitates serious complications, including liver cirrhosis, hepatocellular cancer, cardiomyopathy and diabetes.

Common signs and symptoms include arthralgia, fatigue, cardiac arrhythmias, abdominal pain and skin pigmentation. After the onset of symptoms, hemochromatosis is initially identified by assessing transferrin saturation and ferritin levels. Transferrin saturation > 45% and high serum ferritin concentrations (> 200 ng/mL) indicate hemochromatosis. Next, genetic tests can confirm the specific subtype of the disorder.

This condition is more common in men because women with the abnormal gene are protected in some way by menstrual and fertile events. Treatment options of these patients mainly involve periodic withdrawal of large amounts of blood (regular bloodletting of ~ 450 mL), where the iron is contained in haemoglobin, or iron chelation therapy. The iron chelation therapy is indicated for individuals who cannot support frequent blood donation, for example, patients with heart failure and/or anaemia, or patients with acute iron toxicity. Three common iron chelators for clinical use are deferoxamine, deferiprone and deferasirox.

Porphyrias

Porphyrias are a group of rare inherited or acquired metabolic disorders along the haem biosynthetic pathway (Fig. 3.3.1) that may manifest with neurovisceral and/or cutaneous symptoms, depending on the defective enzyme. There are different porphyrias (Table 3.3.1), each associated with a specific enzyme deficiency leading to the accumulation of a haem precursor.

Table 3.3.1. Porphyrias.

Disease	Tissue	Enzyme	Activity	Organ pathology
Acute intermittent porphyria	Liver	ALA synthase Porphobilinogen deaminase Δ^4-5α-Reductase	Increased Decreased Decreased	Nervous system
Hereditary coproporphyria	Liver	ALA synthase Coproporphyrinogen oxidase	Increased Decreased	Nervous system and skin
Variegate porphyria	Liver	ALA synthase Protoporphyrinogen oxidase	Increased Decreased	Nervous system and skin
Porphyria cutanea tarda	Liver	Uroporphyrinogen decarboxylase	Decreased	Skin, induced by liver disease
Hereditary protoporphyria	Bone marrow	Ferrochelatase	Decreased	Gallstones, liver disease and skin
Erythropoietic porphyria	Bone marrow	Uroporphyrinogen III cosynthase	Decreased	Skin, appendages and reticuloendothelial system
Lead poisoning	All tissues	ALA dehydrase Ferrochelatase	Decreased Decreased	Nervous system, blood and others

Porphyrias can be divided into cutaneous non-bullous (erythropoietic protoporphyria and X-linked protoporphyria), cutaneous bullous (porphyria cutanea tarda, hepatoerythropoietic porphyria and congenital erythropoietic porphyria) and neurovisceral (acute intermittent porphyria and ALA dehydratase deficiency porphyria). The remaining porphyrias may have cutaneous bullous and visceral symptoms. Of these, hereditary coproporphyria is mainly neurovisceral, while variegated porphyria is mainly cutaneous.

Cutaneous symptoms are a consequence of elevated porphyrins in the bloodstream. These porphyrins react to light; therefore, areas exposed to the sun are affected, producing fragile erosive skin lesions in porphyria cutanea tarda or symptoms of scarring stings and burns in erythropoietic protoporphyria.

Neurovisceral porphyrias are characterized by acute attacks, in which excessive haem production is induced after exposure to a trigger. An acute attack usually presents with severe abdominal pain, vomiting and tachycardia. Other symptoms that may appear include hypertension, hyponatremia, peripheral neuropathy, and mild mental symptoms. In severe attacks, there may be severe symptoms including seizures and psychosis. If left untreated, the attack can become very severe, affecting the peripheral, central, and autonomic nervous systems, leading to paralysis, respiratory failure, hyponatremia, coma, and even death. From a biochemical point of view, acute attacks are involved with increasing precursor levels in the haem biosynthetic pathway (Fig. 3.3.1) to the deficient stage. Of these precursors, ALA is considered neurotoxic. For example, acute intermittent porphyria (Table 3.3.1) is a disease in which the activity of ALA synthase is increased and porphobilinogen deaminase (or hydroxymethylbilane synthase) is decreased, also with excessive urine excretion of porphobilinogen.

Hyperbilirubinemia

Hyperbilirubinemia can result from an increase in bilirubin production or to problems in its elimination at the hepato-biliary level. Depending on the production mechanism, different types of bilirubin will predominate in blood.

With the accelerated destruction of red blood cells (that happens in haemolytic anaemias), a big production of bilirubin (Fig. 3.3.2) occurs that, since it still did not cross the liver, belongs to the nonconjugated type. This bilirubin circulates in blood attached to albumin, preventing to cross the glomerular filter, and consequently there is no bilirubin in urine. However, the big production of bilirubin, that will be conjugated in the liver (Fig. 3.3.3) leads to an increased production of urobilinogen in the intestine, that, after reabsorption, will be sent to the urine. In this way, urine will present increased levels of urobilinogen, but no bilirubin.

On the other hand, whenever the biliary ducts are obstructed, compromising biliary excretion, conjugated bilirubin, not being able to cross the obstacle present in the biliary tract, returns to the liver, and flows to blood. Hyperbilirubinemia is of the conjugated type and since it is hydrosoluble can be eliminated at the renal level. If the obstruction is total, since bilirubin does not reach the intestine, no urobilinogen is produced, and consequently will be absent from urine and faeces. Therefore, in these conditions, faeces are colourless (faecal acholia) and urine has colour of Oporto's wine (coluria). If the obstruction is intermittent, some bilirubin still reaches the intestine and faecal and urinary urobilinogen fluctuate.

The name indirect and direct bilirubin comes from the chemical method used for bilirubin measurement. Non-conjugated bilirubin (linked to albumin), to react with sulphanilic diazotinizated acid, needs to be previously treated with methanol (according with the Van Der Bergh's description) or dimethyl sulfoxide (DMSO), to cut the bond with albumin. On the other hand, conjugated bilirubin reacts directly with the reagent. For this reason, the first one is called indirect bilirubin and the second direct.

In generic terms, there is a hyperbilirubinemia of the direct type, when conjugated bilirubin represents more than 50% of total and hyperbilirubinemia of the indirect type when indirect bilirubin corresponds to at least 75% of total bilirubin. In the remaining cases a mixed type of hyperbilirubinemia exists.

Hyperbilirubinemia exists if bilirubin is above 1 mg/dL. The excess of bilirubin can result from an overproduction or a decreased excretion of bilirubin due to diminished liver uptake, decreased conjugation with glucuronic acid (Fig. 3.3.3), decreased excretion by biliary ducts or obstruction of excretion channels. If bilirubin is above 1,5 mg/dL the individual is in a sub-icteric state and jaundice will be evident if it is above 2,5 mg/dL.

Non-conjugated bilirubin is liposoluble and crosses the hematoencephalic barrier. When it reaches high levels in blood, it can damage the central nervous system (more common in newborns) leading to a clinical condition called Kernicterus, with hyperbilirubinemia close to 20 mg/dL. Conjugated bilirubin is hydrosoluble and only this one can appear in urine.

Jaundice

Jaundice is the yellow colouring of skin and mucosa, resulting from bilirubin deposition, that occurs during strong increases of bilirubinaemia, with values above at least 3 times reference values. The maximum normal concentration of bilirubin is 1 mg/dL (17 µmol/L). If levels rise above 1.8 mg/dL (30 µmol/L) skin becomes yellow (jaundice). In this way, bilirubin deposition, direct or indirect, in skin and sclerotic leads to icteric coloration or jaundice.

Jaundice can be classified according with the predominant type of bilirubin. In cases of jaundice resulting from retention, there is an increase in non-conjugated bilirubin and in cases of jaundice from regurgitation conjugated bilirubin increases. Jaundice can also be classified according with urine colour. In acholuric jaundice there is an increase in non-conjugated bilirubin by retention while in choluric jaundice conjugated bilirubin enhances by regurgitation.

Jaundice can still be classified according with the nature of the lesion that leads to it. Pre-hepatic jaundice results from the increased production of bilirubin (Fig. 3.3.2), for example during haemolysis (haemolytic anaemia, effect of toxins), inadequate erythropoiesis, massive transfusion (in a blood transfusion, erythrocytes have a short lifetime), or absorption of big haematomas. In these conditions, plasma concentration of non-conjugated bilirubin (indirect bilirubin) increases.

Intra-hepatic jaundice is caused by a specific defect in bilirubin uptake by liver cells (Gilbert's syndrome), conjugation defect (neonatal jaundice, Crigler-Najjar's syndrome) (Fig. 3.3.3) or defect in bilirubin secretion in the biliary duct (Dubin-Johnson's syndrome, Rotor's syndrome). In the first two defects, there is an increase in non-conjugated plasma levels. On the other hand, in the secretion defect the conjugated form rises. All these processes can be affected by liver diseases, such as hepatitis, alcohol abuse, effect of drugs of abuse, liver congestion or intoxication.

In post-hepatic jaundice, extra-hepatic biliary ducts are blocked, due to for example gallstones, tumour (pancreas carcinoma) or during pancreatitis. In these conditions, plasma concentration of conjugated bilirubin rises.

3.3.3. **Clinical cases**

Clinical case 3.3.1. *Iron-deficiency anaemia*

A 3-year-old boy was taken to the hospital with mild anaemia. The mother revealed that anaemia was detected eight months ago. Ferrous sulphate was administered for 6 months without appreciable improvement. He had been without medication for two months. The boy's diet was regular, eating meat at least 4 times a week. On physical examination it was active, with no visible discoloration of the mucous membranes. Some laboratory tests were requested, the results of which are shown in the table below.

Table 3.3.2. Results obtained in the patient's blood tests.

Parameter	Patient values	Reference values
Red blood cells	2900000/mm^3	3900000-5300000/mm^3
Hb	10 g/dL	11.5 –13.5 g/dL
Haematocrit	30%	34–40%
VCM	65 fL	75–87 fL
HbCM	20 pg	24–30 pg

Observations: Faecal examination: Parasitological: presence of numerous *Necator americanus* eggs; Occult blood search: positive ++.

Identify the type of anaemia found in this boy and its probable aetiology, justifying.

Clinical case 3.3.2. *Effect of 2,3-bisphosphoglycerate*

Acute anaemia in homozygous haemoglobin S leads to a high level of 2,3-bisphosphoglycerate in its erythrocytes. Discuss whether this is a beneficial effect for these patients.

Clinical case 3.3.3. *Haemochromatosis*

A 54-year-old man complained of weakness, lassitude and moderate weight loss (20 kg in the last 7 months). The liver was firm but moderately enlarged and the spleen palpable. The patient stated that his skin had become darker over the years, a change attributed to time spent outdoors. Laboratory results were as shown in the table below.

Table 3.3.3. Results obtained in the patient's blood tests.

Parameter	Patient values	Reference values
Fasting plasma glucose	5.6 mmol/L	4.4-6.1 mmol/L
Urinary glucose	Normal	
Haematocrit	52%	34-40%
Plasma iron	43 mol/L	9.8-27.0 mol/L
Transferrin saturation	77%	20-50%
Total serum bilirubin	20 mol/L	1.7-18.8 mol/L

Based on these observations a liver biopsy was performed. Microscopic examination revealed hepatocyte vacuolation and moderate hemosiderin deposits in the cell cytoplasm. The diagnosis was made as hemochromatosis and iron overload was subsequently confirmed by the desferrioxamine differential test.

a) What is haemochromatosis?

b) What foods are rich in iron? Should this patient be warned to avoid them?

c) How is iron absorbed in the gastrointestinal tract? How is this absorption regulated?

d) How is iron transported in the blood? What proteins are involved in iron transport and storage?

e) Explain why repeated phlebotomy is used to reduce iron overload.

Clinical case 3.3.4. *Deficiency of hydroxymethylbilane synthase or porphobilinogen deaminase (Acute intermittent porphyria)*

A 7-year-old male has enlarged liver and spleen, mild anaemia, mild mental deficiency, brownish-yellow teeth, and dark red urine. Urinary levels of ALA, porphobilinogen and uroporphyrin were excessively high. Hepta-, hexa-, penta- and copper (I) porphyrins were also greatly increased in urine. Porphobilinogen deaminase activity in erythrocytes was decreased by 2-4%. Justify the diagnosis of acute intermittent porphyria.

Clinical case 3.3.5. *Haem catabolism*

You have certainly noticed what happens when we get a "bruise" after a trauma: first it is purple, then greenish and finally yellow until it disappears. Given the degradation of haemoglobin, try to explain that phenomenon.

Clinical case 3.3.6. *Jaundice*

A 49-year-old woman had an 8-day history of anorexia, nausea, and flu-like symptoms. She noticed that her urine had turned dark in the last 2 days. A physical examination revealed that the upper right quadrant of the abdomen was soft. Blood tests showed the values below.

Table 3.3.4. Results obtained in the patient's blood tests.

Parameter	Patient values	Reference values
Bilirubin	63 μmol/L	3-22 μmol/L
AST	936 U/L	12-48 U/L
ALT	2700 U/L	3-55 U/L
Alkaline phosphatase	410 U/L	80-280 U/L
γ-GT	312 U/L	<36 U/L
Total proteins	68 g/L	62-82 g/L
Albumin	42 g/L	40-52 g/L

a) Comment on the results.
b) What should the diagnosis be?

Clinical case 3.3.7. *Jaundice*

A 60-year-old patient had epigastric pain, fever and vomiting. Four days ago, after eating fatty foods, she had colic pain. After a few hours the pain became accentuated, continuous and radiated throughout the abdomen. She reported that for about ten years she had been experiencing liver cramps at varying intervals from months to one year, with colic pain usually lasting from a few minutes to half an hour. She felt an improvement sometimes spontaneously, sometimes with the use of Buscopan. She also reported intolerance to fatty foods and occasional nausea since that time. Complementary diagnostic tests were performed, which presented the results of the table below.

Table 3.3.5. Results obtained in the patient's blood tests.

Parameter	Patient values	Reference values
Direct bilirubin	2.2 mg/dL	0.1-0.4 mg/dL
Indirect bilirubin	1.3 mg/dL	0.1-0.6 mg/dL
Total bilirubin	3.5 mg/dL	0.2-1 mg/dL
Prothrombin time	16 s	12 s
AST	54 U/L	12-48 U/L
ALT	80 U/L	3-55 U/L
Alkaline phosphatase	100	80-280 U/L
Serum amylase	1200 U/dL	30 U/dL

Urine analysis revealed bile pigments and bile salts as abnormal elements.
a) Comment on the results.
b) What should the diagnosis be?

Clinical case 3.3.8. *Deficiency of bilirubin UDP-glucuronosyltransferase (Gilbert's syndrome)*

One 20-year-old patient has total bilirubin of 2.1 mg/dL (reference value: 0.2-1 mg/dL) and direct bilirubin of 0.1 mg/dL (reference value: 0.1-0.4 mg/dL) on a routine examination. The patient is asymptomatic and healthy, and the remaining tests (blood count with reticulocytes, transaminases, alkaline phosphatase, γ-GT and partial urine) are normal. Which are the most probable diagnostics? Justify.

Clinical case 3.3.9. *Obstructive jaundice due to biliary lithiasis*

A 45-year-old woman shows pain in her right hypochondrium (upper right abdomen), a colic-like pain that began three days earlier but worsened on that day. From the day before it began with bitter vomiting (gall flavour), yellow skin and eye colour and coloured urine. Objective examination showed moderate dehydration; jaundice skin and mucous membranes; apyretic; abdomen with pain on palpation of the hepato-biliary zone; and no other changes. Complementary diagnostic tests were performed, which presented the following results:

Table 3.3.6. Results obtained in the patient's blood tests.

Parameter	Patient values	Reference values
AST	80 U/L	12-48 U/L
ALT	150 U/L	3-55 U/L
Total bilirubin	7.0 mg/dL	0.2-1 mg/dL
Direct bilirubin	5.0 mg/dL	0.1-0.4 mg/dL
Alkaline phosphatase	1200 U/L	80-280 U/L
γ-GT	125 U/L	< 36 U/L

An abdominal ultrasound showed gallstones in the gallbladder and dilated main bile duct. No changes suggestive of cholecystitis. Summary urine examination showed large amounts of bilirubin but no bile pigments. The final diagnosis presented was obstructive jaundice due to biliary lithiasis.

a) From the clinical history how do you interpret changes in the colour of stools and urine?

b) How do you interpret changes in blood biochemistry? Would it be expected an increase in indirect bilirubin?

Clinical case 3.3.10. *Deficiency of bilirubin UDP-glucuronosyltransferase (Crigler-Najjar's syndrome)*

One female newborn had jaundice at 3 days of age. After 72 hours her total bilirubin was 24 mg/dL (reference value: 0.2-1 mg/dL), with a predominance of indirect bilirubin. She underwent light therapy up to 25 days of age, phenobarbital and 8 hours/day of home light therapy, with good response. At 8 months, with an indirect bilirubin of 5.5 mg/dL (reference value: 0.1-0.6 mg/dL), luminotherapy was discontinued. Subsequent indirect bilirubin values ranged from 6-8 mg/dL under phenobarbital treatment. Crigler-Najjar´s syndrome was diagnosed. Explain why.

Clinical case 3.3.11. *Dubin-Johnson´s syndrome*

A 28-year-old male patient had conjunctival jaundice. Complementary diagnostic tests revealed a hyperbilirubinemia with predominance of the conjugated fraction (total bilirubin: 2.0 mg/dL, reference value: 0.2-1.0 mg/dL; direct bilirubin: 1.4 mg/dL, reference value: 0.1-0.4 mg/dL, indirect bilirubin: 0.6 mg/dL, reference value: 0.1-0.6 mg/dL), with the remaining biochemical parameters within normal limits. Dubin-Johnson´s syndrome was diagnosed. Explain why.

3.3.4. **Further questions**

Question 3.3.1.
In deoxyhaemoglobin (HHb), how is Fe^{2+} coordinated?

Question 3.3.2.
What are the similarities between haemoglobin and myoglobin?

Question 3.3.3.
Why is a protein structure such as present in myoglobin or haemoglobin needed for the transport of O_2 in the blood?

Question 3.3.4.
The affinity of haemoglobin to O_2 is affected by various allosteric effectors. Indicate the effects of each of them, and the benefits to the organism arising from those effects.

Question 3.3.5.
When isolated, haemoglobin subunits have much higher affinity for oxygen than the intact molecule. Explain why.

Question 3.3.6.
Explain the different physiological functions of haemoglobin and myoglobin as a function of the O_2 dissociation curves for these proteins. Justify these differences at the molecular level.

Question 3.3.7.
Do you agree that myoglobin has no quaternary structure? Justify.

Question 3.3.8.
What are the differences in oxygen fixation between isolated haemoglobin and the 2,3-bisphosphoglycerate (BPG) haemoglobin complex?

Question 3.3.9.

State and explain whether the following sentences are true or false:

a) By lowering the pH, the haemoglobin O_2 dissociation curve changes to the right.

b) The acidic environment of a working muscle allows haemoglobin to bind O_2 more tightly.

c) Haemoglobin affinity for O_2 is decreased at high CO_2 concentrations.

d) In the lungs, the presence of high concentrations of H^+ and CO_2 allows haemoglobin to become oxygenated.

e) In the lungs, the presence of high O_2 concentrations promotes the release of CO_2 and H^+.

f) The quaternary structures of myoglobin and haemoglobin are very similar.

g) Haemoglobin and myoglobin are subunits that establish hydrogen bridges and nonpolar interactions with other subunits.

h) Haemoglobin and myoglobin bind 1 haem group per globin chain.

i) Haemoglobin and myoglobin can bind 1 O_2 per haem.

Question 3.3.10.

Which of the following statements completes the sentence correctly? Haemoglobin differs from myoglobin…

a) Haemoglobin is multimeric while myoglobin is monomeric.

b) Haemoglobin binds O_2 more strongly than myoglobin to any O_2 concentration.

c) Haemoglobin binds CO_2 more efficiently than myoglobin.

d) O_2 binding by haemoglobin depends on CO_2, H^+ and BPG concentrations, whereas O_2 binding by myoglobin does not.

e) The dissociation curve of oxygen to myoglobin is sigmoidal, while that of haemoglobin is hyperbolic.

f) The affinity of haemoglobin for O_2 is regulated by organic phosphate, while the affinity of myoglobin for O_2 is not.

g) Haemoglobin has higher affinity for O_2 than myoglobin.

h) The affinity of myoglobin and haemoglobin for O_2 is independent of pH.

Question 3.3.11.

Significant contributors to total blood carbon dioxide include bicarbonate ion, dissolved CO_2, carbaminohaemoglobin and/or H_2CO_3?

Question 3.3.12.

A variant of haemoglobin (known as *Hb Little Rock*) exhibits a decreased level of BPG binding. What is(are) the most likely consequence(s) of this mutation?

Question 3.3.13.

To create a "super mouse" a scientist genetically manipulates a rat, causing its haemoglobin to bind to oxygen a few dozen times more strongly than the haemoglobin of normal mice. In a race between this mouse and normal mice, who will be faster and why?

Question 3.3.14.

The desire to breathe results from a high level of CO_2 in the blood. Divers often hyperventilate (inhale quickly and deeply for several minutes) just before a prolonged dive believing this will increase the amount of O_2 in their blood. This belief stems from the fact that hyperventilation decreases the amount of CO_2 in the blood. Considering what you know about the properties of haemoglobin, do you think hyperventilation will be helpful? Explain.

Question 3.3.15.

Normally, gaseous CO_2 is efficiently exhaled through the lungs. Under certain conditions, such as obstructive pulmonary disease, exhalation is difficult. The resulting excess CO_2 in the body can lead to respiratory acidosis, in which excess acid accumulates in body fluids. Explain why excess CO_2 can lead to respiratory acidosis.

Question 3.3.16.

Crocodiles, which can stay under water for almost 1 hour, kill their prey by drowning. One adaptation that helps them is that they can use almost all the O_2 that circulates in their blood, whereas humans can only use about 65%. Crocodile haemoglobin does not bind BPG. However, it is very strongly bound to CO_2. How does this help the crocodile get its dinner?

Question 3.3.17.

After storage longer than one week in normal medium, red blood cells are depleted in BPG. Discuss the merits of using fresh blood *vs.* blood one week into transfusions.

Question 3.3.18.

Acute anaemia in homozygous haemoglobin S leads to a high level of BPG in its erythrocytes. Discuss whether this is a beneficial effect for these patients.

Question 3.3.19.

An anaemic individual whose blood has halved haemoglobin content may appear in good health. However, an individual exposed to an amount of carbon monoxide capable of occupying half of the available haem sites becomes disabled. Explain why.

Question 3.3.20.

The half-life of red blood cells is 120 days. What is the fate of Hb carried in them?

Question 3.3.21.

Complete the following sentence: In intestinal absorption of iron…

a) The presence of a reductant such as ascorbic acid increases the availability of iron.

b) Low stomach pH inhibits absorption because it favours Fe^{3+}.

c) Transferrin binds Fe^{2+}.

d) Ferritin transports Fe^{3+} into plasma.

Question 3.3.22.

Consider the haem group:

a) What is required for its synthesis?

b) List the reactions, enzymes and cofactors involved in haem catabolism.

c) Can the product of haem catabolism be excreted? How? Indicate the reactions, enzymes and cofactors involved.

Question 3.3.23.

Does haem biosynthesis require propionic acid, succinyl-CoA, glycine and/or Fe^{2+}?

Question 3.3.24.
Haem biosynthesis cannot occur in which of the following: enterocytes, hepatocytes, mature erythrocytes or adipocytes?

Question 3.3.25.
Complete the following sentence: Iron-chelatase…
a) Is an iron chelating compound.
b) Releases haem iron in haemoglobin degradation.
c) Binds iron to sulphite ions and cysteine residues.
d) Is involved in the mitochondrial stage of haem group synthesis.

Question 3.3.26.
Does haem oxygenase need molecular oxygen, produce bilirubin, produce CO_2, or does it need NADH?

Question 3.3.27.
Complete the following sentence: Bilirubin is…
a) Always water-soluble.
b) An intermediate in haemoglobin synthesis.
c) One nucleotide.
d) From the haem group catabolism.

Question 3.3.28.
In the liver is bilirubin, which is insoluble in water, transformed into a water-soluble compound by reduction, oxidation, dehydrogenation or conjugation?

Question 3.3.29.
Does a liver disease lead to a higher elevation in blood level of haem, biliverdin, bilirubin or bilirubin diglucuronide?

Question 3.3.30.
Does a biliary obstruction lead to a higher elevation in the blood level of conjugated bilirubin, bilirubin, haem or biliverdin?

4. NUCLEOTIDE METABOLISM

CHAPTER CONTENTS

4.1. **Purine nucleotides**

4.1.1. **Short review**

Purine nucleotide synthesis

Nucleotide synthesis is important not only for the crucial role those nucleic acids have in protein synthesis and genetic information, but also for the role of nucleotides like FAD, NAD(P)H, CoASH, cAMP and UDP-glucose in metabolism.

Synthesis of purine nucleotides starts with the synthesis of phosphoribosylpirophosphate (PRPP) by PRPP synthetase (Fig. 4.1.1). The substrate for this reaction (ribose-5-phosphate) is a product of the pentoses-phosphate pathway.

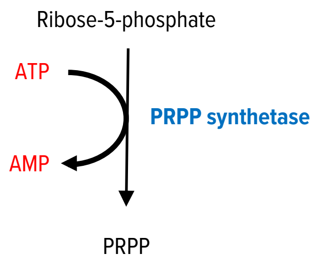

Fig. 4.1.1. Synthesis of phosphoribosylpyrophosphate (PRPP) catalysed by PRPP synthetase.

PRPP is then converted into inosine monophosphate (IMP) via a 10-step metabolic pathway. This pathway needs the hydrolysis of 4 ATP. In the first step of this pathway, PRPP is converted to 5-phosphoribosylamine by the glutamine PRPP amidotransferase enzyme (Fig. 4.1.2).

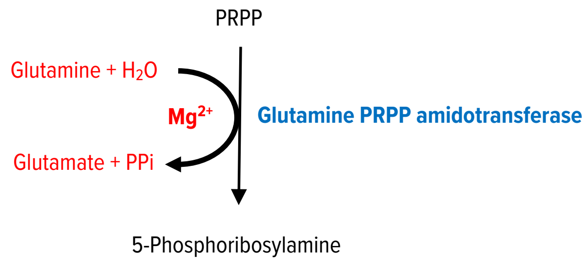

Fig. 4.1.2. Conversion of phosphoribosylpyrophosphate (PRPP) to 5-phosphoribosylamine by the action of glutamine PRPP amidotransferase.

Then, the conversion of IMP to AMP or guanosine monophosphate (GMP) requires two reaction steps.

The efficiency of normal metabolism is controlled by the presence of two distinct salvage pathways. One of these uses the nitrogenic bases, hypoxanthine, guanine and adenine, as substrates, while the other uses preformed nucleosides as substrates. Each pathway is specific for the recovered nitrogenic base or nucleoside. These salvage pathways require the activity of phosphoribosyl transferases. Hypoxanthine-guanine phosphoribosyl transferase (HGPRTase) and adenine phosphoribosyl transferase (APRTase) catalyse the reactions represented in Fig. 4.1.3.

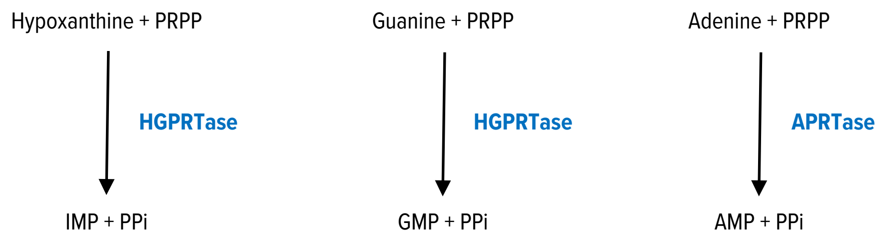

Fig. 4.1.3. Salvage pathway of purine bases. PRPP: Phosphoribosylpyrophosphate; HGPRTase: hypoxanthine-guanine phosphoribosyl transferase; APRTase: adenine phosphoribosyl transferase; IMP: inosine monophosphate; GMP: guanosine monophosphate; AMP: adenosine monophosphate.

Production of AMP and GMP through these phosphoribosyl transferase catalysed reactions effectively inhibits the *de novo* pathway in the amidotransferase catalysed step. First, PRPP is consumed, decreasing the rate of formation of 5-phosphoribosylamine. Second, AMP and GMP serve as feedback inhibitors of the amidotransferase step.

Purine nucleotide degradation

Uric acid is the end-product of an exogenous and endogenous pool of purines. The exogenous pool significantly changes with diet, and animal proteins contribute significantly to this purine pool. The endogenous production of uric acid occurs mainly in the liver, intestines and other tissues like muscles, kidneys and the vascular endothelium. Degradation of purine nucleotides, nucleosides and nitrogenic bases is channelled through a common pathway leading to the synthesis of uric acid.

In muscular degradation of purine nucleotides, AMP is initially converted into IMP by AMP deaminase and later, IMP is hydrolysed to inosine by 5´-nucleotidase activity (Fig. 4.1.4A). In remaining tissues, AMP is hydrolysed by 5´-nucleotidase producing adenosine and later deaminated to form inosine by the action of adenosine deaminase (Fig. 4.1.4B).

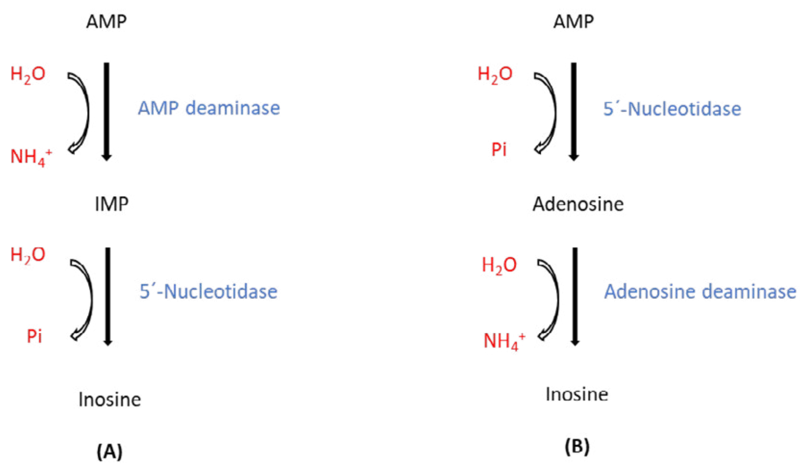

Fig. 4.1.4. Conversion of adenosine monophosphate (AMP) to inosine in muscle (A) and in remaining tissues (B).

GMP and xanthosine monophosphate (XMP) are hydrolysed by 5´-nucleotidase, to form guanosine and xanthosine, respectively (Fig. 4.1.5).

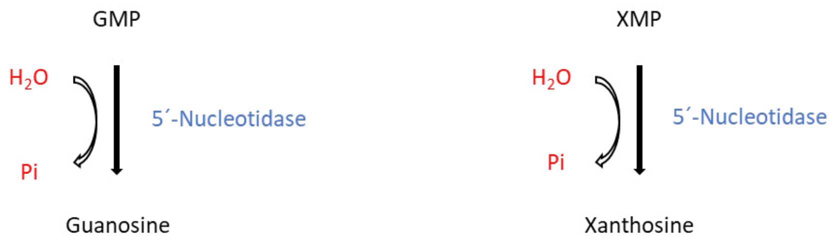

Fig. 4.1.5. Conversion of guanosine monophosphate (GMP) and xanthosine monophosphate (XMP) to guanosine and xanthosine, respectively.

The purine nucleoside phosphorylase enzyme converts inosine, guanosine and xanthosine into hypoxanthine, guanine and xanthine, respectively, with the release of ribose-1-phosphate. Furthermore, hypoxanthine is oxidised into xanthine by xanthine oxidase and guanine is deaminated to xanthine by guanase (Fig. 4.1.6).

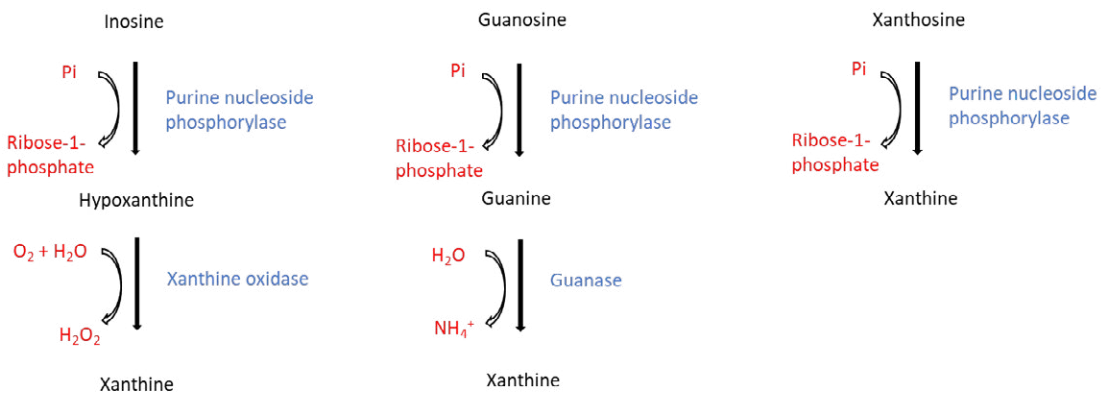

Fig. 4.1.6. Conversion of inosine, guanosine and xanthosine to xanthine.

Xanthine is then oxidised to uric acid by xanthine oxidase (Fig. 4.1.7).

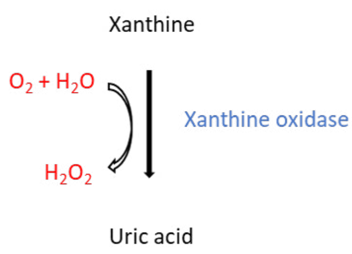

Fig. 4.1.7. Conversion of xanthine to uric acid.

4.1.2. **Pathologies**

Gout

Gout is characterised by high levels of uric acid in blood and urine due to abnormalities in purine synthesis pathway that result in overproduction of purine nucleotides and its degradation product, uric acid.

Studies on gout patients have shown that multiple and heterogeneous defects can be the cause of overproduction of uric acid. Examples of biochemical defects that result in increased purine nucleotide synthesis include increased PRPP synthetase activity and partial HGPRTase (Fig. 4.1.3) activity. HGPRTase deficiency is one of the most common inborn errors of purine metabolism. Factors that increase the rate-limiting step in purine biosynthesis, lead to its increased synthesis and degradation to uric acid (Fig. 4.1.7).

At physiological pH, uric acid is a weak acid and exists mostly as urate, the salt of uric acid. The normal reference range of uric acid in human blood is 1.5 to 6.0 mg/dL in women and 2.5 to 7.0 mg/dL in men. When the level of uric acid is above 6.8 mg/dL, crystals of monosodium urate are formed. These crystals deposit in joints of the extremities and in the renal interstitial tissue, since humans cannot oxidise uric acid to the more soluble compound allantoin, due to lack of uricase enzyme. Usually, most daily elimination of uric acid occurs through kidneys.

There are different approaches used in the treatment of gout including colchicine, antihyperuricemic drugs and allopurinol. Allopurinol and its metabolite, alloxanthine, are effective inhibitors of xanthine oxidase and cause a decrease in uric acid levels. At the same time, an increase in hypoxanthine and xanthine levels occurs, decreasing the synthesis of purine nucleotides.

Lesch-Nyhan´s syndrome

Lesch-Nyhan´s syndrome is clinically characterised by hyperuricemia and excessive production of uric acid, together with neurological and behavioural problems, which may include dystonia, choreoathetosis, ballism, spasticity, hyperreflexia, mental disability, aggressiveness, impulsivity and self-mutilation.

This disorder is associated with a very severe or complete deficiency of HGPRTase activity (Fig. 4.1.3). The gene coding for HGPRTase is present in the X chromosome, so the deficiency is mainly manifested in men. This defect also leads to the excretion of hypoxanthine and xanthine.

Lack of HGPRTase activity, involved in the conversion of hypoxanthine and guanine into nucleotides, explains the hyperuricemia and excessive uric acid production. Hypoxanthine and guanine are not recovered, leading to increased intracellular pools of PRPP and reduced levels of IMP or GMP. These two factors promote the synthesis of purines without adequate regulation of this pathway.

Treatment of Lesch-Nyhan´s patients with allopurinol decreases the amount of uric acid formed, relieving some of the symptoms caused by sodium urate deposits. However, since patients have a marked reduction in HGPRTase activity, hypoxanthine and guanine are not recovered, PRPP is not consumed and consequently purine nucleotide synthesis is not disrupted.

No sustained drug therapy has been uniformly effective for the treatment of neurological problems associated with the Lesch-Nyhan´s syndrome. These patients usually die of renal failure resulting from elevated sodium urate deposits.

4.1.3. **Clinical cases**

Clinical case 4.1.1. *Gout*

A 45-year-old patient presents with foot pain, especially in the big toe. He has always been in good health and reports in his history of a previous episode of pain in his left wrist which had disappeared without treatment. The patient's plasma uric acid concentration was determined to be 10.2 mg/dL (reference value: 3.5-7.0 mg/dL).
a) Explain the clinical case presented.
b) Describe the clinical manifestations of gout and the inflammatory mechanism caused by uric acid.
c) Explain the biochemical mechanisms responsible for the onset of hyperuricemia.
d) Comment on other metabolic changes that may cause an increase in the plasma concentration of uric acid.
e) Describe the most appropriate treatment for gout.
f) Describe the mechanisms of action of the drugs used in the treatment of gout.

Clinical case 4.1.2. *HGPRTase deficiency*

Gout is a pathology that may be associated with a decrease in HGPRTase activity.
a) How do you justify that a deficiency of HGPRTase results in an increase in the synthesis of uric acid?
b) What symptoms do you expect to see in a patient with HGPRTase enzyme deficiency?

Clinical case 4.1.3. *Change in PRPP synthetase activity*

Gout is a pathology that may be associated with an increase in PRPP synthetase activity.
a) Explain.
b) What metabolic processes are directly affected if there is inability to synthesize PRPP?

4.1.4. **Further questions**

Question 4.1.1.
State the origin of the carbon and nitrogen atoms in the synthesis of purine nucleotides.

Question 4.1.2.
Is the enzyme that catalyses the rate-limiting step of purine nucleotide synthesis a multifunctional protein? Does it use PRPP as a substrate? Does it require AMP? Is it primarily controlled by substrate availability?

Question 4.1.3.
How many moles of ATP are needed to synthesize a purine?

Question 4.1.4.
If a cell is unable to synthesize PRPP, which of the following processes is directly affected: FAD synthesis, NAD synthesis, CoA synthesis, ribose-5-phosphate synthesis, or dTMP synthesis?

Question 4.1.5.
Complete the following sentence: When converting PRPP into IMP, there is the need of…
a) 2 ATP, 2 glutamine, 1 glycine and 1 aspartate.
b) 2 ATP, 2 asparagine, 1 glycine and 1 glutamate.
c) 4 ATP, 2 glutamine, 1 glycine and 1 aspartate.
d) 4 ATP, 2 asparagine, 1 glycine and 1 glutamate.

Question 4.1.6.
Can the conversion of adenine to hypoxanthine be caused by caffeine, nitrous acid, ultraviolet radiation, or dimethyl sulphate?

Question 4.1.7.
Can the conversion of guanine to xanthine be caused by caffeine, nitrous acid, ultraviolet radiation, or dimethyl sulphate?

Question 4.1.8.
In nucleotide biosynthesis, glutamine plays a key role. Justify this statement.

Question 4.1.9.
Which of the following antitumour agents works by affecting purine synthesis: acyclovir (acycloguanosine), 5-fluorouracil, methotrexate, hydroxyurea, or allopurinol?

Question 4.1.10.
Which of the following therapeutic agents works by affecting the formation of tetrahydrofolate: acyclovir (acycloguanosine), 5-fluorouracil, methotrexate, or hydroxyurea?

Question 4.1.11.
In a cancer patient, often used drugs cause hair loss, immune deficit and other unpleasant changes. One such drug is methotrexate, which is a powerful inhibitor of dihydrofolate reductase. Explain the interest of methotrexate in the treatment of cancer as well as some of its side effects.

Question 4.1.12.
Why can azaserine, a glutamine analogue, be used as a nucleotide synthesis inhibitor?

Question 4.1.13.
In humans the purine ring cannot be degraded. Explain how it is excreted, indicating the reactions involved.

Question 4.1.14.
Is uric acid formed from xanthine in the presence of O_2, is it a degradation product of cytidine, is it deficient in gout, is it a competitive inhibitor of xanthine oxidase, or is it oxidized in humans before being excreted in the urine?

Question 4.1.15.
In humans, is uric acid the final product of the metabolism of amino groups, purine bases, protamines or amino acids?

Question 4.1.16.
Which of the following molecules gives rise to uric acid when degraded: DNA, FAD, CTP, PRPP, alanine, urea, NAD^+? Explain.

Question 4.1.17.
Caffeine is excreted as uric acid. Explain why.

Question 4.1.18.
How do you justify that a deficiency in HGPRTase results in an increase in uric acid synthesis?

Question 4.1.19.
Does HGPRTase deficiency cause hyperglycaemia, hyperlacticaemia or hyperuricemia?

4.2. **Pyrimidine nucleotides**

4.2.1. **Short review**

Pyrimidine nucleotide synthesis

Pyrimidine nucleotide synthesis, via a 6-step metabolic pathway, is simpler than purine nucleotide synthesis. This pathway needs the hydrolysis of 2 ATP. Carbon and nitrogen atoms are derived from bicarbonate, aspartate and glutamine.

The pyrimidine ring is first formed. In the last 2 reactions of the metabolic pathway, orotate is attached to ribose-5-phosphate (from PRPP), being converted to orotidine-5´-monophosphate (OMP) by orotate phosphoribosyl transferase (OPRTase), and then OMP is converted to uridine monophosphate (UMP) by OMP decarboxylase (Fig. 4.2.1). In higher eukaryotes, UMP synthase (UMPS) harbours both enzymatic activities on a single polypeptide chain. The formed UMP is then the precursor for other pyrimidine nucleotides.

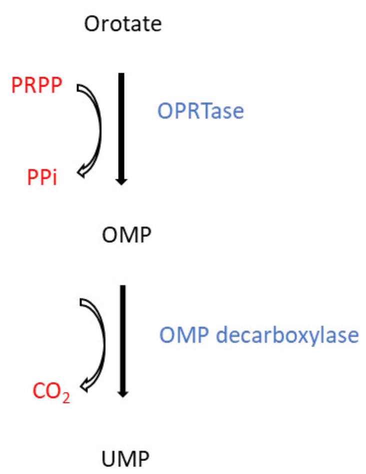

Fig. 4.2.1. Conversion of orotate to uridine monophosphate (UMP). OPRTase: Orotate phosphoribosyl transferase; OMP: Orotidine-5´-monophosphate.

Pyrimidine nucleotide degradation

In humans, the purine ring cannot be degraded, but the pyrimidine ring can be. Pyrimidine nucleotides are degraded to β-amino acids. Uracil and thymine are degraded to β-alanine and β-aminobutyrate, respectively, in parallel pathways in the liver (Fig. 4.2.2).

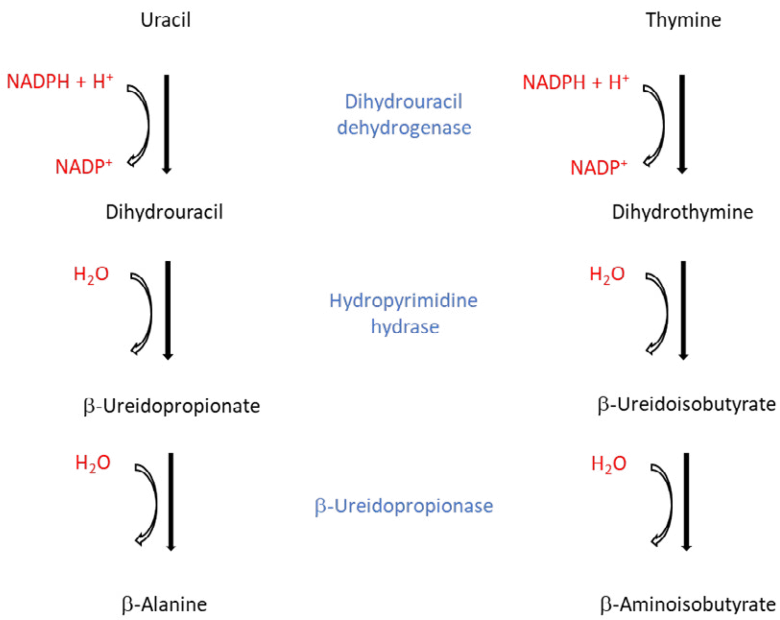

Fig. 4.2.2. Degradation of uracil and thymine to β-alanine and β-aminobutyrate, respectively.

4.2.2. **Associated pathologies**

Hereditary orotic aciduria

Hereditary orotic aciduria is a very rare inborn error of pyrimidine metabolism with autosomal recessive inheritance. It is the only known enzyme deficiency of the pyrimidine biosynthetic pathway, resulting from a deficiency in one or both activities, OPRTase or OMP decarboxylase, of the bifunctional enzyme UMPS encoded by the *UMPS* gene. The presumed cause of hereditary orotic aciduria is biallelic missense mutations resulting in decreased levels of the enzyme and impaired substrate binding. To date, only about twenty patients with this condition have been described.

UMPS defects lead to the accumulation of orotate and/or OMP, because of the inability to convert orotate to UMP. The hallmarks of the disease are a megaloblastic bone marrow that is refractory to hematinic therapy, accompanied by a markedly increased excretion of orotic acid in the urine. Immunodeficiency, developmental delay and failure to thrive have been observed in these patients.

4.2.3. **Clinical cases**

Clinical case 4.2.1. *OPRTase or OMP decarboxylase deficiency (Hereditary orotic aciduria)*

A 10-year-old girl had a learning delay. Routine biochemical and haematological examinations were normal, but a urine analysis revealed elevated levels of orotic acid (18.5 mmol/mmol creatinine, reference value < 1.2 mmol/mmol creatinine). Postprandial glutamine was normal around 765 mmol/L, and fasting orotic acid remained high. The remaining purines and pyrimidines in the urine were analysed and were found to be normal. The diagnosis of hereditary orotic aciduria was made. Explain why.

Clinical case 4.2.2. *OPRTase or OMP decarboxylase deficiency (Hereditary orotic aciduria)*

A 7-year-old girl had anaemia, leukopenia, megaloblastic bone marrow, crystalluria, and normal growth and intelligence. The diagnosis of hereditary orotic aciduria was made. Uridine administration produced a rapid hematologic recovery. The urinary orotic acid/creatinine ratio varied inversely with the uridine dose. Enzyme studies in haemolyzed and fibroblast cultures from the patient revealed reduced levels of OPRTase and OMP decarboxylase. Explain the administered treatment.

4.2.4. **Further questions**

Question 4.2.1.
State the origin of the carbon and nitrogen atoms in pyrimidine nucleotide synthesis.

Question 4.2.2.
Does the degradation of dCMP yield a ketoacid, an amino acid, uric acid or urea?

Question 4.2.3.
Which of the following results from the degradation of dTMP: a keto acid, an amino acid, uric acid or urea?

Question 4.2.4.
Explain why the drug 5-fluorouracil is an indirect inhibitor of DNA synthesis.

5. METABOLIC INTERRELATIONS

CHAPTER CONTENTS

5.1. **Fasting**

5.1.1. **Short review**

Prolonged fasting and starvation can occur in several situations such as a starving child, an anorectic adolescent, an AIDS patient or an individual in starvation strike. The human organism responds in the same way to all these challenges.

The deprivation of food that some individuals purposely do to "clean" their bodies is an illusion. This fasting situation causes lipophilic environmental toxic compounds (DTT, benzene, etc.) that are accumulated in body fat (where they remain harmless) due to the catabolism of fats, to be released into the bloodstream. Under these conditions, the detoxification that is performed by the liver may not be sufficient and these substances may damage cells.

There are several problems that the organism must solve in a starvation condition, such as which biomolecules (sugars, lipids or proteins) should be metabolized in the first place; which tissues can be sacrificed to ensure survival; and how to preserve energy stores.

The main concern of the organism is to preserve tissues that depend exclusively on glucose such as erythrocytes and neurons. However, in a period of a few hours, carbohydrate stores are depleted. This causes the body to sacrifice some circulating amino acids to produce glucose and ATP. The second concern of the body is to maintain muscle mass.

Although triglycerides are the largest energy reserve of the human body, they are a very poor source of glucose. In fact, a small amount of glucose can be obtained from glycerol: 2 glycerol molecules that result from the degradation of 2 triacylglycerol molecules allow the production of only 1 glucose molecule. However, only fatty acids having odd number of carbons can be used to produce glucose, and once again 2 of these molecules are required to reach 1 glucose molecule.

Thus, the body's strategy is to use fatty acids and ketone bodies to produce ATP. Over time, some brain cells adapt to the use of ketone bodies as a source of energy.

Steps in prolonged fasting

Early hours (12-48 hours)

The early hours are like the period between dinner and breakfast. As glucose concentration decreases, the secretion of the glucagon hormone, that stimulates glycogenolysis, increases. Gluconeogenesis of the liver becomes active. The liver releases glucose into the bloodstream to maintain its levels for vital organs. Meanwhile, fatty acids are being used by muscle cells to meet their energy needs. After 12 hours of fasting, the body's battle to maintain a steady supply of glucose to the blood leads to almost complete reduction in carbohydrate stores.

First days (2-24 days)

During the first days, fats and proteins constitute the body's main energy reserve. To preserve structural proteins, especially those associated with muscle mass, the body first catabolizes easily metabolizable amino acids. Some amino acids are used to produce ATP, while glucogenic amino acids are used in gluconeogenesis. These amino acids, especially alanine, provide about 90% of body glucose. Since the body continues to use triglycerides, glycerol contributes with the remaining 10%.

After several weeks (more than 24 days)

Some energy preservation strategies are initiated at this stage, namely lowering body temperature and heart rate, lowering blood pressure and basal metabolism. The individual becomes lethargic, reducing his physical activity. The individual begins to show signs of vitamin deficiencies.

If the body continued to rapidly degrade proteins, it would survive less than 3 weeks. To prevent such rapid protein degradation, its speed decreases by about 2/3. In contrast, the rate of fat catabolism increases considerably. Thus, the ketogenic pathway becomes fundamental at the liver level by releasing ketone bodies into the bloodstream. At this stage ketone bodies can be used by most brain cells (after about 10 days of fasting). Plasma glucose comes from the catabolism of glucogenic amino acids.

The susceptibility to infections increases. A normal individual has fat stores for about 3 weeks. Once the fat stores have been exhausted, the body metabolises proteins again, the only existing reserve. Normally, the body would catabolize 30 to 55 g of protein/day; At this stage, the speed of protein catabolism is accelerated (hundreds g/day).

Final phase

In a final phase of protein depletion, the body deteriorates rapidly, presenting severe muscle atrophy. Under these conditions, muscle is sacrificed to preserve brain structure and functionality. In the late stages, the liver and intestine are highly damaged, the heart and kidney are moderately damaged, the nervous system is also affected, although to a lesser extent.

The body remains alive until about 50% of the body proteins are lost. During a complete fast, death occurs after 1 to 3 months. However, some individuals can survive for a longer period of fasting, depending on the initial percentage of body fat, age, gender and energy expenditure.

5.1.2. **Clinical cases**

Clinical case 5.1.1. *Fasting*

Explain what happens during fasting.
a) What hormones are produced and how do they work?
b) Why is there an increase in urea excretion?

Clinical case 5.1.2. *Fasting*

Prolonged fasting results in increased blood levels of fatty acids and ketone bodies. Comment on this statement and refer to the major sources of brain, muscle and liver energy in this situation.

Clinical case 5.1.3. *Fasting and diabetes*

Explain how a person with diabetes can develop hyperglycaemia during fasting.

Clinical case 5.1.4. *Kwashiorkor´s disease*

Kwashiorkor's disease is a nutritional disease caused by a very protein deficient diet. It is common in some very poor populations where it is known as the "disease the older child catches when the next child is born". It comes when the older child stops breastfeeding and is exposed to a very low-protein, high-carbohydrate diet. It is a nutritional disease with several very serious consequences that pose a real threat to the lives of these children: decreased protein synthesis and weakened immune systems are examples of these consequences. For a child on a diet low in all nutrients, what differences in metabolic adaptation (e.g., fat utilization and synthesis, muscle mass preservation) would you expect to find? Justify.

Clinical case 5.1.5. *Fasting*

The figure below shows the change in exogenous glucose during prolonged fasting (time was counted from the last meal).

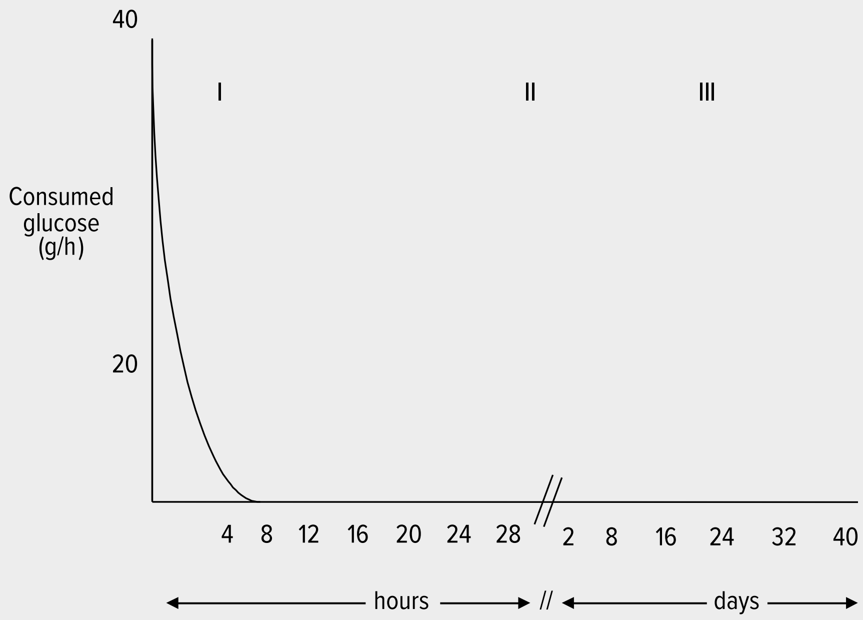

Fig. 5.1.1. Exogenous glucose change during prolonged fasting.

a) Show in the figure the variations in glycogenolysis and gluconeogenesis during fasting.
b) How do the activities of specific carbohydrate metabolism enzymes, including hepatic glycolysis, glycogenolysis and gluconeogenesis during prolonged fasting vary?
c) Are fatty acids possible sources of carbon chains for gluconeogenesis?
d) What is the relative importance of glycogen stores in glycaemic replenishment?
e) What happens at the metabolic level when, after prolonged fasting, food is eaten?
f) Covalent modification of enzymes, particularly by phosphorylation, is an important way of enzymatic regulation. Justify by indicating which enzymes are regulated by this mechanism and which features have (or not) in common?
g) What are the main hormones involved in metabolic control, and what are their effects?
h) What is the mode of action at the target cell level of the hormones referred to in the previous question?
i) How are the changes caused by dietary errors, such as hypoproteic, hypocaloric or hypercaloric regimes, explained at biochemical level?

5.2. **Physical exercise**

5.2.1. **Short review**

Exercise increases the performance of the body and the cell itself as it increases the ability of cells to pick up glucose. In fact, regular exercise can reduce the need for insulin in a diabetic.

The duration and intensity of exercise determines the type of fuel the body chooses to consume. At rest, fats are predominantly used. During a short but high intensity exercise carbohydrates are used. During long-term exercise such as the marathon, runners consume carbohydrates and fat.

ATP, the energy source used by muscle cells for contraction, can be produced from three different pathways (Table 5.2.1): ATP-CP (anaerobic metabolism); lactic acid production (anaerobic glycolysis); oxygen (aerobic metabolism).

During rest, human body stores a very small amount of ATP as a fast source of energy for physical activity. Although small, this amount of ATP can maintain muscle movement for less than a second. In this way, the body stores easily available amounts of energy in the form of high energy compounds such as creatine phosphate or phosphocreatine (CP). In fact, our body does not directly use creatine phosphate, but it regenerates ATP from ADP:

$$\text{Creatine-phosphate} + \text{ADP} + \text{H}^+ \leftrightarrow \text{ATP} + \text{creatine}$$

Although this reaction occurs in the presence of oxygen, it does not require oxygen. Thus, the ATP-CP system is considered anaerobic.

During the first few seconds of intense physical activity (100 m run) creatine phosphate decreases as it is used to maintain constant ATP levels, which together with CTP give off energy for 3-15 seconds. To continue exercise, the body must use other sources of ATP.

Another form of rapid ATP production is the formation of lactic acid of glycolytic origin (anaerobic system). It is the predominant source of ATP during the first minutes of high intensity exercise. In the absence of oxygen, glycolytic pyruvate is used for lactate synthesis by the action of the enzyme lactate dehydrogenase, regenerating NAD^+, which is necessary for the continuation of the glycolytic process. Compared to the aerobic pathway of ATP production (Krebs cycle and respiratory chain), ATP production by glycolysis (2 ATP/glucose) occurs at a higher rate. Glucose stored as glycogen is the first energy source used for lactic acid formation. Thus, muscle cells can utilize thousands of glucose molecules per second to form ATP through anaerobic glycolysis. However, this is not as fast a way to obtain energy as the degradation of CP to form ATP.

Anaerobic glycolysis is an important metabolic pathway, particularly for fast muscle fibres, giving energy for 40 seconds to 2 minutes of physical activity. However, rapid formation of ATP causes lactic acid accumulation and increases the acidity of muscle cells. Not only lactic acid, but also the intermediate compounds of the glycolytic pathway and the degradation of ATP in ADP contribute to the increase in acidity. Acidification has as its main consequence the reduction of the activity of glycolytic enzymes, inhibiting the use of other glucose molecules. In addition, changing pH reduces the binding capacity of Ca^{2+}, an important component of muscle contraction. Increasing [H^+] can cause muscle fatigue and decrease exercise muscle performance.

During exercise, muscle fibres can use energy 200 times faster than during rest. At this rate of use, our ability to exercise would be limited to just a few minutes if we depended only on ATP-CP and lactic acid production. To maintain exercise for a longer period, it is necessary to change the ATP production strategy to an aerobic system.

The Krebs cycle and ATP-forming airway operating aerobic pathway only operates in the cell under conditions where the amount of oxygen is sufficient. In contrast to the two systems described above, the use of oxygen leads to the slow production of large amounts of ATP. Long-term exercise requires the use of ATP produced in the aerobic system ("slow" muscle fibres). After 2 minutes of a long running run, the aerobic system provides about 50% of muscle energy. After 30 minutes of running, the aerobic system provides about 95% of energy; after 2 hours aerobic metabolism provides 98% of the required energy (Table 5.2.1).

Aerobic metabolism primarily utilizes carbohydrates and fats. The presence of sufficient oxygen allows pyruvate to enter the Krebs cycle via acetyl CoA and ATP formation through the respiratory chain. The main source of ATP for muscle contraction is glucose resulting from glycogen degradation. When necessary, the muscle aerobic system can also form ATP through the degradation of triglycerides and fatty acids. For a given oxygen concentration, fat releases more energy than carbohydrates (the carbohydrate reserve is limited). Thus, a 68 kg individual with 10-20% body fat contains carbohydrate reserves (in the form of muscle glycogen, liver glycogen and blood glucose) between 1,800-2,000 kcal. His fat reserves allow him to spend 63,000-120,000 kcal.

Recent data indicate that amino acids contribute with 3-6% of metabolic energy during exercise.

Table 5.2.1. ATP production from different pathways in diverse types of physical activity.

Type of physical activity	Average time	ATP-CP system	Lactic acid production (anaerobic) – muscular glycogen	Aerobic metabolism –muscular glycogen	Aerobic metabolism – liver glycogen and blood glucose	Aerobic metabolism – TG (fatty acids)
Fast use of energy (start of "sprint" or weightlifting)	0-3 s		100%	-	-	-
100-200 m run	5-30 s 10-12 min 20-25 min	50% 25%	50% 65%	10%	-	-
400-800 m run	1-2 s 50-60 s 2-2,5 min	12% 6%	63% 50%	25% 44%	-	-
5-10 km run	13-30 min 15-18 min 32-35 min	* *	12% 3%	88% 97%	-	-
Marathon (42-80 km)	+2 h 2.5-3 h 5.5-7 h	-	-	75% 35%	5% 5%	20% 60%

* Creatine-phosphate is used in the first seconds and, if re-synthesized during the race, is used until the end of the "sprint".

5.2.2. **Clinical cases**

Clinical case 5.2.1. *Physical activity: Marathon and altitude*

To adapt to the altitude of 3700 m (above sea level) a marathoner trained several weeks at the location of his next marathon. Now, a sponsor of another competitor, has invited this marathoner to spend a few days at his beach house, ensuring his return to the venue, the day before the sporting event. Is this a friendly invitation or rather an attempt to hurt this marathon runner's performance? Explain your answer.

Clinical case 5.2.2. *Physical activity: Marathon and prolonged fasting*

A young marathon runner has body fat levels of approximately 4%, while most runners have levels between 12 and 15%. Why in marathon runners can prolonged fasting be so severe?

Clinical case 5.2.3. *Physical activity*

State which neuroendocrine responses contribute to increased energy needs during exercise. Explain.

Clinical case 5.2.4. *Physical activity: Marathon*

A marathon runner entered the stadium for the final lap. At the last minute he was confused, and began to run in the wrong direction, eventually losing consciousness and falling.
a) What happened to this athlete?
b) What organs do we need to maintain with high energy levels permanently?
c) What type of fuel is used during the exercise and what are its sources?
d) What are the contributions of the various organs to maintain the proper functioning of key organs, such as the brain?
e) During the 200 m run the muscle goes into anaerobic metabolism and produces lactate. Is this production advantageous?
f) Which of the athletes will have the highest blood lactic acid levels: a marathon runner or an 800-meter sprinter.
g) What dietary regime should a marathon athlete follow in the 2-3 days prior to the race?

5.3. **Further questions**

Question 5.3.1.
Describe the main metabolic characteristics that give the liver a decisive role in maintaining the body's balance. What metabolic effects would you expect to see from severe liver disease?

Question 5.3.2.
Comment on the statement: "The liver is an altruistic organ".

Question 5.3.3.
Which molecules function as energy reserves in the human organism, and what are their main characteristics?

Question 5.3.4.
Which metabolic pathway(s) produces the majority of ATP through nutrient catabolism?

Question 5.3.5.
In which cellular and metabolic processes is ATP (or equivalent) consumed? Indicate the specific steps of these processes in which this energy expenditure occurs.

Question 5.3.6.
Define anabolism and catabolism. How are both processes related? Give examples of an anabolic pathway and a catabolic pathway.

Question 5.3.7.
What are the important molecules in energy metabolism that participate in oxidation-reduction reactions? Give an example of an oxidation-reduction reaction involving such molecules.

Question 5.3.8.
Is it possible to synthesize glucose in humans from the main product of β-oxidation?

Question 5.3.9.
Name three different vitamin-assisted metabolic reactions, mentioning for each reaction the involved vitamin(s).

Question 5.3.10.
What metabolic pathway(s) might be affected by a low thiamine diet? Explain why.

Question 5.3.11.
What metabolic pathway(s) might be affected by a low biotin diet? Explain why.

Question 5.3.12.
What metabolic pathway(s) might be affected by a low pyridoxine diet? Explain why.

Question 5.3.13.
Thiamine (vitamin B1) is critical for metabolism. Do you agree with the statement? Justify.

Question 5.3.14.
Individuals with thiamine deficiency demonstrate characteristic neurological signs such as loss of reflexes, anxiety, and mental confusion. Suggest a reason why thiamine deficiency is manifested by changes in brain function.

Question 5.3.15.
Under aerobic conditions, which molecules can be used by muscle cells to produce energy?

Question 5.3.16.
Under aerobic conditions, which molecules can be used by neurons to produce energy?

Question 5.3.17.
Why are mammals unable to carry out gluconeogenesis from even-chain fatty acids?

Question 5.3.18.
Why are mammals able to carry out gluconeogenesis from odd-chain fatty acids?

Question 5.3.19.
Why can't fatty acids be used as an energy source under anaerobic conditions?

Question 5.3.20.
Explain, justifying each case, which of the nutrients (glucose, fatty acids and amino acids) can be used as a source of energy by humans under aerobic conditions.

Question 5.3.21.
Explain, justifying each case, which of the nutrients (glucose, fatty acids and amino acids) can be used as a source of energy by humans under anaerobic conditions.

Question 5.3.22.
State which of the following cannot be considered substrates of gluconeogenesis: fatty acids with even number of carbons; fatty acids with odd carbon numbers; lactate; alanine.

Question 5.3.23.
What are the advantages (and/or disadvantages) of using glycogen instead of fatty acids as a form of energy store?

Question 5.3.24.
A patient has the following metabolic changes:

Table 5.3.1. Metabolic changes evidenced by one patient.

Increase	Decrease	Normal
Muscular glycogen	Gliceraldehyde-3-phosphate	Gluconeogenesis
Glucose-6-phosphate	2,3-Biphosphoglycerate	
Fructose-6-phosphate		

What enzyme deficiency could explain such changes? Explain why.

Question 5.3.25.
What is the basis of the hypothesis that athletes' muscles are more heavily buffered than those of untrained individuals?

Question 5.3.26.
What kind of athletes might benefit from having good creatine stores, and why?

Question 5.3.27.
What metabolic pathway is common to glucose and fatty acid metabolism?

Question 5.3.28.
During a heart attack, blood flow to the heart is drastically reduced by a blockage of a coronary artery. What changes in heart metabolism would you expect to see?

Question 5.3.29.
In the first moments of a very intense exercise, where does most of the energy that the muscle needs come from?

Question 5.3.30.
Refer to blood glucose regulation throughout the feeding-fasting cycle.

Question 5.3.31.
In Fig. 5.3.1 the letters in each square correspond to organs or tissues in a post-prandial situation. Indicate the organs or tissues corresponding to the respective letter.

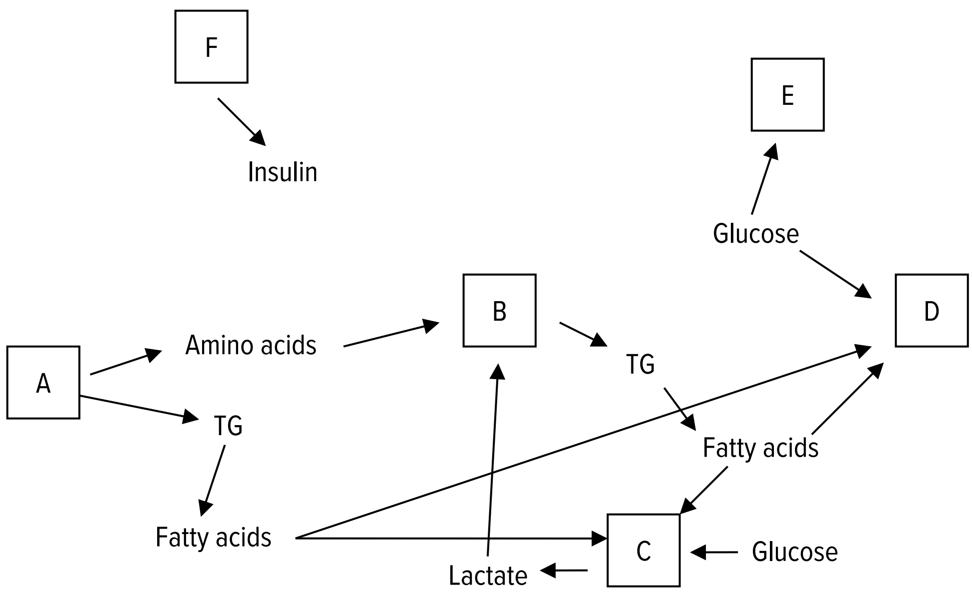

Fig. 5.3.1. Illustrative scheme of the existing metabolic interactions between different organs or tissues of the human organism (TG: triacylglycerols).

Question 5.3.32.
Fill in Fig. 5.3.2:

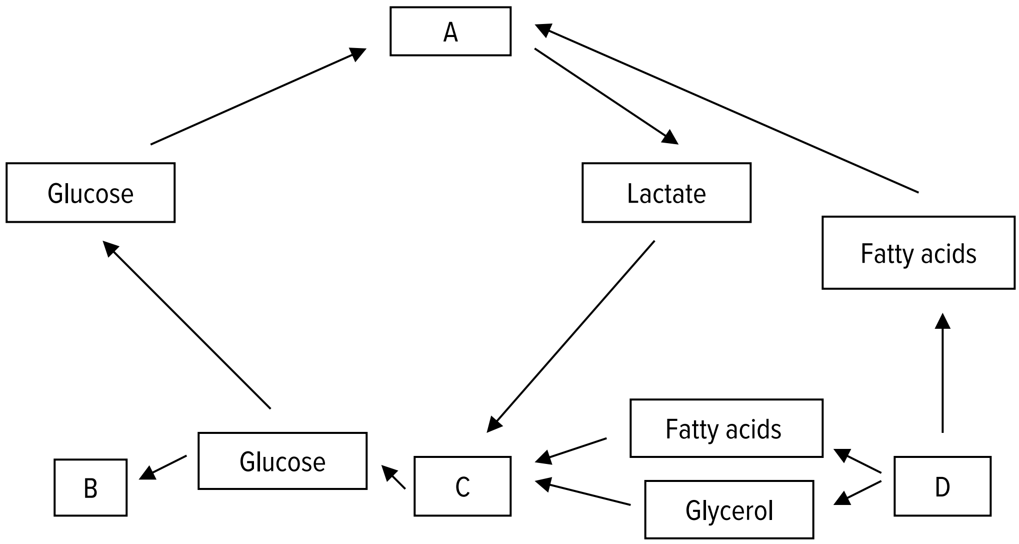

Fig. 5.3.2. Illustrative scheme of the existing metabolic interactions between different organs or tissues of the human organism.

Question 5.3.33.
Consider a patient with citrate synthetase deficiency. To what extent does this situation affect fatty acid metabolism? Explain.

Question 5.3.34.
Describe five possible destinations for amino acids that reach the liver after intestinal absorption.

Question 5.3.35.
Describe five possible fates of glucose-6-phosphate in the liver.

Question 5.3.36.
Describe five possible fates of fatty acids in the liver.

Question 5.3.37.
How is it possible for the liver to use lipids to control blood glucose levels?

Question 5.3.38.
Chronic alcoholics usually have symptoms of malnutrition and eat poorly. Why aren't they hungry?

Question 5.3.39.
In patients with uncontrolled diabetes, symptoms of acidosis and high plasma urea levels often appear.
a) What is acidosis due to?
b) What is the increase in plasma urea due to?

Question 5.3.40.
In patients with renal insufficiency, elevated levels of urea, uric acid and creatinine are observed in the blood. Explain why.

Question 5.3.41.
Explain whether it is possible to survive on a diet consisting solely of carbohydrates. What consequences would it have for the body's metabolism?

Question 5.3.42.
Explain whether it is possible to survive on a diet consisting solely of protein. What consequences would it have for the body's metabolism?

Question 5.3.43.
List the sources and metabolic products of glucose-6-phosphate.

Question 5.3.44.
Describe changes in carbohydrate metabolism caused by prolonged fasting and diabetes.

Question 5.3.45.
What is the role of citrate in the regulation of carbohydrate and lipid metabolism? Explain.

Question 5.3.46.
Consider the basic metabolism studied. What happens to the metabolism of lipids, carbohydrates and proteins after a prolonged fasting period? Explain by providing the appropriate enzymes and controls over the pathways.

Question 5.3.47.
Consider the basic metabolism studied. What happens to lipid, carbohydrate and protein metabolism after a high protein meal? Justify by providing the appropriate enzymes and controls over the pathways.

Question 5.3.48.
Below is a series of statements concerning an individual fasting for two days. Assume liver glycogen is depleted. All statements refer to the hepatocyte. When this is not the case, attention is drawn to it in the text. Make a table, grouping in the left column the letters of the statements that apply to the physiological situation of fasting and in the right column the letters of the statements that do not apply.
a) Urea production is low.
b) Low production or absence of NADPH is verified.
c) The enzyme glycogen synthetase is in its dephosphorylated form, that is, it is active.
d) Glucose-6-phosphatase is active, leading to the production of free glucose.
e) Acetyl-CoA from the intense β-oxidation of fatty acids is being used in the synthesis of ketone bodies.
f) Malonyl-CoA is not found in the cytosol.
g) There are high levels of plasma insulin.
h) Glucose-6-phosphate is metabolized by glycolysis.
i) Intense β-oxidation of fatty acids is taking place such that there is not enough oxaloacetate to place the product of this β-oxidation in the Krebs cycle.
j) Glucose-6-phosphate in the cytosol is used in glycogen synthesis.

Question 5.3.49.
Complete the following statement: …… is an ATP-generating metabolic pathway.
a) Glycolysis.
b) Lipogenesis.
c) Lipolysis.
d) Glycogenesis.

Question 5.3.50.
The regulation of fatty acid synthesis is related to glycolytic activity. Justify this statement.

Question 5.3.51.
For each of the following enzymes, state whether it is most active in the phosphorylated or dephosphorylated form:
a) Pyruvate dehydrogenase.
b) Glycogen phosphorylase.
c) Glycogen synthetase.
d) Pyruvate kinase.
e) Phosphofructokinase.

Question 5.3.52.
One person, after a period of fasting, received a meal rich in carbohydrates. What molecule must rise in plasma in the next half hour?

6. ENDOCRINE SYSTEM

CHAPTER CONTENTS

6.1. **General functioning**

As life forms progressed, from unicellular organisms to more complex ones, the existence and evolution of integrated systems became necessary, to guarantee the transmission of information from one place to another in the organism. Thus, the musculoskeletal system proves to be essential for anatomical integration, the nervous system is highly important in the adaptation of the organism to the external environment and the endocrine system also proves to be fundamental in the coordination of the function of the different organs, to guarantee the correct functioning of cells and organs.

Thus, the functions of the human body, as well as its homeostasis, depend on the precise regulation of different organs and systems. Together, the nervous system and the endocrine system regulate and coordinate the activities of almost every other structure in the body. Currently, an intimate relationship between these two systems is recognized since it is not possible to separate them anatomically and functionally. Some neurons secrete chemical substances (neurohormones) into the bloodstream, while others directly innervate the endocrine glands, influencing their secretory activity. When one of these systems, nervous or endocrine, stops working, homeostasis can be degraded very quickly.

The endocrine system plays extremely important functions in a healthy organism. The term endocrine system derives from the Greek words endo and crino which mean within and separate, respectively, and thus the endocrine glands secrete chemical signals that, in turn, will influence other distant tissues. This system is made up of several glands, each of which can detect minimal hormonal fluctuations in the bloodstream and thus regulating the secretion of these same hormones. The main endocrine glands are the hypothalamus, hypophysis (or pituitary), thyroid, parathyroid, adrenals, pancreas, ovaries and testes.

In general, endocrine glands are made up of aggregates of secretory cells of epithelial origin with intervening connective tissue, rich in blood and lymphatic capillaries. Cells of the endocrine system have prominent nuclei and abundant cytoplasmic organelles, especially mitochondria, endoplasmic reticulum, Golgi complexes, and secretory vesicles. All these glands of the endocrine system are actively involved in physiological processes of daily life. In addition to the endocrine glands, some organs, whose endocrine activity was thought to be null, secrete hormones, such as the secretion of leptin by adipose tissue and angiotensin by blood vessels.

The hypophysis or pituitary is the main gland of the endocrine system, located at the base of the skull below the brain, and directly or indirectly influences the production and release of other hormones.

Hormones, from the Greek hormon, are defined as a chemical signal produced by the endocrine glands and/or endocrine cells of other organs, which are released directly into the bloodstream and transported to the target cells. In the target tissue, hormones bind to protein receptors on the cell, thus exerting the desired signal and effect.

Based on their molecular structures, there are two types of hormones, which directly influence the way they are transported in the bloodstream and their mechanism of action once they reach the target tissue. Lipophilic hormones (steroid and thyroid hormones) must be transported in the bloodstream associated with proteins. Hydrophilic hormones (peptides and catecholamines) can circulate freely in plasma.

Also, the location of cell receptors for these two types of hormones is different. Lipophilic hormones bind to intracellular receptors (cytoplasmic or nuclear), as they can cross the membrane phospholipid bilayer. Hydrophilic hormones bind to plasma membrane receptors, triggering the formation of a secondary messenger that will activate a signalling cascade that culminates in the cellular response. Different hydrophilic hormones act through different secondary messengers, activating their own signalling cascades. These cascades involve phosphorylation of cellular proteins and consequent enzymatic activation/inhibition. The half-life of these hormones is short, and they trigger a fast response. Lipophilic hormones do not need to act through the formation of secondary messengers as they can trigger the cellular response directly through the formation of the hormone-receptor complex. This complex acts at the level of nuclear deoxyribonucleic acid (DNA), activating the transcription of certain genes. Therefore, these hormones have a longer half-life, and their effect takes longer to be noticed.

The main hormonal signalling pathways are: the adenyl cyclase pathway, in which cAMP is the secondary messenger; the phospholipase C pathway, in which IP3 and DAG are the second messengers; the guanyl cyclase pathway, with cyclic guanosine monophosphate (cGMP) as a secondary messenger; the tyrosine kinase pathway; and the steroid hormone pathway.

In the adenyl cyclase pathway, the binding of the hormone to the corresponding receptor (located in the cell membrane) activates the G protein (membrane-bound protein that couples hormone receptors to effector enzymes) which will lead to the activation of the membrane enzyme adenyl cyclase. This enzyme converts ATP into the cAMP secondary messenger. The increase in intracellular cAMP activates the protein kinase that phosphorylates the phosphorylase kinase, converting it into its active form, triggering the cellular response through enzymatic phosphorylations.

In the phospholipase C pathway, the G protein will activate the membrane enzyme phospholipase C, responsible for the formation, from a membrane phospholipid, of the secondary messengers IP3 and DAG, which will trigger the cellular response. IP3 binds to an endoplasmic reticulum receptor leading to the opening of channels that allow calcium ions (Ca^{2+}) to escape from the reticulum and increase their concentration in the cytosol. At the same time, there is depolarization of the membrane, opening of calcium channels and consequent entry of this ion into the cell. The increase in the cytosolic concentration of Ca^{2+}, associated with calmodulin, triggers the exocytosis of the hormone storage vesicles and consequent release of the hormone by the gland. DAG activates protein kinase which, by phosphorylation, will trigger specific cell responses.

In the tyrosine kinase pathway, the activation of the receptor mediates, through enzymatic phosphorylation, an intracellular signal transduction pathway. For example, in the insulin signalling pathway, the insulin receptor, belonging to a family of growth factor receptors, has intrinsic tyrosine kinase activity. This receptor is in the cell membrane and is composed of 2 subunits, α and β, which associate forming an α2β2 tetramer. The α subunit is the insulin-binding subunit, while the β subunit, transmembrane, has tyrosine kinase activity. After insulin binding, the receptor undergoes autophosphorylation at multiple tyrosine residues. This results in receptor kinase activation and consequent tyrosine phosphorylation of a family of insulin receptor substrates (IRS). Like other growth factors, insulin uses phosphorylation and protein-protein interactions as essential tools to transmit the signal. These protein-protein interactions are critical to transmit the receptor signal towards the final cellular effect. Tyrosine kinase-associated receptors do not have intrinsic tyrosine kinase activity but are non-covalently associated with proteins that do.

Hormones have an important role in the proper functioning of the body. For example, even before waking up in the morning, blood levels of hormones start to rise, as is the case of cortisol (a hormone that helps with waking up). Cortisol, secreted by the adrenal gland, helps to maintain normal blood pressure and glucose levels, to normalize electrolyte levels and thus the state of vigilance and alertness. Later, at the end of the day, and even during sleep, cortisol levels drop, making it possible to rest. The endocrine glands continue their surveillance with an unconscious and involuntary monitoring of the organism throughout the day, thus controlling the release of the respective hormones according to the individual's needs.

The secretion of most hormones does not occur at a constant rate, but in a pulsatile manner, that is, most endocrine glands increase and decrease their secretory activity drastically overtime. The rate of secretion of each hormone is controlled by a negative feedback mechanism. There are three main models of hormone regulation: the action of another substance on the endocrine gland in addition to the hormone; the neuronal control of the gland; and the control of the secretory activity of an endocrine gland by the hormone or neurohormone secreted by another gland.

Endocrine disorders can be divided into three types of conditions: hormone excess, hormone deficiency and hormone resistance. In any of these conditions, the homeostasis of the organism is compromised.

6.2. **Hypothalamus and Hypophysis**

6.2.1. **Short review**

Structure, secretion and regulation

Hypophysis is located below the hypothalamus and establishes connections with it by the infundibulum. The pituitary measures approximately 1 cm in diameter, weighs 0.5 to 1.0 g, and is situated in the sella turcica of the sphenoid bone. It is an organ that is divided into posterior lobe or neurohypophysis, and anterior lobe or adenohypophysis.

Lobes have a different embryonic origin, which is manifested in their structure and function. The neurohypophysis forms during embryonic development from the lower part of the brain, and so its axons extend from the lower part of the hypothalamus. The secretions of this gland are called neurohormones. The adenohypophysis is formed from the embryonic tissue of the oral epithelium, without neural connections with the hypothalamus. It can produce its own hormones, but their secretion is regulated by the hypothalamus. The pituitary diverticulum or Rathke's pouch contains the anterior pituitary and establishes relationships with the buccal cavity of the embryo. Near the neurohypophysis, this pouch loses its connection with the oral cavity and with the anterior pituitary.

The hypothalamus is related to the anterior pituitary due to the blood portal system, where the arterial blood supply to the median eminence and the pituitary is ensured by the superior medial and inferior pituitary arteries. The magocellular neurons of the supraotic nuclei and paraventricular nuclei have long axons, which terminate in the neurohypophysis.

The hypothalamus controls the secretory activity of the adenohypophysis, and the adenohypophysis controls the secretory activity of the thyroid, adrenal, and sex gonads. In turn, the secretory activities of these organs influence the secretory activity of the pituitary gland and, consequently, of the hypothalamus. The activity of the endocrine system is regulated by a feedback mechanism. Negative feedback (inhibitor) occurs when the concentration of hormones secreted by a gland (thyroid, adrenals and sex gonads) reaches a concentration greater than necessary, thus leading to the interruption of hormone secretion and the arrest of this mechanism. There will be positive feedback when the concentration of the hormone decreases, resulting in the need for the gland to produce it to ensure physiological activity.

Nerve stimulation of the hypothalamus occurs through at least four mediators: noradrenaline or norepinephrine, dopamine, serotonin or 5-hydroxytryptamine and γ-aminobutyric acid (GABA). Other substances, such as oligopeptides, may also have similar actions.

The hypothalamic-pituitary bundle is a nerve pathway made up of axons, which extends from the hypothalamus, through the infundibulum, to the neurohypophysis. In the hypothalamic magnocellular neurons, located in the cell body, the synthesis and maturation of hormones like ADH (or vasopressin) and oxytocin occur in the RER. In the Golgi apparatus, these hormones are packaged into granules and transported through the axons of the hypothalamic-pituitary tract, where hormonal processing takes place. Neurohormones (ADH and oxytocin precursor peptides) produced in the hypothalamus reach the neurohypophysis in the form of neurosecretion vesicles. These vesicles contain biologically active peptides, oxytocin and ADH, and other peptide products resulting from hormonal processing, neurophysins, which are released from nerve terminals to the neurohypophysis. The influx of calcium through voltage-gated channels causes depolarization and opening of calcium channels, thus exocytosis of the contents of the neurohypophysis vesicles into the systemic circulation (through the cavernous sinus and the internal jugular vein). These hormones have a short half-life, so they are rapidly cleared from the circulation by the kidneys, and to a lesser extent, by the liver and brain.

The hypothalamic regulatory hormones are: corticotropin-releasing hormone (CRH), which stimulates the secretion of adrenocorticotrophic hormone or corticotropin (ACTH); thyrotropin-releasing hormone (TRH), which stimulates the secretion of thyroid-stimulating hormone or thyrotropin (TSH) and prolactin; gonadotropin-releasing hormone (GnRH), which stimulates the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH); growth hormone releasing hormone (GHRH), which stimulates the secretion of growth hormone (GH) also called somatotropin; somatostatin or growth hormone-inhibiting hormone (GHIH), which inhibits the secretion of GH, TSH and prolactin; prolactin releasing factor (PRF) and dopamine (DA) or prolactin inhibiting factor (PIF), which stimulate or inhibit, respectively, prolactin secretion.

In turn, the adenohypophysis (Fig. 6.2.1) is responsible for the secretion of TSH, which acts on the thyroid with the production of thyroid hormones; secretion of ACTH, which acts on the adrenals to produce hormones such as cortisol, aldosterone and androgens; secretion of GH, hormone responsible for growth, bone and muscle differentiation, and production of insulin-like growth factors (IGF) or somatomedins; secretion of FSH and LH, which act on the sexual gonads (ovaries and testes) with production of hormones such as testosterone, progesterone and oestrogen; secretion of prolactin, which acts on the mammary glands triggering the production of breast milk.

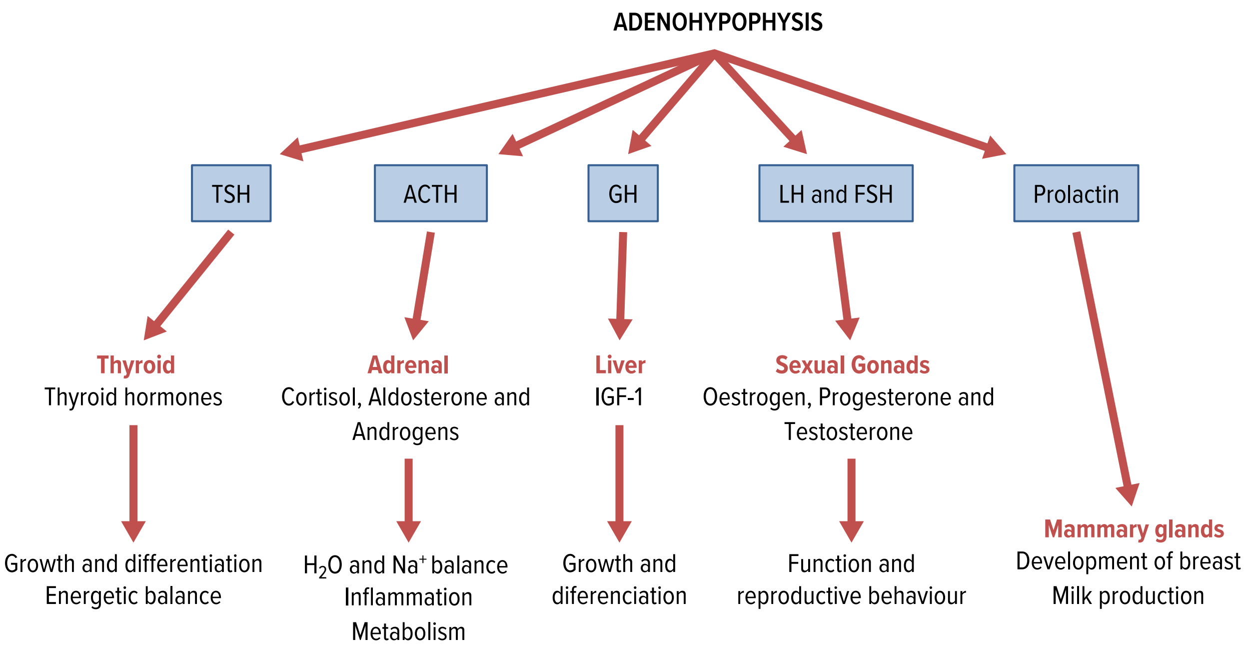

Fig. 6.2.1. Hormones secreted by adenohypophysis, target tissues and physiological effects.

Action on metabolism

Antidiuretic hormone

ADH is synthetized by cell bodies of neurons, in the supraotic nuclei of the hypothalamus, and transported by axons from the hypothalamic-pituitary bundle to the neurohypophysis, being stored at the terminals of the axons. Subsequently, ADH is released from these terminals into the blood and transported to the kidneys. This is a hydrophilic hormone, binding to membrane receptors on kidney cells. Its signalling cascade involves the synthesis of the secondary messenger cAMP, via adenylcyclase, and leads to the increased water retention in kidneys and consequent reduction on urine volume.

The secretion of ADH varies as a function of changes in osmolality and blood volume. The increase in osmolality leads to an increase in solutes concentration in solution. In this way, when blood osmolality increases, the frequency of action potentials in osmoreceptor neurons (specialized hypothalamic neurons that respond to osmotic pressure differences) and neurosecretory cells increases, enhancing ADH secretion in the hypothalamus and its release from the neurohypophysis. As ADH causes an increase in water retention by the kidneys, consequently there is a decrease in the volume of produced urine and a decrease in blood osmolality.

The decrease in blood osmolality can also be caused by excess water consumption. In turn, it decreases the frequency of action potentials in osmoreceptor neurons, decreases the amount of ADH synthesized in the hypothalamus, as well as the amount of hormone released in the neurohypophysis, and decreases renal reabsorption. Consequently, in minutes or a few hours, the volume of water eliminated in the form of urine increases.

ADH plays an important role in urine formation and in maintaining osmolality and extracellular fluid volume within normal values. There is a decrease in plasma volume and a decrease in blood pressure, which in turn increases the frequency of action potentials in the neurosecretory cells of the hypothalamus and increases ADH secretion. Consequently, it increases the release of ADH from the neurohypophysis into the bloodstream, and thus increases water retention. As water in the urine is extracted from the blood, as renal reabsorption increases, the amount of ADH in the blood volume decreases. If blood pressure or plasma volume increases, this phenomenon manifests itself in the reverse order. It can thus be said that ADH has the function of increasing constriction of blood vessels and increasing blood pressure.

Oxytocin

Oxytocin is synthesized by the cell bodies of neurons in the paraventricular nuclei (PVN) of the hypothalamus, and is transported along the axons to the neurohypophysis, being stored in the axon terminals. Being a hydrophilic hormone, its physiological effects are triggered by its binding to oxytocin receptors, located in the plasma membrane, and expressed in the uterus, mammary glands and brain. Its signalling cascade involves the synthesis of the secondary messengers IP3 and DAG, via phospholipase C.

The release of oxytocin stimulates contraction of uterine smooth muscle cells during childbirth and contraction of mammary myeloepithelial cells, which surround the alveoli of the mammary glands, during lactation. Distention of the cervix at the end of pregnancy, the end of stimulation of the mammary glands and the release of milk, constitute physiological signals that are transmitted to the supraoptic nuclei (SON) and PVN of the hypothalamus, terminating the release of oxytocin. The response of uterine muscle cells is positively influenced by the increase in the number of gap junctions between smooth muscle cells and the increase in prostaglandin synthesis.

Thus, whenever sensory neurons receive physiological information from the uterus and nipples, they transmit action potentials from these organs to the spinal cord, and then to the PVN and SON of the hypothalamus. If the concentration of oxytocin decreases, its production is stimulated in the hypothalamus through the mechanism of positive feedback, and action potentials are transmitted to the axons of the hypothalamic pituitary bundle up to the neurohypophysis, where the release of oxytocin by the hypothalamus occurs. There is also an increase in plasma oxytocin in non-pregnant women during menstruation, allowing the expulsion of the uterine epithelium, and smaller amounts of blood during menstruation and during sexual intercourse, which may interfere with the path of spermatozoa through the uterus.

The action of oxytocin in man is still not fully understood. However, it is known that the concentration of this hormone can decrease in situations of anxiety, and increase, improving libido and social relationships in men and women.

Thyroid stimulating hormone

TSH is a glycoprotein made up of α and β subunits, which binds to thyroid gland membrane receptors. Its function is to stimulate the synthesis and secretion of thyroid hormones by the thyroid gland. TRH, from the hypothalamus, binds to a membrane receptor located in the anterior pituitary. Its signalling cascade involves the synthesis of the secondary messengers IP3 and DAG, via phospholipase C, which will trigger the cellular response and consequent release of TSH by the anterior pituitary. Then, after binding to the membrane receptor, TSH initiates its signalling cascade that involves the synthesis of the secondary messenger cAMP, via adenyl cyclase.

TSH reaches higher levels at night and stimulates the thyroid gland to produce triiodothyronine (T_3) and tetraiodothyronine or thyroxine (T_4), hormones that stimulate cellular metabolism. In this way, TSH secretion is controlled by TRH, and also by hormones produced by the thyroid gland (T_3 and T_4). Moreover, thyroid hormones T_3 and T_4 inhibit the secretion of TRH and, consequently, TSH. Additional factors that inhibit TSH secretion are dopamine, somatostatin and glucocorticoids.

Growth hormone

GH is an important hormone in the regulation of growth and metabolism. As a peptide hormone, it binds to membrane receptors associated with tyrosine kinase, activating its signalling cascade. This hormone enhances the entrance of amino acids in cells that will be used in protein synthesis; increases lipolysis (degradation of triacylglycerols) and the release of fatty acids by adipocytes, which are used as primary energy sources; increases tissue glycogen synthesis and storage, using glucose as a secondary energy source; regulates blood nutrient levels after meals and during periods of fasting.

Indirectly, GH increases the production of polypeptides by the liver, skeletal muscles and other tissues. IGFs are polypeptides produced in response to GH. IGFs are transported in the blood to target tissues and by binding to the appropriate receptors stimulate the growth of cartilage and bones and increase protein synthesis in skeletal muscles. Examples are IGF-1 (also called somatomedin C), responsible for growth, muscle development, decreased levels of body fat and increased protein synthesis, and IGF-2 (somatomedin A) responsible for foetal growth and the ovarian cycle. They are both liver-produced polypeptides that have a structure identical to insulin.

GH release is regulated by the secretion of two neurohormones released by the hypothalamus, GHRH, which stimulates GH production, and somatostatin or GHIH, which inhibits GH secretion. Stimuli that influence GH secretion act on the hypothalamus to increase or decrease GHRH or GHIH production. Thus, GH secretion is stimulated by decreased blood glucose levels and stress and is inhibited by increased blood glucose levels. Elevated blood levels of some amino acids also increase GH secretion. GH secretion is higher during deep sleep and in children. In addition to GH, there are other factors that influence growth, such as genetics, nutrition and sex hormones. After adolescence, GH secretion decreases, but never ceases, allowing the dimensions of soft and hard tissues to be maintained.

Adrenocorticotrophic hormone

ACTH is a hormone derived from a molecular precursor, pro-opiomelanocortin, that acts on the adrenal glands. Upon stimulation of the hypothalamus, production of CRH occurs, which stimulates the cAMP cascade via adenylcyclase, that culminates in ACTH secretion in the anterior pituitary. ACTH is released into the systemic circulation and binds to membrane receptors, activating the adrenal cortex adenyl cyclase pathway, with cAMP synthesis, which, through activation of the signalling cascade, stimulates the production and release of glucocorticoids (cortisol), mineralocorticoids (aldosterone) and to a lesser extent androgen precursors.

The release of CRH and ACTH is punctually stimulated by stress, infection, hypoglycaemia, physical exercise, surgery or trauma. Circadian ACTH secretion occurs in a pulsatile manner, with higher intensity in the morning and lower during the night. The serum cortisol reaches maximum level between 6 and 8 am, during sleep, just before awakening. A negative feedback controls hormonal secretion. Thus, when plasma cortisol concentration is high, cortisol inhibits ACTH secretion in the anterior pituitary and of CRH in the hypothalamus. Cortisol is the anti-stress, anti-inflammatory and anti-insulin hormone.

Luteinizing and follicle-stimulating hormones

Gonadotropins are hormones that act on the sex gonads and include LH and FSH. These hormones are secreted by the anterior pituitary and regulate reproduction. The secretion of these hormones is ensured by the release of another hormone produced in the hypothalamus, GnRH, which acts on the anterior pituitary cells. The mechanism of action of GnRH involves the production of the secondary messengers IP3 and DAG, via phospholipase C, with activation of exocytosis and release of gonadotropins into the circulation. LH and FSH are released into the bloodstream and bind to membrane receptors, activating the intracellular synthesis of cAMP, whose signalling cascade via adenylcyclase culminates with the production of gametes (sperm in the testes and eggs in the ovaries).

Feedback control by the hypothalamic-pituitary system regulates the secretion of these hormones. If the concentration of steroid hormones (testosterone in men and oestrogen in women) increases, there is negative feedback in the anterior pituitary, decreasing the production of FSH and LH, and negative feedback in the hypothalamus, decreasing the production of GnRH.

During pregnancy (2nd to 5th month) the plasma concentration of gonadotropins is very low. During childhood, this secretion is null, occurring its reactivation in adolescence, with an increase in FSH secretion relative to LH. In reproductive age, women have monthly cyclic increases in LH relative to FSH, which is not seen in men. For both sexes over 50 years of age, the production of gonadotropins increases, with FSH levels being higher than LH. This increase in FSH in relation to LH is more pronounced in women than in men, corresponding to the time of menopause.

FSH and LH act during the woman's menstrual cycle and have a pulsatile and synchronized secretion. FSH stimulates follicular development in the ovaries and the secretion of oestrogens, thus constituting the follicular phase. Oestrogen is responsible for the development and maintenance of female secondary sexual characteristics (fat deposits on the breasts, buttocks and thighs; growth of pubic and axillary hair; development of the mammary glands and pelvis enlargement). LH controls the maturation of Graafian follicles, ovulation, formation and maintenance of the corpus luteum during the luteal phase and stimulates the secretion of progesterone and oestrogens. Progesterone is responsible for preparing the endometrium for implantation of the embryo and the mammary gland for lactation. If fertilization occurs, the embryo produces chorionic gonadotropin, which maintains the corpus luteum. If not, the corpus luteum ends up degenerating, and menstruation occurs. If the plasma concentrations of oestrogen and progesterone increase, a negative feedback control at the anterior pituitary level will occur.

In men, FSH, together with testosterone, stimulates spermatogenesis in the cells of the seminiferous tubules (Sertoli cells). LH stimulates the production of testosterone by Leydig cells or interstitial cells. Testosterone is responsible for tissue development of the testes, prostate, and secondary sexual characteristics (such as muscle and bone development, growth of facial, axillary and pubic hair, increased basal metabolism, decreased body fat, and deep voice development).

Prolactin

Prolactin is a hormone produced by the anterior pituitary that plays an important role in the normal development of breast tissue and in the production of milk by the mammary glands in breastfeeding women. TRH is a hypothalamic neurohormone that, in addition to interfering with TSH secretion, is also responsible for the release of prolactin by the anterior pituitary. Prolactin binds to membrane receptors associated with tyrosine kinase activating its signalling cascade via tyrosine kinase. The release of prolactin from the anterior pituitary is subject to the positive control of hypothalamic PRF and the negative control of hypothalamic dopamine. Nipple sucking stimulates the release of prolactin, which in turn inhibits its own production by stimulating the release of dopamine from the hypothalamus.

Prolactin can also increase the number of receptor molecules for FSH and LH in the ovaries, enhancing their secretion. After ovulation, it can also stimulate increased secretion of progesterone by the ovary.

Men have lower levels of prolactin, and its physiological effect is not yet fully understood.

6.2.2. **Pathologies**

Hyperpituitarism

Acromegaly and gigantism

Clinical manifestations of acromegaly result from excess GH and/or IGF-1, as well as from the effect of the pituitary adenoma on structures adjacent to the sella turcica or pituitary fossa. Ectopic GHRH secretion may be related to the presence of acromegaly and gigantism in patients with carcinoid or islets of Langerhans tumours in the pancreas. This disorder may also be characterised by abnormal release of GH by the action of TRH and GnRH. Chronic GH hypersecretion is caused by the stimulation and release of IGF-1. IGF-1 is related to the proliferation of bones, cartilage and soft tissues and to the increase in size of other organs, triggering classic manifestations of acromegaly. GH secretion, although high, has a random prevalence throughout the day. It turns out that glucose suppression does not occur, and in turn, there is no GH stimulation by hypoglycaemia.

The incidence of acromegaly is approximately equal in both sexes, with a higher prevalence in the age group of 40 years. Symptoms are characterized by an acromegalic face with localized and excessive bone growth of the skull and mandible; enlargement of the extremities ("sausage toes"); arthralgia and carpal tunnel syndrome; sleep apnoea; changes in glucose metabolism (with insulin resistance, glucose intolerance and diabetes mellitus); skin symptoms (thickening of the skin, increased oiliness and skin papilloma's); cardiovascular symptoms (systemic arterial hypertension, heart failure, arrhythmias, cerebrovascular disease); respiratory symptoms (sleep apnoea); psychological symptoms (mood change, depression); endocrine symptoms (hyperprolactinemia, diabetes mellitus); symptoms associated with visceromegaly, due to excessive organ growth (goitre, hepatomegaly, splenomegaly, cardiomegaly); fatigue, weakness and lethargy.

Gigantism presents similar manifestations to acromegaly. However, this pathology appears more frequently in children and adolescents. Patients with gigantism may develop acromegaly if GH hypersecretion persists into adulthood or is not treated.

GH-secreting pituitary adenomas are a consequence of the chronic hypersecretion of GH, which, in turn, causes an increase in IGF-1 (mediator of most of the effects of GH). Patients have associated hypogonadism that delays epiphyseal metabolism. The combination of excess IGF-1 with hypogonadism may also increase linear bone growth. These adenomas often arise after the existence of prolactinomas. Although these are not malignant, they present a clinical picture resulting from hyperprolactinemia, such as visual deficits and headaches.

Classic, cyclic and ectopic Cushing syndrome

Classic Cushing's syndrome results from inappropriate exposure to high levels of cortisol. For this reason, this syndrome is also called hypercortisolism. This syndrome is characterized by hypersecretion of ACTH, with bilateral adrenocortical hyperplasia and hypercortisolism. There is also abnormal negative feedback of glucocorticoids on ACTH secretion. It was also observed abnormal response of GH, TSH and gonadotropins produced after stimulation.

Corticotropic adenomas are responsible for the hypersecretion of ACTH and are obtained from a pituitary adenoma that develops spontaneously. Hypercortisolism suppresses the normal functioning of the hypothalamic-pituitary axis and the release of CRH, thus nullifying the hypothalamic regulation of circadian variation and stress responses.

The disease is variable in terms of gender, age, duration and severity, having an incidence of about 13 cases per million inhabitants. In 45-70% of patients, dyslipidaemia (increased LDL and decreased HDL) and glucose intolerance are observed.

Symptoms may develop after months or years of cortisol hypersecretion. The most significant and concordant signs and symptoms are capillary weakening, emotional disturbances, full moon face, red cheeks, buffalo hump/fat pockets, osteoporosis, thin and wrinkled skin, cardiac hypertrophy, thin arms, adrenal tumour or hyperplasia, centripetal obesity, stretch marks, weak legs, muscle weakness and skin ulcers that are difficult to heal. In men, kidney stones and erectile dysfunction are also common. In women, loss of libido and menstrual irregularities are usually present. At the neurophysiological level, there are also cases of depression, emotional irritability, sleep disorders, cognitive deficit, mania and anxiety. Less specific manifestations are hirsutism, acne, decreased wound healing speed, arterial hypertension, glucose intolerance with hyperglycaemia and diabetes mellitus, psychiatric disorders, hypogonadism, amenorrhea and impotence.

The increase in glucocorticoid levels in the body also has an influence on the immune system, reducing cellular immunity. This factor may be important since these patients are more susceptible to opportunistic infections.

This pathology can develop because of endogenous or exogenous factors, the most common being exogenous (e.g., uncontrolled and poorly supervised administration of glucocorticoids). These substances are mainly used in the treatment of inflammation, autoimmune diseases or neoplastic disorders. It can also result from the use of substances containing prednisone (a substance that has a similar effect to cortisol), other injectable corticosteroids used for bone and joint pain, inhaled steroids for the treatment of asthma or creams for skin conditions.

The disease caused by endogenous factors has an incidence of 2-3 cases per million people, with a higher incidence in females in the 5th decade of life. This type of Cushing's syndrome can have several aetiologies, whether at the level of the pituitary, adrenal or even an ectopic tumour. The signs and symptoms are very variable and only occur when there are episodes of hypercortisolism, being like classic Cushing's syndrome symptoms.

Cyclic Cushing's syndrome is one of the variants of this syndrome that is characterized by the occurrence of episodes of hypercortisolism, which are alternated by periods with normal cortisol secretion. The most frequent cause is the exogenous administration of glucocorticoids.

Ectopic Cushing's syndrome stands out from the classic syndrome as it is associated with rare causes such as primary pigmented nodular hyperplasia of the adrenal or micronodular hyperplasia, macronodular hyperplasia and ectopic secretion of CRH. It can also be caused by the appearance of tumours that release excessive amounts of ACTH, the most common being small cell lung cancer. However, it can be due to other types of neoplastic pathologies such as different types of carcinomas (thymus, bronchial, pancreatic, gastrointestinal, thyroid), neuroendocrine tumours or pheochromocytoma.

In ectopic Cushing's syndrome, acute symptoms of hypercortisolism also appear, related to the advanced phase of the syndrome, such as hypertension, hypokalaemia, oedema, psychosis and opportunistic infections. As adenomas are smaller in size, clinical symptoms related to the primary ACTH-secreting tumour (e.g., headache or visual impairment) are rarely seen. The clinical course of this syndrome varies according to the type of tumour. An example is the syndrome caused by less aggressive tumours, which manifests characteristics very similar to the pituitary-dependent Cushing's syndrome, causing a later diagnosis. In these cases, symptoms such as severe hypertension that persists despite treatment, hypokalaemia and metabolic alkalosis must be considered. The usually affected age group is 20-40 years, although it can also manifest in infants and patients over 70 years of age and prevails in males.

Hyperprolactinemia

The aetiology of prolactin hypersecretion can be physiological, pathological or idiopathic. It has a physiological aetiology when it occurs during pregnancy, lactation, stress or during sleep.

On the other hand, the pathological aetiology occurs: when it is an endocrine abnormality resulting from hypothalamic-pituitary disorders, such as tumours and trauma; due to pituitary hypersecretion in cases of prolactinomas (microadenoma and macroadenoma), metastatic tumours, infections such as tuberculosis, sarcoidosis, histocytosis, acromegaly, Cushing's syndrome; systemic disorders such as renal failure, hypothyroidism, cirrhosis and hypersecretion induced by drugs such as dopamine receptor blocking agents, histamine receptor antagonists, selective serotonin reuptake inhibitors (SSRIs) and calcium channel blockers.

This condition may also have an idiopathic cause.

Hyperprolactinemia is more common in patients with prolactinomas. These are pituitary tumours that can be classified into microadenomas (with dimensions smaller than 10 mm), present in premenopausal women, and macroadenomas (with dimensions equal to or greater than 10 mm), common in men or postmenopausal women. The distribution by gender is approximately equal. Prolactinomas most often arise from the lateral wings of the anterior pituitary, but progress and fill the sella turcica, compressing the remaining anterior and posterior lobes.

Clinically, the predominant physiological consequence of hyperprolactinemia is hypogonadotropic hypogonadism, which results from GnRH suppression. Clinical manifestations depend on age, sex and the amount of excess prolactin. Clinical manifestations occur earlier in women than in men, with symptoms of oligomenorrhea, amenorrhea, galactorrhoea, decreased libido, infertility and decreased bone mass. In postmenopausal women, galactorrhoea is rare. Hypoestrogenism due to prolonged hyperprolactinemia can result in osteopenia. Women with hyperprolactinemia and normal menses have normal bone marrow density. Hyperprolactinaemic women may show signs of chronic hyperandrogenism, such as hirsutism and acne. In males there is erectile dysfunction, decreased libido, infertility, gynecomastia, decreased bone mass and rarely galactorrhoea.

Overtime, the patient may experience lower energy, reduced muscle mass, and an increased risk of osteoporosis. Neurological symptoms may also occur, caused by pressure from a tumour mass of a macroprolactinoma. Symptoms include headaches, visual field loss, cranial neuropathies, hypopituitarism, and seizures.

Syndrome of the inadequate secretion of the antidiuretic hormone

This syndrome is characterized by excess ADH that causes water retention, hypervolemia and inhibits the action of the renin-angiotensin-aldosterone system (RAAS) increasing thirst and natriuresis. Consequently, hyponatremia, decreased plasma osmolality (Posm) and increased urinary osmolality (Uosm) occur.

Etiologically, this pathology is characterized by neoplasms that are the main cause of this disease, disorders of the central nervous system (CNS) due to lesions in the neurohypophysis or pathological activation of the hypothalamic-pituitary-adrenal axis (trauma, tumours, infections, hydrocephalus, bleeding associated with hyponatremia), lung diseases (pneumonia, tuberculosis, aspergillosis and bronchiectasis) and use of drugs that can stimulate the release of ADH (e.g. non-steroidal anti-inflammatory drugs, tricyclic antidepressants, SSRIs, neuroleptics, thiazide diuretics and ecstasy). SSRIs are the drugs that are most effective in stimulating the release of ADH.

Individuals infected with human immunodeficiency virus (HIV) may also experience syndrome of inappropriate antidiuretic hormone secretion, due to *Pneumocystis jiroveci* pneumonia and CNS infections.

The main clinical manifestations are related to hyponatremia (decreased level of sodium ion in the serum). Hyponatremia is euvolemic, and results in an inability to dilute urine in the presence of plasma hypoosmolality. The clinical picture is variable, depending on the speed with which the syndrome develops and the intensity of the hyponatremia. Hyponatremia must be diagnosed in less than 48 hours, as clinical conditions can progress to seizures and coma.

The more severe the hyponatremia, the more severe the symptoms. Initially, patients are asymptomatic. Mild symptoms appear when the serum sodium concentration is between 125-130 mEq/L, with nausea, headache, myalgia, malaise, hyperoxia and mental confusion. If the sodium concentration varies between 115-125 mEq/L, mild symptoms such as lethargy, disorientation, agitation, depression and psychosis begin to appear. Severe symptoms develop when the sodium concentration is less than 115-120 mEq/L, with respiratory arrest, convulsions and coma. Cerebral oedema may also occur.

Thyroid-stimulating hormone-secreting pituitary adenoma

TSH-secreting pituitary adenomas are rare tumours that manifest as hyperthyroidism with goitre in the presence of elevated TSH. They are larger chromophobic adenomas, characterized by presenting symptoms resulting from tumour compression, such as headaches and visual changes. These tumours appear with similar frequency in both sexes and can occur in a mixed or plurihormonal form, with tumour hypersecretion of TSH and co-secretion of GH and/or prolactin or, more rarely, LH and FSH.

Hypopituitarism

Kallmann´s syndrome

Kallmann´s syndrome is a congenital form of idiopathic hypogonadotropic hypogonadism. This pathology, caused by a defect in neuronal migration, involves the GnRH-producing cells and the neurons of the olfactory bulbs. Thus, it is characterized by the presence of an olfactory disorder, anosmia or hyposmia, and renal agenesis, synkinesia, cleft palate and syndactyly.

Decreased release of GnRH affects different gonadotropins (FSH and LH), and consequently leads to delayed puberty with a higher incidence in males that show a decrease in testicular volume. Females may have absence of pubertal development, amenorrhea, and menarche. Most individuals with puberty delay have no underlying endocrine pathology, showing only a delay in growth and maturation. There are also psychological and emotional disturbances, due to hypogonadotropic hypogonadism, and bone demineralization. In males, cryptorchidism and micropenis are frequent clinical manifestations of hypogonadotropic hypogonadism in infancy/childhood.

Although most detected cases are sporadic, there may be a hereditary aetiology, and the mode of transmission may be autosomal dominant, autosomal recessive or X-linked recessive. Mutations occur in genes that regulate GnRH migration in neurons and affective bulb morphogenesis. To date, seven genes involved in the development of this pathology have been identified: *KAL1*, *FGFR1*, *PROK2*, *PROKR2*, *FGF8*, *CHD7* and *WDR11*. Patients with mutations in the *KAL1* gene have severe hypogonadism and renal impairment. On the other hand, mutations in the *FGR1*, *FGF8* or *CHD7* genes lead to the development of cleft lip, cleft palate and high-arched palate, tooth agenesis and facial asymmetry. Furthermore, mutations in the *KAL1*, *FGFR1*, *FGF8*, *PROKR2* or *CHD7* genes can cause sensorineural deafness and mutations in the *KAL1*, *FGFR1*, *PROK2* and *PROKR2* genes may provoke synkinesis. Less frequently, musculoskeletal, neurological or cardiac disorders, obesity and oculomotricity may also occur.

Sheehan´s syndrome

Sheehan's syndrome is characterized by postpartum hypopituitarism, which can lead to pituitary necrosis. It results from hypotension or severe shock, due to bleeding during or after delivery. This disease is common when delivery care is inadequate. Clinical manifestations can occur postpartum or several months or even years after delivery.

In the acute phase of the disease, headaches, vision changes, decreased lactation and postpartum amenorrhea appear. The chronic phase is characterized by a deficiency of GH, LH, FSH, ACTH and TSH hormones and leads to symptoms such as asthenia, muscle weakness, dry skin, anaemia, nausea, vomiting, hair loss and infertility.

Other frequent clinical manifestations in patients with this syndrome are psychiatric disorders such as postpartum depression, weight loss, syncope, epigastric pain, drowsiness, sweating, inability to respond to verbal stimuli, premature wrinkles, acute circulatory collapse, congestive heart failure, hypoglycaemia and diabetes insipidus.

Pituitary dwarfism

Pituitary dwarfism is characterized by a deficiency in the production of GH by the anterior lobe of the pituitary gland and consequently reduced growth in childhood and adolescence. The deficiency of this hormone can be total or partial and can occur in isolation or associated with deficiency of other hormones such as TSH and ACTH.

Pituitary dwarfism causes a decrease in the growth rate of bones and soft tissues, with maximum heights of approximately 1.50 m. Etiologically, in most cases, this type of dwarfism is due to a congenital intracranial tumour, usually of the craniopharyngioma type, which disrupts the functioning of the pituitary gland, causing a decrease in hormone production. Other possible causes are infections and pituitary lesions.

Clinical signs of this disorder are round skull and face, small jaw, prominent font, small nose with a cell root, exophthalmos, short neck, small larynx with a thin voice, small hands and feet, thin hair, increased fat in the trunk and cryptorchidism. Less frequently, patients may also show decreased hairiness, lower basal metabolism in children, insulin hypersensitivity, premature aging and risk of cardiovascular failure.

6.2.3. **Clinical cases**

Clinical case 6.2.1. *Blockage of norepinephrine action*

A chemical agent that blocks the action of norepinephrine was injected directly into the hypothalamus of a laboratory animal and a change in the secretion rate of various hormones in the anterior pituitary was observed. Explain this observation since norepinephrine is not a pituitary hormone.

Clinical case 6.2.2. *Diabetes insipidus*

ADH was given to four individuals marked in the graph (1, 2,3 and 4).

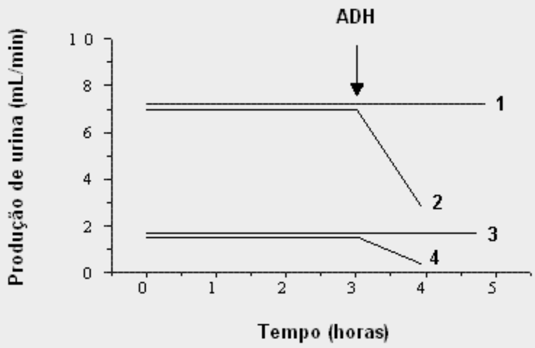

Fig. 6.2.2. Urine production after ADH administration.

a) Which is the normal individual, and which one suffers from hereditary diabetes insipidus? Explain.
b) What is the symptom(s) present in cases of inappropriate ADH secretion syndrome?

Clinical case 6.2.3. *Cushing syndrome*

Cushing's syndrome may be caused by a tumour in the adenohypophysis or adrenal cortex. Explain how these two possibilities can be distinguished by measuring blood levels of ACTH and cortisol.

Clinical case 6.2.4. *Cushing syndrome*

Suppose a patient blood test reveals very high levels of ACTH, leading to suspicion of the presence of a hormone-secreting tumour. What is the possible location for that tumour? Explain.

Clinical case 6.2.5. *Cushing syndrome*

In one patient there are elevated levels of ACTH. What metabolic processes are affected? What is the effect on glucose levels (increase or decrease)?

Clinical case 6.2.6. *Adenohypophysis dysfunction*

A 35-year-old window cleaner complained of muscle weakness, difficulty in climbing stairs and getting up. He had truncal obesity, violet abdominal striae, "full moon" face, and "buffalo neck". His blood pressure was 180/110 mmHg. The results of patient blood tests are shown in the following table:

Table 6.2.1. Results obtained in patient blood analysis.

Parameter	Patient values	Standard values
Na^+	136 mmol/L	135-145 mmol/L
K^+	3.2 mmol/L	3.4-4.9 mmol/L
HCO_3^-	33 mmol/L	21-28 mmol/L
Glucose	7.5 mmol/L	2.8-6.0 mmol/L
Cortisol (9:00 hours)	930 nmol/L	140-690 nmol/L
Cortisol (24:00 hours)	900 nmol/L	100 nmol/L
ACTH (9:00 hours)	130 ng/L	10-80 ng/L
Urinary cortisol	840 nmol/24h	< 300 nmol/24h

The patient underwent the dexamethasone suppression test, and the following results were obtained for cortisol (9:00 hours): low dose - 880 nmol/L; high dose - 320 nmol/L. Explain the presented case.

6.3. **Thyroid and Parathyroid**

6.3.1. **Short review**

Structure, synthesis and regulation of secretion

Thyroid

The thyroid is a highly vascularized endocrine gland, with a butterfly shape because it consists of two lobes that are connected by a narrow bridge of thyroid tissue called the isthmus. The lobes are found laterally juxtaposed to the upper half of the trachea, immediately below the larynx. The thyroid is one of the largest endocrine glands weighing approximately 15-25 g. This gland is heavier in women, and even increases in weight during pregnancy. It is surrounded by a fibrous capsule, from which thin connective tissue septa extend into the gland itself, dividing it into its constituent lobes.

The thyroid contains several small spheres, called thyroid follicles, whose walls are composed of a layer of cubic epithelial tissue cells, delimited by a basal membrane. Between the follicles is a delicate network of loose connective tissue with numerous capillaries. Parafollicular cells are dispersed between follicles and follicle wall cells.

The thyroid is an endocrine gland with a particular characteristic that makes it unique compared to other endocrine glands, since it can store large amounts of hormones in an inactive form, inside extracellular compartments (in the centre of the follicles). When inactive, thyroid cells are simple, flattened or cubic cells, but when they are synthesizing hormone, the epithelial cells are taller, with more cytoplasm and large palely stained nuclei, thus reflecting their increased activity.

The thyroid produces two hormones, T_3 and T_4, the latter being the most synthesized (Table 6.3.1). Both are released into the bloodstream and transported to target tissues. There, T_4 is converted into T_3, with T_3 being the active hormone that influences target cells, with high affinity for the respective nuclear receptors.

Table 6.3.1. Properties of the hormones T_3 and T_4.

Hormonal property	T_3	T_4
Serum concentrations		
Total hormone	0.14 μg/dL	8 μg/dL
Free hormone	6×10^{-12} M	21×10^{-12} M
Half-life	0.75 days	7 days
Production rate	32 μg/day	90 μg/day
Intracellular hormonal fraction	~ 70%	~ 20%
Relative metabolic power	1	0.3

The biosynthesis of T_3 and T_4 requires a complex enzyme system, involving several sequential steps. The most abundantly expressed protein in the thyroid gland is thyroglobulin (Tg), involved in the storage of the inactive form of thyroid hormone and iodine, as well as in the synthesis of thyroid hormones. Tg is composed by tyrosine and is synthesized in the follicular cells.

Considering the chemical structure of T_3 and T_4, the presence of iodine in the body is essential, especially in the thyroid. To initiate the production of thyroid hormones, iodide ions (I^-) are actively transported into the cells of the thyroid follicles. Inside the follicular cells, I^- are oxidized to iodine, and one or two iodine atoms are chemically bonded to each of the tyrosine units of Tg. This last step occurs simultaneously with the secretion of Tg by exocytosis into the lumen of the thyroid follicle. In the follicle lumen, two diiodotyrosine molecules from Tg combine to form T_4, or, alternatively, a monoiodotyrosine molecule combines with a diiodotyrosine molecule to form T_3. After their synthesis, the T_3 and T_4 hormones are stored in large quantities inside the follicles as components of Tg.

When needed, Tg is transported to follicular cells, where lysosomes fuse with endocytic vesicles. In lysosomes, proteolytic enzymes degrade Tg to release the hormones T_3 and T_4, which in turn diffuse from the follicular cells into the interstitial spaces and finally into the capillaries of the thyroid gland. Tg constituting amino acids are recycled for new Tg synthesis.

Once released into the bloodstream, these hormones are transported to target cells by plasma proteins. About 70 to 75% of the T_3 and T_4 hormones circulate in the bloodstream associated with thyroxine-binding globulin (TBG), while the remaining percentage is bound to other plasma proteins, such as albumin. Being lipophilic hormones, the mechanism of action of the T_3 and T_4 hormones involves binding to an intracellular receptor.

Regarding the production of T_3 and T_4 by the thyroid follicles, it is essential to highlight the importance of TSH produced by the adenohypophysis. This hormone is responsible for activating the synthesis of T_3 and T_4 hormones. In fact, without TSH stimulation, at the level of the thyroid follicles, the production and consequent release of T_3 and T_4 hormones into the bloodstream does not occur.

The TRH produced by the hypothalamus will stimulate the anterior pituitary to produce TSH. The increase in the production of TRH by the hypothalamus may occur due to some stimuli such as exposure to cold or stress, while prolonged fasting decreases the production of TRH. Just as TRH stimulates the production of TSH, TSH also stimulates, at the thyroid level, the production of T_3 and T_4, through hyperplasia and hypertrophy of thyroid cells. When the release of TRH decreases, the secretion of TSH also decreases and consequently the hormones T_3 and T_4 decrease. Due to this relationship between the different hormones, it can be said that the thyroid is controlled by a negative feedback mechanism. That is, the hormones T_3 and T_4, when in excess in the bloodstream, have an inhibitory effect on the secretions of TRH by the hypothalamus and TSH by the anterior pituitary (Fig. 6.3.1).

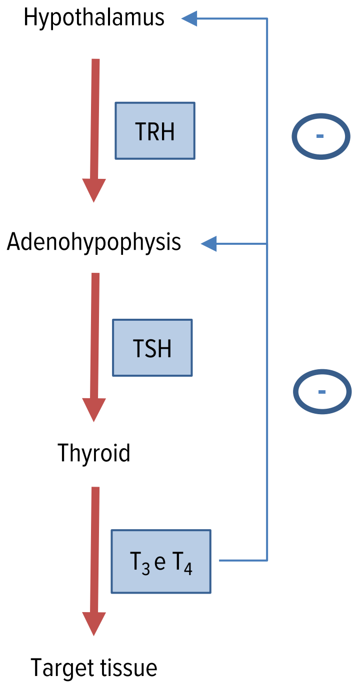

Fig. 6.3.1. Regulation of T_3 and T_4 hormones secretion.

The thyroid gland also produces the hormone calcitonin in parafollicular cells. This hormone is a polypeptide consisting of 32 amino acids, involved in calcium metabolism. For maximum biological activity, the presence of the complete structure of the hormone, that is, its 32 amino acids, is required. Calcitonin has a half-life of approximately 5 min, being metabolized by the liver and eliminated by the kidneys.

Calcitonin is produced by the parafollicular or C cells of the thyroid gland and later stored in secretory granules. This hormone is part of the family of peptides such as amylin and other peptides that are distributed in different peripheral tissues, thus inducing several biological effects, such as potent vasodilation, reduced nutrient intake and, finally, decreased bone resorption.

The calcitonin signalling cascade involves, after binding to the respective receptors located in the kidney and bone, the stimulation of adenyl cyclase activity and consequent production of cAMP that will trigger the cellular response.

Calcitonin interferes with serum calcium ion concentrations, with bone being its main target organ. This hormone interacts with the membrane receptors of osteoclasts, decreasing their activity and increasing the lifespan of osteoblasts, that is, calcitonin decreases bone resorption while increasing calcium deposition in bones. This bone activity results in a decrease in blood calcium levels. Calcitonin inhibits parathyroid hormone-induced osteoclastic bone resorption and rapidly blocks the release of calcium and phosphate from bones. This hormone also influences the kidney, by binding to its receptors in the cortical ascending portion of the loop of Henle.

Parathyroid

The parathyroid glands are located on the posterior surface of each thyroid lobe, being included in it. There are four of them and their cells are organized in densely packed masses or cords. These glands secrete parathyroid hormone or parathormone (PTH), involved in the regulation of plasma calcium levels.

The amount of calcium and phosphate ions in the blood results from the absorption of these ions coming from the diet in the small intestine, the deposition of these ions in the bone and their release from the bone. The skeleton serves, among other functions, as a reserve of calcium and phosphate in the form of calcium phosphate crystals called hydroxyapatite. Osteoblasts are involved in the removal of calcium and phosphate from the blood and their deposition in bone. On the other hand, osteoclasts promote the dissolution of hydroxyapatite crystals, thereby releasing calcium and phosphate ions into the blood.

PTH secretion is regulated by plasma calcium levels. Fluctuations of this ion are detected by the calcium-sensitive parathyroid receptor. It is a receptor located in the plasma membrane of the cells of the parathyroid glands, which is coupled with the G protein. Thus, when plasma calcium levels are high, this ion binds to the calcium-sensitive parathyroid receptor. Activation of the receptor leads to the activation of the G protein which triggers the activation of phospholipase C and consequent activation of phospholipase A2. The latter activates the arachidonic acid cascade and production of leukotrienes that lead to the degradation of PTH, decreasing its release into the bloodstream. On the other hand, whenever plasma calcium levels are low, PTH secretion occurs. This will lead to an increase in plasma calcium which, through negative feedback, promotes inactivation of the parathyroid gland. Also, the active form of vitamin D leads to inhibition of PTH secretion. However, in this case the decrease in PTH secretion results from reduced expression of the PTH encoding gene.

PTH secretion is also regulated by plasma levels of phosphate and magnesium. Thus, in the presence of high plasma levels of phosphate, there is a decrease in the activity of the phospholipase A2 enzyme and a consequent reduction in the production of arachidonic acid. Consequently, inhibition of the secretion of PTH ceases, thus increasing its secretion. Hyperphosphatemia can also indirectly affect PTH production, since it leads to a decrease in plasma calcium levels and activates vitamin D. On the other hand, hypophosphatemia leads to a decrease in PTH production by repressing the transcription of its encoding gene. Plasma magnesium levels regulate PTH secretion in the same way as calcium levels.

Action on metabolism

Thyroid

Thyroid hormones affect almost every tissue in the body. However, not all tissues respond in the same way. In general, these hormones cause an increase in metabolic activity and are essential for the normal process of growth and maturation of the organism.

Thyroid hormones can alter the number and activity of mitochondria, thus resulting in increased ATP production. These hormones increase the activity of the Na^+ and K^+ ion exchange pump, thus contributing to an increase in body temperature. Thus, a hypersecretion of thyroid hormones increases basal metabolism, while hyposecretion leads to a decrease in basal activity, body temperature, muscle strength, among others. Basal metabolism can increase by 60 to 100% when thyroid hormones are high in the bloodstream.

The basal metabolism depends on the adequate supply of thyroid hormones. In carbohydrate metabolism, thyroid hormones accelerate the absorption of glucose from the gastrointestinal tract and stimulate the uptake and oxidation (glycolysis) of glucose in most cells and synthesis of glucose (glycogenolysis and gluconeogenesis) in liver. This influence occurs through the ability of the T_3 and T_4 hormones to increase glycolysis, gluconeogenesis and insulin secretion.

Thyroid hormones also influence lipid metabolism by leading to the mobilization of lipids from adipose tissue, thus increasing the plasma concentration of free fatty acids. In this way, they increase the cellular oxidation of free fatty acids and stimulate the synthesis and biliary secretion of cholesterol. Thyroid hormones have a greater effect on the expression of the gene coding for HMG-CoA reductase, a major limiting and controlling point in cholesterol biosynthesis. On the other hand, thyroid hormones also control the production of LDL receptors at the level of hepatocytes. That is, in hepatocytes, thyroid hormones stimulate increased production of LDL receptors, which absorb LDL cholesterol from the blood plasma.

In protein metabolism, thyroid hormones activate the transcription of nuclear genes, which stimulates protein synthesis, resulting in an increase in the functional activity of the organism. The growth and maturation of organs also depend on thyroid hormones. Bones, hair, teeth, connective tissue and nervous tissue require thyroid hormones for normal growth and development. These hormones also promote brain growth and development, which leads to hyperexcitability. The effect of these hormones at the level of the cardiovascular system is to cause vasodilation in all tissues, which results in an increase in heart rate and contraction and cardiac output. At the level of the respiratory system, these hormones cause an increase in the frequency and depth of breathing. In the gastrointestinal system they cause an increase in appetite, in the speed of secretion of digestive juices and in motility. Furthermore, thyroid hormones act on the endocrine system, causing an increase in the rate of hormone secretion and in the need for hormones by the tissues. They also cause an increase in muscle reaction.

Regarding the hormone calcitonin, its production rate and consequent release/secretion increase because of the increase in blood calcium levels. Calcitonin release is regulated by plasma calcium levels through a calcium receptor present on parafollicular cells. Plasma calcium levels greater than 9 mg/dL stimulate the release of calcitonin. Other hormones such as glucagon, gastrin and serotonin also increase calcitonin secretion. After meals, produced gastrin induces the secretion of calcitonin, that will regulate postprandial calcium levels.

Individuals with medullary thyroid carcinoma can show an increase in the serum concentration of calcitonin. However, calcitonin levels undergo concentration fluctuations throughout life, showing a decrease with age, especially in women.

Recently, non-thyroid sources of calcitonin have been identified, such as bronchial cells of the lung, prostate and brain. However, to date, its physiological role is not known.

Excessive or deficient production of calcitonin does not result in an obvious disturbance in calcium balance. The importance of calcitonin in the balance of calcium homeostasis is not yet clear, since normal serum calcium concentrations are observed in individuals who have undergone a thyroidectomy (removal of the thyroid) and therefore also removal of parafollicular cells.

Parathyroid

The main target tissues for PTH are bone, kidney and intestine. In bone, PTH stimulates osteoclast activity and may even cause an increase in the number of osteoclasts. The increased resorption and rate of bone destruction causes the release of calcium and phosphate, increasing blood calcium levels.

In the kidneys, PTH induces calcium reabsorption so that less calcium is excreted in the urine. This hormone also causes an indirect stimulation of Ca^{2+} reabsorption by the gut, increasing vitamin D through stimulation the C-1 hydroxylation of 25-hydroxy-vitamin D within the kidney.

In the small intestine, PTH causes an increase in the production of active vitamin D, which results in an increase in the absorption of calcium and phosphate by the intestine and, consequently, an increase in blood calcium levels. In this way, these ions can be deposited in the bone.

In these target tissues, three types of PTH receptors have been identified (PTHR1, PTHR2, PTHR3) all coupled to G protein. PTHR1 is responsible for the physiological effects of this hormone. On the other hand, the physiological importance of receptors 2 and 3 has not yet been identified. The binding of PTH to PTHR1 leads to the activation of the adenyl cyclase pathway responsible for the synthesis of cAMP. PTHR1 can also activate other signalling cascades such as that of phospholipase C that culminates in an intracellular increase in calcium levels.

6.3.2. **Pathologies**

Throughout life some functional problems may occur in the endocrine system. If the complex feedback regulation system that tells the human body how much and when certain hormones should be produced, shows deficiencies, serious endocrine disease, and even death can occur. Thyroid disorders affect 1 in 200 adults, being more common with increasing age particularly in women.

Hypothyroidism

Hypothyroidism is an abnormal physiological condition characterized by decreased production and secretion of thyroid hormones. In this disorder, it is possible to verify abnormally low serum levels of T_3 and T_4, which lead to a decrease in basal metabolism (about 40% below the reference values).

Hypothyroidism can be classified according to the origin of the problem: primary hypothyroidism when the dysfunction originates from the thyroid itself; and secondary hypothyroidism when the origin comes from the pituitary gland, with low serum concentrations of the hormone TSH.

Primary hypothyroidism is relatively common and affects 4-8% of the population, being even more prevalent in women than in men, at a ratio of approximately 10 to 1. This dysfunction can be divided into subclinical and clinical. In subclinical hypothyroidism, gland failure is minimal, showing a slight decrease in thyroid hormones, with serum values in the normal range. However, due to high pituitary sensitivity, there is a slight increase in TSH levels. Most of the time, subclinical hypothyroidism is asymptomatic, being detected only by biochemical tests. On the other hand, clinical hypothyroidism is characterized not only by low levels of thyroid hormones but also by visible clinical manifestations, which reflect the inherent decrease in metabolic rate.

In adults, primary hypothyroidism may be associated with a decrease in thyroid tissue resulting from an autoimmune disease, surgery, or radioactive iodine treatment (Table 6.3.2). However, not all cases of hypothyroidism are related to a reduction in the size of the gland and may also be associated with an enlargement of the thyroid gland, resulting from lymphocytic infiltration such as Hashimoto's thyroiditis or dietary iodine deficiency.

As for secondary hypothyroidism, as already mentioned, it is characterized by low TSH secretion and consequent reduction in the production and release of thyroid hormones. This dysfunction results from a disorder of the anterior pituitary or hypothalamus, such as pituitary or hypothalamic neoplasia, congenital hypopituitarism or pituitary necrosis (Sheehan's syndrome) (Table 6.3.2).

Table 6.3.2. Causes of hypothyroidism.

Causes of hypothyroidism	**Primary**	Hashimoto disease
		Post-radiotherapy
		Post-surgery
		Deficiency of iodine in food
		Pharmaceutical products (example: amiodarone, lithium, interferon)
	Secondary	Hypothalamus or hypophysis neoplasm
		Post-radiotherapy of the head
		Hypophysis necrosis (Sheehan´s syndrome)

In general, hypothyroidism shows non-specific signs and symptoms such as fatigue, lethargy, depression, cold intolerance, weight gain, constipation, menorrhagia, hoarseness, cognitive changes/dementia and myalgias. The most evident signs are bradycardia, dry skin and hair, non-pitting oedema, cerebellar ataxia, slow reflexes, peripheral neuropathy, and there may also be goitre or signs of congestive heart failure.

In older adults, hypothyroidism can be confused with Alzheimer's disease or other dementias. In women, hypothyroidism is often confused with depression.

Hashimoto thyroiditis

Thyroiditis encompasses a diverse group of disorders characterized by inflammation of the thyroid gland, such as Hashimoto's thyroiditis, a chronic lymphocytic thyroiditis. Hashimoto's thyroiditis was first reported in 1912 by Hakuro Hashimoto, who described four women initially asymptomatic, but that progressed to hypothyroidism, with thyroid alterations characterized not only by a diffuse and painless enlargement of the gland but also by an intense lymphocytic infiltrate. Since then, the disease has been of interest in several studies.

Hashimoto's thyroiditis is the most common cause of hypothyroidism in adults, especially in women. Individuals with this pathology often have a family history of thyroid disease. This Hashimoto's condition is also common in individuals with chromosomal disorders such as Turner's, Down's or

Klinefelter's syndromes or can be associated with autoimmune diseases such as Addison's disease, hypoparathyroidism and diabetes.

As any autoimmune disease, this condition is characterized by the immune system attacking the body itself, more precisely the thyroid, thus inevitably interfering with the normal functioning of the gland. Therefore, this disease is usually accompanied by anti-Tg, anti-thyroid peroxidase (anti-TPO) and anti-thyrotropin receptor antibodies (anti-TRAb). With the autoimmune response, cytokines are released, followed by inflammation that causes glandular destruction and consequent clinical hypothyroidism.

The autoimmune process is thought to start with the activation of CD4 T lymphocytes specific for thyroid antigens. During Hashimoto's thyroiditis, lymphocytes recruit B cells and CD8 T cells into the thyroid. The progression of the disease leads to the death of thyroid cells, resulting in the appearance of hypothyroidism. The reason why lymphocytes are activated is still unknown. However, there is the theory of viral or bacterial infection with proteins very similar to the thyroid protein.

In the early stages of Hashimoto's thyroiditis, the gland is diffusely enlarged, firm, indurated, and nodular. As the disease progresses, the gland becomes smaller. In advanced stages the gland is atrophic and fibrotic. Microscopically, the destruction of thyroid follicles and lymphocytic infiltration with lymphoid follicles are evident.

Signs and symptoms of Hashimoto's thyroiditis are like hypothyroidism described above. Among the signs and symptoms already mentioned are dry skin and hair, fatigue, weight gain, constipation, menorrhagia, hoarseness, cognitive disorders/dementia, myalgia, among others. One of the typical features that is most evident in Hashimoto's thyroiditis is a swollen face with swollen eyelids.

Euthyroid disease syndrome

The term euthyroid disease syndrome identifies abnormalities in tests to assess thyroid function in individuals with non-thyroid systemic disease and in those undergoing surgery. The term non-thyroid disease syndrome has been used to describe these same anomalies.

In general, abnormalities in thyroid function assessment tests can result from several situations, usually reversible, such as disorders in the hypothalamic-pituitary-thyroid axis, in the binding of thyroid hormones to plasma proteins, in the absorption by the tissues of thyroid hormones and/or thyroid hormones metabolism. These changes reduce the bioavailability as well as the activity of thyroid hormones, thus creating a clinical condition of hypothyroidism.

Proper and timely recognition of thyroid function changes in various non-thyroid systemic diseases is of paramount importance, as abnormal thyroid function test results can sometimes mimic or mask the biochemical changes seen in patients with intrinsic thyroid disease.

Hyperthyroidism

The term hyperthyroidism refers to any condition in which there is an excess production of T_3 and T_4 hormones. In other words, the thyroid gland is overactive. The physiological condition of hyperthyroidism is a form of thyrotoxicosis, that is, a clinical condition that results from inadequate action of the thyroid hormones, usually due to their inadequate excessive levels. Hyperthyroidism affects approximately 2% of women and 0.2% of men worldwide.

In this condition, there is an increase in basal metabolism of about 60-100% above the reference values. Therefore, in hyperthyroidism the main symptoms are heat intolerance, palpitations, tremor, anxiety, fatigue, weight loss, muscle weakness, diarrhoea, difficulty sleeping, irritability and hot, moist skin. The most visible signs are tachycardia, fixed gaze, eyelid retraction, goitre and hyperreflexia. The rarest manifestations, which may occur in less than 1% of individuals with hyperthyroidism, are localized dermopathy, i.e., pretibial myxoedema, and swelling of the fingertips, called thyroid acropachy.

The main causes of hyperthyroidism include Graves' disease (autoimmune disease), benign or neoplastic adenoma (which rarely promotes hormone hyposecretion), viral infection (thyroiditis), pituitary tumour, thyroid storm (sudden release of large amounts of thyroid hormones triggered by surgery, stress, infection or unknown cause).

Graves' disease

Thyrotoxicosis is a clinical condition triggered by an excess of circulating thyroid hormones in the human body. The main aetiology of thyrotoxicosis is hyperthyroidism caused by Graves' disease (in about 60-80%), toxic adenomas, among other clinical situations.

The prevalence of Graves' disease varies in the population depending mainly on iodine intake. Studies confirm that an increase in iodine intake promotes an increased prevalence of Graves' disease. This clinical condition is rarely diagnosed before adolescence, that is, Graves' disease typically appears between 20 and 50 years of age and may also appear at an older age.

Graves' disease is an autoimmune pathology in which there appears to be a deficiency in thyroid-specific T lymphocytes, which somehow allows the formation of thyroid-stimulating immunoglobulin G (IgG) antibodies. That is, T lymphocytes become sensitized to antigens present in the thyroid and end up stimulating B lymphocytes to synthesize antibodies against these same thyroid antigens. The antibodies eventually target and bind to the TSH receptor on the surface of thyroid cells, stimulating the growth and function of thyroid cells. Therefore, it is normal and recurrent to verify the presence of anti-TPO and anti-Tg antibodies in the bloodstream of individuals with Graves' disease.

There are certain factors that can stimulate the autoimmune process, including pregnancy, excess iodine, treatment with iodine, viral and bacterial infections and the suspension of treatment with corticosteroids.

The signs and symptoms evidenced in Graves' disease include their own characteristics, as well as others like any other situation of thyrotoxicosis. The clinical presentation depends on the severity of thyrotoxicosis, the duration of the disease, the age of the individual and their susceptibility to high concentrations of thyroid hormones. The most evident symptoms are hyperactivity, irritability, dysphoria, sweating, heat intolerance, palpitations, fatigue, weakness, weight loss with increased appetite and diarrhoea, among others. Signs of this condition are tachycardia, tremor, goitre, muscle weakness and eyelid retraction.

In Graves' disease, the thyroid gland is enlarged, usually symmetrically, due to hypertrophy and diffuse hyperplasia of the thyroid follicular cells, which may or may not be associated with infiltrative ophthalmopathy, thyroid acropachy and, more rarely, with localized myxoedema.

Infiltrative ophthalmopathy is a problem present in about 40% of individuals with Graves' disease. Its onset begins with the period of hyperthyroidism and is thought to be a consequence of antibody-mediated inflammation and infiltration. This ophthalmopathy is described by the increase in the dimensions of the extraocular muscles, due to the inflammatory infiltrate, which consequently leads to proptosis when the eye is displaced anteriorly and orbital oedema when compressing the orbital veins. This condition causes eye discomfort, itching, excess tear production and photophobia.

Pretibial myxoedema is an immunologically less common process that occasionally affects patients with Graves' disease. Its onset occurs years after the treatment of hyperthyroidism, usually in patients with ophthalmopathy. This condition is characterized by the appearance of slightly scaly erythematous (hard but not painful) plaques limited to the skin of the ankles and pretibial area. They usually resolve spontaneously.

Goitre

The term goitre describes any enlargement of the thyroid gland, which occurs in 4 to 10% of the adult population. This clinical situation can be caused by a single nodule, multiple nodules, or a regular enlargement of some or all the glands.

Goitre can be classified according to its epidemiology, aetiology, anatomical and morphological characteristics, functional status or even the association of these different characteristics. Goitre can present two types of morphology, diffuse or multinodular, and can still be considered endemic, sporadic or familial, taking into account its epidemiology. Etiologically, goitre can arise in situations of iodine deficiency, Graves' disease, Hashimoto's thyroiditis and neoplasia. It may also exhibit retrosternal or cervical anatomy and a state of function toxic or euthyroid.

Goitre can be simple, that is, non-toxic, as well as toxic. The toxic goitre is usually nodular and associated with hyperthyroidism, while simple goitre can be diffuse or multinodular.

The main events or factors that can trigger the development of goitre are hereditary deficiency of enzymes of the T_4 biosynthetic pathway, inflammatory diseases (such as Hashimoto's thyroiditis and Graves' disease), thyroid tumours, iodine deficiency and other goitrogenic agents.

Overtime, there has been a higher prevalence of goitre in women, which increases with age until adulthood, where a decrease in prevalence begins, more evident in males.

Osteoporosis

Osteoporosis means "porous bone" and is characterized by a deterioration of bone tissue, leading to a reduction in bone mass and consequent bone fragility.

Osteoporosis can be classified as primary or secondary. The concept of primary osteoporosis represents two conditions: type I osteoporosis, which is characterized by loss of trabecular bone due to a lack of oestrogen; and type II osteoporosis in which cortical and trabecular bone loss occurs in men and women due to prolonged inefficiency of remodelling, dietary deficiency, and activation of the parathyroid axis with age. Secondary osteoporosis is caused by systemic diseases or by certain medications such as glucocorticoids or phenytoin.

Osteoporosis is a public health disease. Studies suggest that one in two women and one in four men aged 50 and over will experience a bone fracture due to osteoporosis. Fracture ratios between females and males are 7:1 at the spine, 1.5:1 at the distal forearm, and 2:1 at the hip.

All postmenopausal women and men 50 years of age and older should be clinically evaluated for osteoporosis risk to determine the need for mean bone density (BMD) testing. In general, the greater the number of risk factors present in the individual, the greater the actual risk of fracture. Osteoporosis is both preventable and treatable, but since there are no warning signs before a fracture, many people are not diagnosed in time to receive effective therapy during the early stages of the disease. Many factors have been associated with the risk of osteoporosis-related fractures (Table 6.3.3).

Table 6.3.3. Risk factors of osteoporosis.

Lifestyle	Pharmaceutical products	Other disturbances
Low calcium consumption	Anticoagulants (heparin)	Cystic fibrosis
Vitamin D insufficiency	Lithium	Haemochromatosis
Excess of vitamin A	Cyclosporin	Homocystinuria
Immobilization	Aromatase inhibitors	Riley-Dale syndrome
Thinness	Barbiturates	Intestinal mal absorption
Alcohol	Anticonvulsants	Lupus
Tobacco	Glucocorticoids	Arthritis rheumatoid
High caffeine consumption		Celiac disease

Hypoparathyroidism

Classic hypoparathyroidism

This hypoparathyroidism is characterized by inappropriate secretion of PTH. This PTH hyposecretion can be congenital when associated with immune deficiency or acquired. There are several possible causes for the acquired form such as neoplasms, surgical removal of the parathyroid glands, magnesium deficiency, autoimmune, hemochromatosis or idiopathic.

Clinical features include hypocalcaemia and hyperphosphatemia. A frequent complication is cataracts, which can result from high phosphate levels leading to calcium phosphate precipitation in the eye lens. A classic clinical sign is called Chvostek's sign, which is characterized by spasm or contraction of facial muscles in response to facial nerve percussion. Patients may also be asymptomatic.

In cases of hypoparathyroidism, plasma calcium levels normalize after PTH administration.

Pseudo-hypoparathyroidism

Contrary to the above, pseudohypoparathyroidism does not result from low PTH secretion. Instead, it superficially resembles hypoparathyroidism, but in this case the plasma levels of PTH are high. This type of hypoparathyroidism arises from an abnormal response to PTH.

There are two types of pseudohypoparathyroidism, both very rare hereditary diseases: type 1 and type 2. In type 1, there is a congenital defect in the G protein that is normally activated by the PTH receptor. As a result, there is no activation of adenyl cyclase and consequently there is no formation of cAMP. This leads to no cellular response to the presence of PTH. Patients with type 1 hypoparathyroidism have abnormal skeletal features such as a round face, short stature, shorter fourth and fifth metacarpals and metatarsals, and a tendency to form exostoses. These patients may have learning difficulties.

In type 2 hypoparathyroidism, cAMP formation occurs but responses to this second messenger are blocked.

In cases of pseudohypoparathyroidism, plasma calcium levels do not normalize after PTH administration.

Hyperparathyroidism

Primary hyperparathyroidism

Primary hyperparathyroidism is the excessive production of PTH. This condition has a prevalence of 1 case in 1,000 individuals and can occur in men or women of any age, however, it is more common in post-menopausal women. The most frequent cause of this hypersecretion is a parathyroid adenoma. The second most frequent cause is diffuse hyperplasia of the parathyroid glands. Less often, it results from a parathyroid carcinoma. Parathyroid adenomas can be multiple and sometimes have a familial character, resulting from some type of syndrome.

As a result of this excessive production of PTH, clinical manifestations will occur, such as increased plasma calcium levels accompanied by urinary excretion of this same ion, which leads to increased production of kidney stones. Another characteristic symptom is the decrease in plasma phosphate levels.

Although patients with hyperparathyroidism may be healthy for many years and asymptomatic, they are at an increased risk of developing osteoporosis and kidney failure.

Secondary and tertiary hyperparathyroidism

Patients with chronic renal failure or vitamin D deficiency often develop the so-called secondary hyperparathyroidism. In both cases, there is a decrease in calcitriol synthesis that leads to hypocalcaemia. Consequently, there will be an increase in PTH secretion.

In the initial phase of renal failure, there is a decrease in the plasma levels of vitamin D and calcium, which leads to an increase in PTH secretion. At a more advanced stage, there is a reduction in the number of calcium and vitamin D receptors in the parathyroid glands, which makes them resistant to regulation by retro-inhibition of PTH secretion. In this way, the plasma calcium level continues to rise, without inhibition of PTH secretion.

Patients who have undergone kidney transplantation may develop hypercalcemia, as they are able to metabolize vitamin D normally. This is called tertiary hyperparathyroidism.

6.3.3. **Clinical Cases**

Clinical case 6.3.1. *Hypothyroidism*

An individual with symptoms of hypothyroidism has low plasma concentrations of T_3, T_4 and TSH. After TRH injection, the concentrations of these three hormones increased. Where is the disability leading to hypothyroidism?

Clinical case 6.3.2. *Hypothyroidism*

Blood tests of a 63-year-old woman with stress angina revealed the results in the table below.

Table 6.3.4. Results obtained in patient blood tests.

Parameter	Patient values	Standard values
TSH	96 mU/L	0.35-5.0 mU/L
T_4	23 nmol/L	55-144 nmol/L
Cholesterol	9.3 mmol/L	< 6.5 mmol/L
Creatine kinase	290 U/L	< 150 U/L
AST	35 U/L	12-48 U/L

An electrocardiogram showed some evidence of ischemia but was not indicative of myocardial infarction. How should these results be interpreted?

Clinical case 6.3.3. *Thyrotoxicosis*

A 28-year-old woman with thyrotoxicosis has had two treatments with carbimazole (anti-thyroid drug). The results obtained in your latest blood tests are in the table.

Table 6.3.5. Results obtained in patient blood tests.

Parameter	Patient values	Standard values
TSH	< 0.05 mU/L	0.35-5.0 mU/L
T_4	210 nmol/L	55-144 nmol/L

a) What happened?
b) Which other biochemical tests can be useful in this case?

Clinical case 6.3.4. *Hyperthyroidism*

A 7-year-old female attended an endocrinology consultation due to enlargement of the anterior neck. After examination, she was prescribed propylthiouracil. After a month of medication, she noted a significant increase in goitre. Levothyroxine was then associated with propylthiouracil at a dose of 100 mg/day. Justify the applied treatments.

Clinical case 6.3.5. *Production of an abnormal substance that works as TSH*

A healthy individual is said to have normal thyroid function when the rate at which thyroid hormones are secreted remains within normal concentration limits. However, in some individuals, the immune system initiates the production of an abnormal substance that functions like TSH. Describe the effect this substance will have on thyrotropin and thyroid hormone secretion rates.

Clinical case 6.3.6. *Thyroxin supplementation*

If a person takes a high dose of thyroxine tablets for an extended period, blood thyroxine levels will be elevated, TSH secretion will be low and thyroid secretion will be reduced. Explain why.

Clinical case 6.3.7. *Thyroid nodule*

A 49-year-old woman on hormone replacement therapy found a thyroid nodule. No lymphadenopathy was detected and clinically appeared to be euthyroid. It was found to be a "cold" nodule and ultrasound indicated it was cystic. Blood biochemical results were as shown.

Table 6.3.6. Results obtained in patient blood tests.

Parameter	Patient values	Standard values
T_4	172 nmol/L	55-144 nmol/L
TSH	0.40 mU/L	0.35-5.0 mU/L

a) Why is T_4 level so high.
b) What other tests should be done?

Clinical case 6.3.8. *Thyroid cancer*

A 25-year-old woman sought medical attention due to the recent appearance (six months) of a lump in the cervical region. On clinical examination, there was suspicion of a thyroid nodule, and the patient underwent a biopsy (fine needle) that found no evidence of malignancy. She was submitted to a surgery to remove the thyroid nodule with preservation of the remaining gland. Pathological examination revealed a benign adenoma. About a year after the surgery, the patient detected a new cervical nodule, soon followed by others, and again underwent surgery to remove only the nodules since the biopsy showed no signs of malignancy. After the anatomopathological examination, showing signs of malignancy, the patient underwent a "near-total" thyroidectomy. Whole-body iodine-131 scanning performed after two months showed a large amount of iodine-capturing tissue in the right thyroid lobe region and in both lung fields. The patient underwent surgery to complement thyroidectomy. She was also submitted to radioiodine therapy at the dose of 200 mCi (milliCuries) of iodine-131. Whole body scan showed two iodine-foci in lung fields and serum thyroglobulin dosage was 165 ng/mL (reference value < 10 ng/mL). She was subjected to a new dose of 200 mCi iodine-131. Whole body scan showed no iodine-capturing area and serum thyroglobulin dosage was 1.5 ng/mL. Justify the serum thyroglobulin values obtained under both circumstances.

Clinical case 6.3.9. *Multinodular goitre*

A 65-year-old male patient has 12 years of asymptomatic slow, progressive, anterior cervical tumour. Physical examination revealed an asymmetric cervical mass of 20x10 cm, which occupies the entire thyroid gland, with a predominance of the left lobe, of firm consistency, uneven, non-painful surface, poorly mobile with swallowing, limiting cervical mobility. TSH and T_4 values were normal. Fine-needle aspiration biopsy revealed benign cytology. Cervical ultrasound revealed multinodular goitre. The patient underwent total thyroidectomy. Anatomopathological study revealed adenomatous hyperplasia. The patient evolved favourably, without dysphonia and without clinical signs of hypocalcaemia. He is currently taking levothyroxine (100 μg/day). Justify the clinical procedures adopted.

Clinical case 6.3.10. *Hashimoto's thyroiditis (Autoimmune thyroiditis)*

A 58-year-old patient reports that for the past two months he has been very forgetful. He had two episodes of loss of sense with body tremors, which soon improved. Sometimes he has difficulty viewing at night-time. This patient has been treating thyroid disease for 5 years, but for 5 months has been taking the medicine irregularly. On physical examination he presents with a slow and thick voice and answers questions in a confused way. The skin as well as the hair are dry and brittle, and the tongue is thick and bulky. He has a blood pressure of 100/65 mmHg, bradycardic heart (40 bpm) and decreased tendon reflexes. The diagnosis was Hashimoto's thyroiditis (autoimmune thyroiditis). Justify.

Clinical case 6.3.11. *Graves' disease (Autoimmune disease)*

Limited cutaneous scleroderma was diagnosed by histopathology in a 12-year-old female patient. After three years of treatment with diltiazem (40 mg/day), she had heat intolerance, increased skin oiliness, tachycardia, tachypsychism and enlarged thyroid gland. Thyroid hormone assay showed hyperthyroidism and antibodies: thyroid anti-peroxidase 140 U/mL (reference value <35 U/mL), anti-microsomal 6400 U/mL (reference value <100 U/mL) and anti-thyroglobulin 1600 U/mL (reference value <100 U/mL). Graves' disease (autoimmune disease) was diagnosed and treatment with anti-thyroid drugs and radioactive iodine was prescribed. The response was excellent, and the patient evolved euthyroid for 3 years without any anti-thyroid drug, maintaining positive autoantibodies. Justify the diagnosis and the prescribed treatment.

Clinical case 6.3.12. *Hypocalcaemia*

In a situation of hypocalcaemia, what happens to the rate of secretion of hormones that regulate plasma calcium concentration?

6.4. **Adrenal**

6.4.1. **Short review**

Structure, secretion and regulation

The adrenal glands (also known as suprarenal glands) are situated at the top of each kidney and are composed of a central medulla and an outer cortex, each portion being derived from distinct embryonic tissues. They have a pyramidal shape and can measure 1-1.5 cm in height and 7-8 cm in length.

The adrenal cortex is divided into three zones (glomerulosa, fasciculata, and reticularis), responsible to produce three types of hormones: mineralocorticoids, glucocorticoids and gonadocorticoids. These hormones are lipophilic, and in this way act by binding to intracellular receptors.

In the glomerulosa zone, the outermost region of the adrenal cortex, mineralocorticoids are produced, with aldosterone being the hormone produced in greater amount. This hormone is primarily responsible for the reabsorption processes of sodium and excretion of potassium and hydrogen ions.

The fasciculata zone, the intermediate zone, is responsible for the secretion of glucocorticoids, cortisol being the main one. Fig. 6.4.1 shows how the process of regulating cortisol secretion occurs. Neurons in the hypothalamus release CRH upon stimulation by stress or hypoglycaemia. In the adenohypophysis, CRH binds to cells, stimulating the secretion of ACTH. In turn, ACTH will bind to the membrane receptors of cells in the adrenal cortex, thus stimulating cortisol secretion. In turn, it is cortisol that inhibits the secretion of CRH and ACTH by negative feedback.

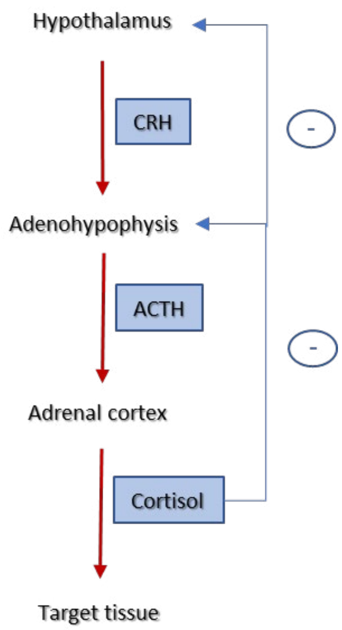

Fig. 6.4.1. Regulation of cortisol secretion.

In the innermost part, called the reticularis zone, gonadocorticoids (sex hormones) are produced, being androgens the male sex hormones, especially testosterone, and oestrogens the female sex hormones produced here.

The adrenal medulla derives from the same cells that give rise to postganglionic sympathetic neurons, and for this reason secretes neurohormones. Only two hormones are produced in the spinal cord: adrenaline (or epinephrine) (80%) and noradrenaline (or norepinephrine) (20%). Noradrenaline is a precursor of adrenaline, so the relationship between these two hormones is very important. These hormones are catecholamines and are produced in response to stimulation of sympathetic nerves, especially in stressful situations (Fig. 6.4.2).

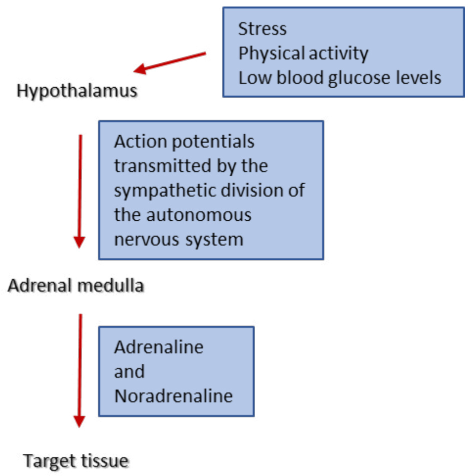

Fig. 6.4.2. Regulation of the adrenal medulla secretions.

Action on metabolism

The adrenal gland releases different hormones essential for the normal functioning of the body. These hormones control numerous vital functions such as daily metabolism (in the regulation of blood glucose), the regulation of water balance, the regulation of inflammation, the control of the response to stress situations or the control of the onset of puberty.

The mineralocorticoid aldosterone has the renin-angiotensin system as its main mechanism for stimulating its secretion: angiotensin produced by the liver is matured by the action of renin secreted by the kidneys. In the form of angiotensin II, it acts on the adrenal cortex triggering the release of aldosterone that increases tubular reabsorption of sodium by the kidneys causing an increase in natremia, and consequently an increased reabsorption of water by the kidneys. This hormone also enhances renal tubular excretion of potassium, leading to decreased levels of potassium in the extracellular fluid and increases the excretion of hydrogen ions into the urine (when present in high concentrations it can cause metabolic alkalosis).

Glucocorticoids, such as cortisol, act on numerous target tissues, thus promoting numerous metabolic responses. Cortisol increases the catabolism of lipids and proteins, decreases the uptake of glucose and amino acids by the skeletal muscle and increases gluconeogenesis, which translates into an increase in blood glucose levels. It also has anti-inflammatory effects.

The effects of adrenaline, a catecholamine, are short-lived because it is rapidly metabolized, excreted or captured by tissues. Adrenaline acts on various tissues, promoting various metabolic changes. The response to this hormone is commonly called "fight or run", as it is produced in various stressful situations. In all these situations, the body needs the rapid production of ATP for effective muscle contraction.

The action of adrenaline on the liver involves its binding to two types of receptors: the α-adrenergic and the β-adrenergic receptors. The α-adrenergic receptor activates the phospholipase C pathway responsible for the synthesis of the two secondary messengers' IP3 and DAG. IP3 will promote glycogen degradation by activating glycogen phosphorylase. At the same time, these two secondary messengers inhibit glycogen synthetase, thereby preventing glycogen synthesis. The β-adrenergic receptor acts by activating the cAMP cascade via adenyl cyclase. The increase in intracellular cAMP leads to the activation of a cascade that culminates with the phosphorylation of glycogen phosphorylase (which becomes active) and glycogen synthetase (which becomes inactive). As a result, liver glycogen will be degraded, allowing glucose to be released into the bloodstream. The existence of two types of adrenaline receptors, which work through different signalling cascades, makes the response to this hormone faster and more effective. A large amount of glucose is released into the plasma which will be used by the muscles to produce the ATP needed for muscle contraction.

In adipocytes, adrenaline stimulates the activation of the cAMP cascade via adenyl cyclase. The increase in intracellular cAMP leads to the activation of the hormone-sensitive lipase that will trigger the degradation of triacylglycerols into glycerol and free fatty acids, releasing them into the bloodstream. Released glycerol can be used in the liver for gluconeogenesis, increasing plasma glucose or for re-esterification with fatty acids. Free fatty acids are used as a substrate or energy source by most tissues, mainly musculoskeletal and liver. In the liver, fatty acids are esterified or can undergo β-oxidation and conversion to ketone bodies.

In addition to increasing the release of glucose by the liver and fatty acids by the adipose tissue, adrenaline increases heart rate, reduces blood flow in the vessels of internal organs and increases blood flow to skeletal muscles and heart, decreases visceral function, and enhances blood pressure and metabolic activity in skeletal muscles.

6.4.2. **Pathologies**

Nelson's syndrome

Nelson's syndrome is characterized by the development of an ACTH-producing pituitary tumour. This syndrome results from a severe complication of a bilateral adrenalectomy performed to treat Cushing's syndrome. The syndrome may have an early development, that is, 2 months after bilateral adrenalectomy, or very late, up to 24 years after surgery, with an average of 6 years.

After performing bilateral adrenalectomy, the following parameters should be taken into account to prevent Nelson's syndrome: perform prophylactic radiotherapy at the pituitary level; administer appropriate steroid replacement treatment; analyse the visual field every 6 months and subsequently decrease the frequency of analysis; periodically analyse plasma ACTH levels (in the first year, every 3 months and later decrease the frequency of analysis); perform a magnetic resonance imaging (MRI) every 6 months for the first year after bilateral adrenalectomy and annually thereafter; and finally, perform an ultrasound to evaluate the testicular tissue.

Elevated ACTH levels and the presence of melanoderma (increased pigmentation due to a pathological problem) are characteristic of this syndrome. Hyperpigmentation can often occur at the level of the scar left by bilateral adrenalectomy.

Congenital adrenal hyperplasia

Congenital adrenal hyperplasia is characterized by the deficiency of one of the enzymes from the metabolic pathway of adrenal hormones production. This genetic defect leads to excessive production of one or more adrenal steroids, thus resulting in an insufficiency of glucocorticoids and/or mineralocorticoids, coupled with an excess or deficiency of sex steroids. It is one of the most frequent endocrinopathies in childhood, resulting in alterations in sexual development, in a clinical picture of saline loss at the neonatal level, and manifestations of hyperandrogenism in adolescence and in oligomenorrhea and fertility disorders in adulthood.

Normal production of adrenal steroids occurs via stimulation by ACTH, which promotes the activity of the steroidogenic acute regulatory protein (StAR). In turn, StAR transports free cholesterol to the mitochondrial membrane where it is converted to pregnenolone. This is then converted into other products depending on the routes. When one of the enzymes is affected, the pathway is compromised leading to an excess or deficiency of products.

There are several types of congenital adrenal hyperplasia, encompassing a diverse group of enzymatic disorders, being the most common (responsible for about 90-95% of cases) the deficiency in 21-hydroxylase. These classic cases contribute to the appearance of prenatal virilization (25% of these cases). In the remaining 75% an aldosterone deficiency occurs, resulting in a high-risk of neonatal onset during salt-wasting crises. These crises have as main signs difficult feeding, weight loss, growth deficit, vomiting, dehydration, hypotension, hyponatraemia, hyperkalaemia and metabolic acidosis with progression to adrenal crises.

In children (< 8 years), excess of androgens can cause pubic hair, apocrine odour, acne and rapid growth. In female children, ambiguous genitalia and clitoral growth are common, and in male children, penis growth is common. In female adults, it is common to observe acne, hirsutism, male pattern baldness, amenorrhea and menstrual irregularities, infertility and deepening of the voice. In adult males it is often asymptomatic, with beard growth and subfertility as the main recorded symptoms. There are also complications such as the development of tumours in the ovaries and uterine ligaments in the case of women and testicular adrenal tumours in the case of men.

Non-classical cases are characterized by an intermediate deficiency of the enzyme, resulting in a postnatal onset with signs of hyperandrogenism, but no female virilization at birth.

In 5% of cases of congenital adrenal hyperplasia, there is a deficiency in 11-β-hydroxylase, which leads to excessive production of androgens, promoting the appearance of virilization in pre or postnatal women, and of mineralocorticoids that can result in hypertension.

Other less common and for that reason called rare types are deficiencies in 3-β-hydroxysteroid dehydrogenase and 17-α-hydroxylase, or even in the cytochrome P450 oxidoreductase causing partial deficiency in 17-α-hydroxylase and 21-hydroxylase.

In the deficiency of the cytochrome-P450 oxidoreductase, there are reports of craniosynostosis, hydrocephalus, choanal stenosis, dysplastic ears, stenotic external auditory channels and Antley-Bixter´s syndrome.

Conn's syndrome

Conn's syndrome is also called primary hyperaldosteronism. It results from an inappropriate endogenous release of a mineralocorticoid, aldosterone, by one or both adrenal glands. This syndrome may arise due to an aldosterone-producing adenoma, adrenal hyperplasia, adrenocortical cancer, or idiopathic bilateral hyperaldosteronism. This syndrome has a higher incidence in females aged between 30 and 60 years.

Recent studies show that this pathology is responsible for about 1-10% of cases of hypertension. Excess aldosterone promotes secondary hypertension syndrome and sodium conservation at the distal convoluted tubule level. Therefore, sodium conservation leads to an increase in water retention. Hypernatremia and metabolic alkalosis may also occur.

The secondary effects of hyperaldosteronism, such as progressive fibrosis at the coronary and renal arterial levels, sympathetic autonomic excitation and endothelial cell dysfunction, cause hypertension to take exacerbated proportions, increasing cardiovascular risk and consequent associated mortality. Weight gain is seen whenever hypertension persists after adrenalectomy.

There is a relationship between obesity and hyperaldosteronism. Aldosterone is also responsible for worsening glucose intolerance through its binding to the mineralocorticoid receptor.

Bartter's syndrome

Bartter's syndrome is an autosomal recessive disorder, evidenced by the inability of the loop of Henle to reabsorb filtered sodium and chloride. At the renal level, it is known that a glomerulus in its healthy state filters a volume of blood through a barrier that is semipermeable to molecules, depending on their size and load. Small molecules, such as water and electrolytes, are easily filtered and enter the renal tubules in massive amounts and must be reabsorbed to maintain homeostasis.

This syndrome is a set of similar tubulopathies, whose general characteristic is the loss of salts. There is an excessive loss of potassium, sodium and chloride in the urine, resulting in an electrolyte imbalance.

This syndrome is divided into three different types. The neonatal type results from deficiencies in the *NKCC2* (type I) and *ROMK* (type II) genes. This is one of the most serious types, manifesting *in utero*, usually in the last trimester of pregnancy, and causes polyuria, polyhydramnios (excess amniotic fluid) and premature birth. In children, polyuria can lead to dehydration, as well as thirst, vomiting, electrolyte imbalance, short stature and growth delay. The foetus may also show calcium deposits in the kidneys, nephroalkalosis and hypercalciuria.

The classic type results from a deficiency in the *CLCNKB* gene. It can be diagnosed at two years of age, but it most often appears in adolescence. Polyuria, increased fluid intake, normal or low blood pressure and increased levels of angiotensin and prostaglandin E2 are also common symptoms. If no treatment is done it can lead to kidney failure and metabolic alkalosis.

The intermediate type is the Gitelman type which is caused by mutations in the *NCCT* gene. It can appear in adolescence or adulthood. This type has the same characteristics as the classic type, the main difference being the loss of serum magnesium. It is often asymptomatic and can be discovered accidentally.

There are also reports of two more types involving mutations in the *BSD* gene. The main difference compared to the previous ones is the associated hearing difficulty, that results from the important role of chloride channels in the ear epithelium.

Addison's disease

Addison's disease may also be called primary adrenal insufficiency. The most common cause of this pathology is autoimmune adrenalitis, which consists of the destruction of the adrenal cortex that leads to a deficiency in the production of glucocorticoids, mineralocorticoids and adrenal androgens.

Other less common causes for this syndrome are infections, bleeding, metastatic cancer, drug use and adrenoleukodystrophy. It is known that in 50% of cases patients develop another autoimmune pathology.

The signs and symptoms developed by patients with this syndrome are very subtle and unspecific. The main signs and symptoms and their prevalence are anorexia (100%), weakness or fatigue (100%), hyperpigmentation (94%), gastrointestinal disorders (nausea, vomiting, abdominal pain, constipation, diarrhoea, etc.) (92%), hypotension (systolic blood pressure < 110 mmHg) (90%), salt appetite (16%), dizziness (12%), vitiligo (10-20%), and muscle and joint pain (10%). There are also reports of anaemia, morning fatigue and hypoglycaemia, sticky skin, headaches and lethargy.

Hyperpigmentation is evidenced in the palmar and plantar region, forehead and buccal mucosa. The increase in ACTH and melanocyte-stimulating hormone levels, as well as their associated peptides, occurs due to the loss of feedback from the hypothalamic-pituitary-cortisol axis, resulting in the appearance of hyperpigmentation.

An acute adrenal crisis, also called an Addisonian crisis, can often occur. This crisis usually occurs after a prolonged period of glucocorticoid and mineralocorticoid loss, when about 90% of the gland is destroyed. This episode is manifested by abdominal pain, weakness, vomiting, fever, low responsiveness, hyponatremia, hyperkalaemia, hypotension, and shock.

6.4.3. **Clinical cases**

Clinical case 6.4.1. *Disturbance of the adrenal cortex*

A 31-year-old woman was admitted to the hospital with a 2-day history of abdominal pain and vomiting. Her blood pressure was 110/65 mmHg and her pulse was regular (88 beats / min). A diagnosis of intestinal obstruction was made. The analyses were as follows:

Table 6.4.1. Results obtained in the patient blood analysis.

Parameter	Patient values	Standard values
Na^+	128 mmol/L	135-145 mmol/L
K^+	6.1 mmol/L	3.4-4.9 mmol/L
Cl^-	92 mmol/L	95-105 mmol/L
HCO_3^-	18 mmol/L	21-28 mmol/L
Urea	10.8 mmol/L	2.5-8.0 mmol/L
Creatinine	180 μmol/L	40-130 μmol/L

She was given 1.5 L of intravenous saline overnight, and the next morning the symptoms had disappeared. Plasma sodium had risen to 134 mmol/L and potassium decreased to 4.8 mmol/L. Checking her history, she had not felt well for months with weight loss and anorexia. She noticed being pigmented. A rapid Synacthen test was performed, and the plasma cortisol was below 60 mmol/L both before and after 0.25 mg Symacthen injection.

a) Suggest a diagnosis.
b) How can changes in sodium and potassium levels be explained?

Clinical case 6.4.2. *Addison's disease*

A 40-year-old man was examined due to severe skeletal muscle pain. The following biochemical results in a plasma sample were unexpected:

Table 6.4.2. Results obtained in patient blood tests.

Parameter	Patient values	Standard values
Na^+	130 mmol/L	135-145 mmol/L
K^+	6.1 mmol/L	3.4-4.9 mmol/L
Cl^-	90 mmol/L	95-105 mmol/L
HCO_3^-	17 mmol/L	21-28 mmol/L
Urea	7.6 mmol/L	2.5-8.0 mmol/L
Creatinine	150 μmol/L	40-130 μmol/L

a) Suggest a diagnosis.
b) What other tests could help in the diagnosis?

Clinical case 6.4.3. *Disturbance of the adrenal cortex*

A 31-year-old woman has had weight gain over the past three months, together with hirsutism, amenorrhea, and hypertension. The cortisol:creatinine ratio in the urine was increased and the daytime plasma cortisol rhythm was absent. Treatment with dexamethasone 0.5 mg did not suppress cortisol, and insulin-induced hypoglycaemia did not lead to increased cortisol. What investigations should be done?

Clinical case 6.4.4. *Hyperaldosteronism*

Hyperkalaemia (high blood K^+) can cause death by producing fibrillation of the heart (a condition that causes the heartbeat to stop). Nevertheless, a healthy person can ingest any potassium-rich bananas they want. Explain how the adrenal cortex makes this possible.

6.5. **Pancreas**

6.5.1. **Short review**

Structure, secretion and regulation

The pancreas is a complex organ formed by an exocrine part and an endocrine part. It consists of a head, located in the curvature of the duodenum, a body and a tail that extends to the spleen. It is an elongated structure, measuring approximately 15 cm in length and weighing about 85 to 100 g.

The exocrine part of the pancreas is made up of acini that produce digestive enzymes. The acini clusters form lobes that are separated by thin septa. The lobes are connected by small intercalated and intralobular canals that form the interlobular canals. The latter bind to the pancreatic duct which, in turn, binds to the common bile duct in the hepato-pancreatic ampulla. These channels are lined by simple cylindrical epithelium and the epithelial cells of the acini are pyramidal. At the junction of the pancreatic ampulla with the pancreatic duct there is a smooth muscle sphincter.

Pancreatic juice is produced in the pancreas and, through the pancreatic channels, is released into the small intestine. This juice is made up of two components, one aqueous and the other enzymatic. The aqueous portion is produced by the cylindrical epithelial cells and consists of sodium and potassium ions in concentrations identical to that of the extracellular fluid, associated with bicarbonate ions responsible for neutralizing the chyme (acid) coming from the stomach. This neutralization will activate the pancreatic enzymes. Bicarbonate ions are secreted by the channel epithelium and water passes by passive diffusion, to make the pancreatic juice isotonic.

Enzymes produced by pancreatic acinus cells allow proper digestion of lipids, proteins and carbohydrates. The main pancreatic proteolytic enzymes are trypsin, chymotrypsin and carboxypeptidase. These enzymes are released in their inactive zymogen form, i.e., trypsinogen, chymotrypsinogen and procarboxypeptidase, and their activation occurs by removing certain peptides from precursor proteins. If these enzymes were produced in their active form, destruction of the tissues that produce them would take place. Pancreatic juice also contains pancreatic amylase, which allows the continued digestion of polysaccharides, previously initiated in the mouth. Moreover, this juice also has pancreatic lipases that will digest lipids, releasing free fatty acids, glycerol and cholesterol.

The regulation of exocrine secretion from the pancreas is done by hormonal and nervous mechanisms. When the chyme reaches the duodenum, it stimulates the release of secretin that will activate the production of the aqueous component of pancreatic juice.

On the other hand, the endocrine portion of the pancreas consists of the islets of Langerhans (pancreatic islets) formed by α, β, δ cells and PP cells and is responsible for the synthesis of hormones. Different cell types secrete different hormones (Table 6.5.1).

The α cells, which make up 20% of the islet of Langerhans, secrete a small polypeptide hormone, glucagon. The β cells primarily produce insulin, but they also secrete C-peptide and amylin. These cells constitute about 60-70% of the pancreatic islet. In smaller amounts in the pancreatic islets there are δ cells (about 5-15%) responsible for the production of somatostatin. There is still a very small percentage (2%) of cells responsible for the secretion of pancreatic polypeptide, the PP cells.

For an adequate paracrine regulation of hormone release, the different cells are grouped in a particular way, that is, the β cells are in the centre of the islets of Langerhans, and the α, δ and PP cells are in the periphery.

Table 6.5.1. Constitution of the Islets of Langerhans.

Cell type	Proportion of cells in the Islet	Produced peptides
A	25%	Glucagon (GLP-1, GLP-2)
β	60-70%	Insulin, C-peptide, Amylin
δ	5-15%	Somatostatin
PP	2%	Pancreatic polypeptide

Blood reaches the pancreas via the splenic artery and the superior and inferior pancreaticoduodenal arteries. The high vascularization allows a rapid passage of hormones into the blood stream according to the body's need, through the vagal nerve, terminal parasympathetic nerves and sympathetic nerves. The direction of blood in the islets is from the centre to the periphery, which means that α and δ cells are more exposed to high concentrations of hormones produced by β cells, such as insulin. Thus, there is inhibition of glucagon secretion due to the high concentration of insulin. After their secretion, pancreatic hormones travel to the liver, which is the main organ of metabolism, where they undergo a first-pass effect and are subsequently released into the bloodstream.

Insulin is the main pancreatic hormone and consists of two polypeptide chains linked by a bisulphite bridge: A chain with 21 amino acids and B chain having 30 amino acids. It was the first protein to be fully sequenced and chemically synthesized. This hormone is synthesized as pre-proinsulin. The pre signal sequence directs the protein to the RER where it will suffer maturation. The pro sequence is essential for hormone exocytosis. Both signal sequences are proteolytically cleaved by proteases in serine residues, giving rise to the active hormone. Proinsulin consists of a terminal β-amino chain and an α-carboxy terminal chain linked by a peptide, the C-peptide. The C-peptide is essential in the synthesis of insulin as it allows the formation of bisulphite bonds. In the Golgi complex there is a new cleavage where insulin and C-peptide are formed, being stored in granules in the cytoplasm of β cells.

Glucose is the primary stimulator of insulin secretion. When the plasma glucose concentration is greater than 5 mmol/L, leads to β-cell degranulation, and consequent insulin release. Glucose enters β cells via a transporter called GLUT2 and is phosphorylated by the enzyme glucokinase. This enzyme detects the entry of glucose, causing insulin to be produced, and consequently leading to an increase in intracellular concentrations of ATP, resulting from its production by glycolysis and the Krebs cycle. As the intracellular ATP concentration is high, ATP-sensitive potassium channels (K_{ATP}) will be blocked, promoting membrane depolarization and the consequent opening of Ca^{2+} channels, leading to an influx of Ca^{2+}. The increase in plasma Ca^{2+} concentration induces insulin secretion only in the presence of amplifying messengers such as DAG and unesterified arachidonic acid. Free arachidonic acid is only released via an ATP-sensitive and calcium-insensitive phospholipase A.

Glucagon is a 29-amino acid polypeptide with a short half-life (5-10 min) and is degraded in the liver. The pro-glucagon which is synthesized in the intestine, is processed differently to produce glucagon-like peptide-1 (GLP-1) in the intestinal cells and glucagon in the α-cells of the pancreas. GLP-1 is produced in the intestine in response to the high concentration of glucose present in the intestinal lumen. This high glucose concentration will stimulate the release of insulin.

The target tissue of glucagon is the liver, acting on membrane receptors. Glucagon receptors are mostly expressed in the liver and kidneys, with smaller amounts found in the β cells of the pancreas, adipose tissue, heart, brain, stomach and adrenal gland. Glucagon binding to the corresponding receptor activates the adenyl cyclase pathway, with formation of the cAMP secondary messenger. This secondary messenger will now trigger the cellular response.

Somatostatin is synthesized in δ cells of the pancreas from its 92-amino acid precursor pro-somatostatin. This precursor is processed by proteolytic cleavage giving rise to a peptide of 14 amino acids (SS14) and another of 28 amino acids (SS28). This hormone has an inhibitory effect on almost all gastrointestinal and pancreatic exocrine and endocrine functions. It is difficult to analyse the regulation of the secretion of this hormone as it is found in smaller numbers in the islets of Langerhans.

The pancreatic polypeptide is made up of 36 amino acids, produced in PP cells and secreted into the bloodstream after food intake, exercise, or vagal stimulation.

Amylin or islet amyloid polypeptide is a peptide with 37 amino acids that belongs to the calcitonin family that includes calcitonin and adrenomedullin. Amylin is synthesized into a precursor form undergoing post-translational modification (amidation). It is stored in β granules and excreted together with insulin and C-peptide.

Action on metabolism

The target tissues of insulin are the liver, muscle and adipose tissue. This hormone acts on a receptor located on the cell membrane, with tyrosine kinase activity which, upon activation, through enzymatic phosphorylation, mediates intracellular signal transduction.

Insulin release occurs in two phases: the initial phase in which there is a rapid release of insulin stored in β cells; the later phase with slower and more continuous release of insulin accompanied by its synthesis. In the liver, insulin stimulates the expression of genes encoding enzymes involved in glucose utilization such as glucokinase, pyruvate kinase, and lipogenic enzymes. At the same time, insulin inhibits the expression of genes encoding enzymes involved in glucose production, such as phosphoenolpyruvate carboxykinase and glucose-6-phosphatase. In liver, insulin stimulates glycogen synthesis, that is, glucose storage, by increasing the activity of phosphoprotein phosphatase which, in turn, leads to the dephosphorylation of glycogen phosphorylase (which becomes inactive) and glycogen synthase (which is converted into active form). In muscles, insulin promotes protein synthesis. Table 6.5.2 presents the effects of this hormone on carbohydrate, lipid and protein metabolism.

Table 6.5.2. Effects of insulin on carbohydrate, lipid and protein metabolism.

Metabolic effects	Insulin stimulates	Insulin inhibits
Carbohydrate metabolism	Glucose transport across the cell membrane in adipose tissue and muscle; Glycolysis in adipose tissue and muscle; Glycogen synthesis in adipose tissue, muscle and liver.	The breakdown of glycogen in muscle and liver; Gluconeogenesis in the liver.
Lipid metabolism	Synthesis of fatty acids and triacylglycerols; Absorption of triacylglycerols from the blood into adipose tissue and muscle; Cholesterol synthesis in the liver.	Lipolysis in adipose tissue, decreasing plasma fatty acid levels; Oxidation of fatty acids in muscle and liver; Ketogenesis.
Protein metabolism	Transport of amino acids in tissues; Protein synthesis in muscle, adipose tissue, liver and other tissues.	Protein breakdown in muscle; Urea formation.

Insulin secretion by the pancreas is stimulated by high plasma glucose levels, among other factors. The effects of insulin are opposite to those of other hormones such as glucagon, adrenaline, glucocorticoids and GH. Blood glucose concentration is the result of a balance between the action of these different endocrine forces.

Insulin secretion is inhibited by the sympathetic nervous system. Adrenaline raises blood glucose via α-adrenergic receptors, present in muscles and liver cells, by inhibiting insulin release and promoting glycogenolysis. Amino acids, mainly arginine and leucine, and fatty acids stimulate insulin secretion. The parasympathetic nervous system and the incretins GLP-1 and gastric inhibitory peptide (GIP) also stimulate insulin secretion.

The main target tissue for glucagon is the liver. The action of glucagon counteracts the action of insulin. To exert its action, glucagon binds to its membrane receptor on hepatocytes. This binding leads to the activation of adenyl cyclase which is responsible for the synthesis of cAMP secondary messenger. This messenger will now promote a cascade of activations that culminate in the final effect of this hormone: stimulating glycogen degradation and gluconeogenesis and inhibiting glycolysis, which leads to an increase in plasma glucose concentration. The activation cascade involves a series of phosphorylations that activate/inhibit enzymatic activities: cAMP activates the protein kinase, this phosphorylates the kinase phosphorylase which is converted into its active form and then the latter will phosphorylate glycogen phosphorylase which, by becoming active, will promote the breakdown of stored glycogen. At the same time, it phosphorylates glycogen synthase, that becomes inactive, and for this reason glycogen synthesis is prevented. In addition to this signalling cascade, glucagon also affects the activity of other enzymes, such as glucose-6-phosphatase, mediating the stimulation of hepatic glucose production and its release into the bloodstream (Table 6.5.3).

Table 6.5.3. Effects of glucagon on glucose metabolism.

Effect on target enzyme	Metabolic response
Increased expression of glucose-6-phosphatase	Releases glucose that will go into the bloodstream
Glucokinase suppression	Decreases the entry of glucose into the glycolytic cascade
Phosphorylation (activation) of glycogen phosphorylase	Stimulates glycogenolysis
Inhibition of glycogen synthase	Inhibits glycogen synthesis
Stimulation of phosphoenolpyruvate carboxykinase expression	Stimulates gluconeogenesis
Suppression of pyruvate kinase activity	Decreases glycolysis

Glucagon secretion by the pancreas is inhibited by high blood glucose levels (hyperglycaemia) and stimulated by low blood glucose levels (hypoglycaemia), among other factors. A carbohydrate-rich meal inhibits glucagon secretion and stimulates the release of insulin from β-cells, through the intestinal release of GLP-1. Somatostatin also inhibits glucagon release. Glucagon promotes glucose mobilization. Another of the effects of glucagon is to shift unesterified fatty acids from triacylglycerol synthesis to β-oxidation, leading to ketogenesis.

Somatostatin is a potent inhibitor of insulin, glucagon and pancreatic polypeptide secretion, but also of GH, TSH and prolactin. Somatostatin also inhibits the release of gastrointestinal hormones such as gastrin, cholecystokinin (CCK), secretin, motilin, vasoactive intestinal peptide (VIP) and GIP. Somatostatin decreases gastric, duodenal and gallbladder motility.

The effects of pancreatic polypeptide are inhibition of pancreatic exocrine secretion, gallbladder contraction, stimulation of glucocorticoid secretion, modulation of gastric and gastrointestinal acid secretion. The polypeptide can cross the blood-brain barrier and reach the CNS.

Amylin binds to a variant of the calcitonin receptor. The modified calcitonin receptor has a higher affinity for amylin. This binding occurs due to transmembrane proteins called receptor activity modifying proteins (RAMPs). Plasma amylin concentrations increase after a meal or glucose infusion. This hormone, together with insulin, regulates plasma concentrations of glucose in the blood, inhibiting postprandial glucagon secretion and decreasing gastric emptying. Amylin inhibits glycogen synthesis and activates glycogenolysis. In individuals with obesity, arterial hypertension and gestational diabetes, the concentration of amylin is increased. In individuals with type 1 diabetes, amylin is low or almost absent.

6.5.2. **Pathologies**

Diabetes mellitus

The most common disease associated with changes in pancreatic hormone secretion is diabetes mellitus. It is a non-hereditary chronic disease that affects both sexes at any age. It is characterized by high blood glucose levels that result from the lack of production and/or inability of insulin to exert its effects. The most common types of diabetes mellitus are type 1, type 2 and gestational diabetes.

Type 1 diabetes, also called insulin-dependent, is characterized by an autoimmune destruction of the β cells of the pancreas, which leads to a complete deficiency in the production of insulin. This condition can result from environmental factors, viral infection, or toxins. Only 2-5% of diabetes cases are type 1, which has a rapid onset and usually appears in young people under 30 and children.

Type 2 diabetes, also called non-insulin-dependent, is characterized by insulin resistance and/or insufficient insulin secretion by the β cells of the pancreas. This type of diabetes is the most common, accounting for about 90% of diabetes cases. It usually appears in obese adults over 40 years of age and is of slow onset, being characterized by mild hyperglycaemia. This type of diabetes is often associated with "insulin resistance syndrome", which is a metabolic syndrome that is manifested by high blood pressure, atherosclerosis and obesity.

Gestational diabetes is manifested by impaired glucose tolerance. It is diagnosed during pregnancy and can continue after delivery or even be transmitted to the foetus.

The pathophysiology of the disease involves an increase in plasma osmolarity, and urinary loss of glucose accompanied by high loss of water and sodium (polyuria). Polydipsia also occurs, which consists of dehydration through compensatory mechanisms such as thirst. Patients may also show polyphagia, which is the inability to use glucose by cells, stimulating hunger, which triggers compensatory responses by activating lipolysis and proteolysis. These compensatory responses will lead to an increase in free fatty acids and glucogenic amino acids in the liver, which will lead to the accumulation of ketone bodies in the blood (diabetic ketoacidosis) and their urinary excretion.

Diabetic ketoacidosis is characterized by high levels of glucose and ketone bodies in the blood resulting from decreased availability of insulin and stimulation of hormones such as glucagon, cortisol and GH. This condition occurs mainly in individuals with type 1 diabetes. Ketonemia results in acidaemia due to the acidic nature of ketone bodies, with a drop in blood pH (metabolic acidosis). Excretion of glucose and ketone bodies leads to dehydration, which will worsen acidaemia. This ketoacidosis can occur due to infection, interruption or inappropriate use of insulin, or surgery. Regular medical monitoring helps in preventing diabetic ketoacidosis. Treatment strategy involves the supply of insulin, hydration and ingestion of potassium, to maintain the ionic balance.

The diagnosis of diabetes is made based on the following parameters and values for venous plasma in the general population:

a. Fasting blood glucose ≥ 126 mg/dL (or ≥ 7.0 mmol/L); or

b. Classic symptoms + occasional blood glucose ≥ 200 mg/dL (or ≥ 11.1 mmol/L); or

c. Blood glucose ≥ 200 mg/dL (or ≥ 11.1 mmol/L) at 2 h, in the oral glucose tolerance test (OGTT) with 75 g of glucose; or

d. Glycosylated haemoglobin (HbA1c) ≥ 6.5%.

In an asymptomatic person, this diagnosis should not be made based on a single abnormal fasting glucose or HbA1c value but should be confirmed in a second analysis after one to two weeks. It is advisable to use only one parameter for diagnosing diabetes. However, if there is simultaneous assessment of fasting glucose and HbA1c, if both are diagnostic values, this is confirmed, but if one is discordant, the abnormal parameter should be repeated in a second analysis.

The diagnostic criteria for diabetic ketoacidosis are capillary blood glucose greater than 200 mg/dL, ketonuria present and pH below 7.3. The main symptoms are thirst, frequent urination, constant tiredness and difficulty breathing.

There is still no cure for diabetes, but it is possible to control it and prevent further damage. A late diagnosis or poor control of diabetes can increase the risk of developing other diseases such as cardiovascular disease, retinopathy, nephropathy and diabetic foot.

Since insulin isolation in 1921 by Frederick Benting and Charles Best, diabetes can be controlled. However, patients' life expectancy is reduced to about 1/3 due to complications. Insulin is the main treatment for type 1 diabetics. In case of diabetic ketoacidosis, oral or intravenous fluid replacement is the first action to implement. Fluid replacement is important to dilute the amount of sugar present in blood.

Insulinoma

Insulinoma involves the presence of a tumour in the β cells of the pancreas. It is characterized by the appearance of signs and symptoms resulting from hypoglycaemia secondary to an uncontrolled hypersecretion of insulin. When there is an insulinoma, glycaemic regulation is altered. The tumour stimulates insulin production even when the glycaemic index is low, which leads to hypoglycaemia.

The main symptoms are confusion, blurred vision, forgetfulness, faint feeling and unconsciousness, hunger, weakness and sweating.

The combination of low blood glucose and high insulin levels is a sign of the presence of insulinoma. For a correct diagnosis, the study of proteins that block the production of insulin, measurements that cause greater release of insulin by the pancreas and other hormones that affect the production of insulin must be carried out. If these tests are positive, a 72-hour fasting test should be performed to monitor the glycaemic index. A computed tomography or magnetic resonance imaging may also be used.

In patients with insulinoma, prolonged periods without glucose intake should be avoided, using carbohydrate-rich meals and/or continuous glucose infusion.

Glucagonoma

Glucagonoma involves the presence of a secretory tumour in the α-cells of the pancreas, which leads to increased levels of glucagon. This disorder is rare, affecting 1 in 20 million individuals. This type of tumour is found in the tail or body of the pancreas, appears sporadically and affects men and women equally.

The main characteristic symptom of this disorder is a rash called necrolytic migratory erythema. Other symptoms are painful glossitis (inflammation of the tongue), cheilitis (inflammation of the lips), stomatitis (inflammation near the corners of the lips), normocytic anaemia, weight loss, mild diabetes mellitus, hypoaminoacidemia, low plasma zinc concentrations, deep vein thrombosis and depression.

Necrolytic migratory erythema is one of the diagnostic factors of this disorder. Typically, this erythema affects the perioral and perigenital region, fingers and lower limbs. This rash starts as a small erythematous lesion and then progresses to form a blister that ulcerates. Thereafter, crushing and consequent scarring with hyperpigmentation occurs. To confirm the diagnosis, skin biopsies are performed. Through radioimmunoassay it is possible to diagnose this disorder, by measuring the fasting glucagon concentration. If its value is greater than 2,000 pg/mL, the disorder is present (normal value is between 120-150 pg/mL).

Pancreatic adenocarcinoma

Pancreatic adenocarcinoma is a very rare tumour, with about 85% of cases arising from the ductal epithelium. The remaining cases result from disorders in the cells of the islets of Langerhans. Tumours resulting from changes in hormone secretion can be functional or non-functional in origin. Functional adenocarcinomas secrete high amounts of insulin or glucagon. Non-functional tumours do not produce enough hormones to keep blood sugar under control. This type of tumour is one of the most aggressive tumours of the gastrointestinal tract, since its diagnosis is usually late, which makes treatment difficult.

The main risk factors for the development of this carcinoma are tobacco, overweight, sedentary habits, chronic inflammation of the pancreas, excessive consumption of fat, and exposure to chemical compounds such as solvents and petroleum derivatives.

Symptoms associated with this disorder are common to many disorders. Among them are loss of appetite, weight loss, abdominal pain, diarrhoea and jaundice. To diagnose this pathology, exams such as resonances, abdominal ultrasounds and biopsy should be performed. This tumour does not cause pain, that is, when it is diagnosed, it is already at a very late stage.

Pancreatitis

Pancreatitis is a gastrointestinal disorder resulting from inflammatory processes. It is characterized by inflammation of the pancreas resulting from the release of pancreatic enzymes within the pancreas itself, initiating the digestion of the organ. It can also arise from changes in insulin and glucagon secretion. If there is excessive release of pancreatic hormones, these will change the pancreas, causing inflammation.

There are two types of pancreatitis: acute and chronic. Acute pancreatitis is a short-lived inflammation. On the contrary, chronic pancreatitis is an inflammation that persists for many years. The main causes are alcohol consumption and gallstones. Other less frequent causes are metabolic disorders and hereditary diseases. Pancreatitis can cause weight loss due to food malabsorption, as there is a decrease in the production of digestive enzymes and destruction of β cells.

This pathology is characterized by intense pain in the upper abdomen that radiates to the back. Usually, the pain starts suddenly and reaches its maximum intensity in a few minutes, remaining constant and intense. Other characteristic symptoms of this disease are vomiting, low blood pressure and jaundice. The diagnosis of pancreatitis is established in patients who present these symptoms, who have gallstones or who have an exaggerated alcohol intake. Another diagnostic method is the detection of high concentrations of pancreatic enzymes such as amylase and lipase. Moreover, abdominal ultrasound, computed tomography and endoscopic study of the bile and pancreatic ducts can also be used.

6.5.3. **Clinical Cases**

Clinical case 6.5.1. *Insulin dependent diabetes mellitus (type 1)*

A 17-year-old diabetic boy did not administer insulin because he felt sick, unable to eat food. However, he developed ketoacidosis and mental confusion. Routine laboratory tests showed what is shown in the table.

Table 6.5.4. Results obtained in patient blood tests.

Parameter	Patient values	Standard values
Glucose	450 mg/dL	70-105 mg/dL
Ketone bodies	20 mmol/L	Not detectable
HCO_3^-	5 mmol/L	18-23 mmol/L
Anionic hiatus	32 mEq/L	7-14 mEq/L
pH	7.0	7.35-7.45

a) Why did severe ketoacidosis arise?
b) The patient has moderate hyperglycaemia. Two strategies may be proposed as adjuvants to insulin therapy to lower blood glucose: inhibition of gluconeogenesis and inhibition of fatty acid oxidation. What are the dangers of this therapeutic action?

Clinical case 6.5.2. *Insulin dependent diabetes mellitus (type 1)*

A 21-year-old girl who had type 1 diabetes 5 years ago, was taken to the hospital in coma. She had not taken insulin for 48 hours, and the smell of acetone could be detected in her breath. She had physical signs of dehydration that could be considered moderate to severe. A blood sample was taken to determine the laboratory parameters shown in the table below.

Table 6.5.5. Results obtained in patient blood tests.

Parameter	Patient values	Standard values
Glucose	990 mg/dL	70-110 mg/dL
Haematocrit	49%	37-47%
Na^+	134 mmol/L	136-145 mmol/L
K^+	5.9 mmol/L	3.5-5.0 mmol/L
Cl^-	94 mmol/L	100-106 mmol/L
Total CO_2	3 mmol/L	18-23 mmol/L
Fatty acids	1200 μmol/L	200-800 μmol/L
Triacylglycerols	450 mg/dL	35-160 mg/dL
Urea	85 mg/dL	15-38.5 mg/dL
Plasma pH	7.05	7.35-7.45

The urine sample was (++++) for glucose and strongly positive for ketone bodies. The patient was rapidly treated with insulin and rehydrated.

a) Explain the causes of high hyperglycaemia present in this patient.
b) How did ketosis develop having a high level of plasma glucose?
c) Explain the causes of dehydration of this patient.
d) Explain the causes of high levels of triacylglycerols, fatty acids and urea.
e) The patient, if not treated, will lose weight. Explain.
f) At admission in the hospital, what would be the expected glycogen levels stored in liver in this patient?
g) Insulin can be considered the strongest known anabolizing agent. Make a scheme showing the signalling transduction pathway for insulin after its attachment to the receptor in the plasma membrane.

Clinical case 6.5.3. *Non-insulin dependent diabetes mellitus (type 2)*

A 62-year-old patient was seen at a diabetic clinic and his main complaints were tiredness, poor eyesight, tingling in his legs, kidney problems, difficulty in healing, increased thirst and urinary frequency. Laboratory tests showed urine glucose and only traces of ketone bodies. Blood tests revealed: 14% glycosylated haemoglobin (reference value: 6%) and triacylglycerol concentration of 450 mg/dL (reference value: 35-160 mg/dL). A glucose tolerance test was performed, and the result was as shown below.

Table 6.5.6. Results obtained in the glucose tolerance test performed to the patient.

Time (minutes)	Patient values (mmol/L)	Standard values (mmol/L)
0 (fasting)	8.9	3.3-5.6
30	13.3	< 10
60	18.1	< 10
90	16.9	< 7.8
120	15.8	< 6.7

a) Explain the biochemical basis responsible for the onset of symptoms and the elevated levels of glucose and triglycerides in the patient's bloodstream.
b) Explain what glycosylated haemoglobin is and the meaning of the high value.
c) Explain the glucose tolerance test.
d) What is the most appropriate treatment for type 2 diabetic individuals?
e) Type 2 diabetic patients may sometimes develop hyperglycaemic, hyperosmolar coma. Explain why?
f) What is considered "well controlled" diabetes? What parameters are currently considered ideal for good diabetes control?
g) What are the consequences of poor diabetes management in the medium and long term?
h) Why is fasting glucose alone not a good indicator of good diabetes control?
i) Why is it important to control postprandial blood glucose?

Clinical case 6.5.4. *Non-insulin dependent diabetes mellitus (type 2)*

A 70-year-old diabetic woman with non-insulin-dependent diabetes (type 2) controlled her diabetes through diet for 20 years. Last week, her family's doctor noticed that she had hypertension and prescribed a thiazide diuretic to lower her blood pressure. After taking the diuretic, she became very thirsty and drank a large amount of apple juice. However, she lost weight and began to complain of dizziness. There was no ketone breath. Routine laboratory tests showed the results below.

Table 6.5.7. Results obtained from the patient's blood tests.

Parameter	Patient values	Standard values
Glucose	900 mg/dL	70-105 mg/dL
Ketone bodies	3 mmol/L	Not detectable
HCO_3^-	25 mmol/L	18-23 mmol/L
Anionic hiatus	14 mEq/L	7-14 mEq/L
pH	7.4	7.35-7.45

a) How do you explain the change in the patient's clinical condition?
b) What is the main goal of the therapy for this patient?
c) This patient does not develop ketoacidosis. Explain why.
d) Why is this patient with such high hyperglycaemia?

Clinical case 6.5.5. *Non-insulin dependent diabetes mellitus (type 2) and deficiency in insulin receptors*

Insulin promotes glucose entry into cells. Many diabetics do not respond to insulin due to a deficiency in their cell receptors.
a) How does this affect circulating glucose levels and the rate of muscle glycogen synthesis after a meal?
b) Describe what happens to insulin and glucagon secretion after a meal, and how these hormones influence metabolism under that condition.
c) Some people may have elevated plasma insulin and glucose levels at the same time. Explain why.

Clinical case 6.5.6. *Hyperinsulinism*

Some malignant pancreatic tumours cause excessive insulin production by β cells. Affected individuals exhibit tremors, weakness, fatigue, and hunger. If this situation continues, brain damage occurs.
a) What is the effect of hyperinsulinism on the metabolism of carbohydrates, amino acids and lipids in the liver?
b) What are the causes of the observed symptoms? Suggest why, if this condition continues, brain damage may occur.

Clinical case 6.5.7. *Obesity*

A 41-year-old man went to check up. No complaints except weight gain in the last 5 years (8 Kg). He had a sedentary lifestyle and drank about 10 shots of whiskey a week. Blood tests showed the results presented in the table.

Table 6.5.8. Results obtained in patient blood tests.

Parameter	Patient values (mg/dL)	Standard values (mg/dL)
Glucose	125	70-110
Total cholesterol	330	< 200
Triacylglycerols	600	< 150
HDL	35	> 45
LDL	185	< 130
Glucose 2 hours after 75g of Dextrosol V.O.	180	< 120

a) How would you rate the patient from the point of view of carbohydrate metabolism?
b) What would your recommendations be to the patient for dextrosol glucose dosing to be considered standardized?
c) Based on your answer to (a) what would be the risks for this patient.
d) What is the most likely diagnosis of this patient?

Clinical case 6.5.8. *Obesity*

A 20-year-old woman sought medical attention for obesity. Most of her excess weight was in the form of triglycerides in adipose tissue. Its activity can be described as sedentary to moderate. Much of her caloric intake consisted of pasta, cookies, cakes, ice cream, soda and beer.
a) How is obesity defined?
b) Does the patient's history provide some clues as causes of her obesity?
c) What mechanisms can cause obesity?
d) What is the enzyme regulating the rate of *de novo* biosynthesis of fatty acids?
e) How does acetyl-CoA produced in mitochondria reach the cytoplasm for use in the fatty acid biosynthetic pathway?
f) How could the carbohydrates ingested by this patient provide the reducing equivalents needed for fatty acid biosynthesis?
g) How is it possible to form excessive amounts of triglycerides in the body if the diet contains predominantly carbohydrates?
h) What is leptin? What is its mechanism of action?
i) What other factors may accompany obesity and increase the risk of mortality?
j) Name the main behaviours involved in treating obesity.

6.6. **Further questions**

Question 6.6.1.
What class of hormones are transported free in the blood? And those bound to plasma proteins? Justify.

Question 6.6.2.
Is thyroxine transported in the blood bound to TBG, bound to thyroglobulin, bound to colloid or dissolved in blood plasma? Justify.

Question 6.6.3.
Contrast the location of receptors for the various classes of hormones.

Question 6.6.4.
Are steroid hormone receptors found on cell membranes, cytosol, ribosomes, mitochondria, or the Golgi apparatus?

Question 6.6.5.
Which of the following organs secrete hormones: kidneys, pancreas and/or brain?

Question 6.6.6.
Which hormones are taken in pills, and which need to be injected? Explain.

Question 6.6.7.
Briefly describe the three different types of hormone action mechanisms.

Question 6.6.8.
Describe the concept of feedback-regulation in hormonal control.

Question 6.6.9.
Considering a particular hormone, state whether the following statements are true or false, justifying:
a) The hormone binds to cell membrane receptors on all cell types.
b) If it is lipid soluble, has an intracellular receptor.
c) It circulates bound to a protein, which shortens its half-life.
d) If it is a small peptide, its receptor is in the nucleus.
e) Steroid hormones are non-polar.
f) Polypeptide hormones are non-polar.
g) Non-polar hormones cannot be taken orally and must be injected.
h) Non-polar hormones cannot enter their target cells.

Question 6.6.10.
Summarize the effects of glucagon, adrenaline and insulin on carbohydrate metabolism in liver, muscle and adipose tissue.

Question 6.6.11.
Describe what happens to insulin and glucagon secretion after a meal, and how these hormones influence metabolism during that period.

Question 6.6.12.
Describe what happens to insulin and glucagon secretion during fasting, and the metabolic changes that occur during that period.

Question 6.6.13.
Explain how a person with diabetes can develop hyperglycaemia while fasting.

Question 6.6.14.
How does adrenaline regulate glycogen metabolism? Provide the main steps. Explain. Compare the end product in liver and muscle.

Question 6.6.15.
Distinguish between insulin-dependent and non-insulin-dependent diabetes.

Question 6.6.16.
How is obesity related to non-insulin dependent diabetes mellitus?

Question 6.6.17.
Explain the differences regarding hypertriglyceridemia and ketoacidosis between a person with insulin-dependent diabetes and a person with non-insulin-dependent diabetes. Justify conveniently.

Question 6.6.18.
In a well-nourished state, what hormone(s) does adipose tissue respond to and what effect does it have?

Question 6.6.19.
One subject was injected with hormone A at time 0 and hormone B after 120 minutes. Table 6.6.1 depicts the change in plasma glucose concentration overtime in this subject's plasma.

Table 6.6.19. Variation of glucose concentration over time in the plasma of an individual injected with hormone A at time 0 and hormone B at 120 minutes.

Time (minutes)	[Glucose] (mg/dL)
0	120
15	100
30	90
45	75
90	125
120	125
145	160
150	140
160	120

a) Identify hormone A and its mode of action.
b) State the reason for the increase in glucose concentration from 45 to 90 minutes.
c) Identify hormone B.

Question 6.6.20.
Starting with the hypothalamus, and diagramming all the hormones involved, explain how hypoglycaemia can affect GH production.

Question 6.6.21.
A healthy individual is said to have normal thyroid function when the rate at which thyroid hormones are secreted remains within normal concentration limits. However, in some individuals, the immune system starts producing an abnormal substance that functions like TSH. Describe the effect this substance will have on the secretion rates of thyrotropin and thyroid hormones.

Question 6.6.22.
Classically, anterior pituitary hormones are divided into three groups according to their chemical structure. Classify these groups, indicating the respective hormones.

Question 6.6.23.
If there is a total absence of neuronal communication between the hypothalamus and the pituitary gland, which hormones will this gland have whose secretion will be altered?

Question 6.6.24.
Consider the following factors and identify which ones stimulate and which inhibit growth hormone secretion: GHRH; SS; Fasting; Hyperglycaemia; Chronic stress.

Question 6.6.25.
In a laboratory animal, sympathetic preganglionic fibbers innervating the adrenal medulla were cut. What happens to plasma epinephrine concentration during rest and stress?

Question 6.6.26.
Construct the diagram for the following hormonal sequence: CRH-ACTH-cortisol, considering stress as the main stimulus.

Question 6.6.27.
Name four physiological functions of calcium.

Question 6.6.28.
Taking the increase in osmolality as a stimulus, refer to the physiological consequences on the renin-angiotensin-aldosterone system.

Question 6.6.29.
In a situation of hypocalcaemia, what happens to the rate of secretion of hormones that regulate plasma calcium concentration?

Question 6.6.30.
Complete the following table:

Table 6.6.2. Function and place of hormonal production.

Function	Hormone	Produced by...
Decreases glycemia		
Increases glycemia		
Decreases plasma Ca^{2+} concentration		
Increases plasma Ca^{2+} concentration		
Stimulates thyroid		
Decreases water excretion		
Causes uterine contractions during childbirth		
	Oxytocin	
Stimulates milk production		
Increases basal metabolic rate		
Stimulates body growth		
Decreases sodium excretion		
Causes epiphyses to close		
Stimulates the adrenal cortex		

Question 6.6.31.
Using the following terms – anabolism, catabolism, liver, muscle, adipose tissue, plasma, glycogen, glucose, proteins, amino acids, lipids, fatty acids, gluconeogenesis and glycolysis – distinguish the period of absorption and post-absorption in terms of hormonal regulation of nutrients and stimulation of insulin secretion.

Question 6.6.32.
Indicate whether the following statements are true or false, justifying your answer.
a) Sleep is important for the physical growth of children.
b) Sex hormones have contradictory effects on growth.
c) The occurrence of a goitre indicates the lack of iodine in the diet.
d) Lack of T_3 increases sensitivity to cold.
e) Cortisol administration causes an increase in bone mass.
f) Sex hormones bind to membrane receptors and cause an increase in the intracellular concentration of the secondary messengers cAMP, cGMP and Ca^{2+}.
g) Cortisol is administered to patients undergoing transplants.
h) Maintenance of the corpus luteum requires the existence of adequate levels of LH in the bloodstream.

Question 6.6.33.
Why, when we have a cold, food doesn't taste good to us?

Question 6.6.34.
What are the hormones produced by the adrenal cortex and their effects?

Question 6.6.35.
What are the hormones produced by the adrenal medulla and their effects?

Question 6.6.36.
What are the hormones produced by the thyroid and their effects?

Question 6.6.37.
What are the hormones produced by the parathyroid glands and their effects?

Question 6.6.38.
What are the hormones produced by the endocrine pancreas and their effects?

Question 6.6.39.
What are the hormones produced by the ovaries and their effects?

Question 6.6.40.
What are the hormones produced by the testes and their effects?

Question 6.6.41.
What are the hormones produced by the placenta and their effects?

Question 6.6.42.
What are the hormones produced by the anterior pituitary lobe and their effects?

Question 6.6.43.
What are the hormones produced by the posterior lobe of the pituitary and their effects?

Question 6.6.44.
Name the hypophysiotropic hormones and their effects.

Question 6.6.45.
What are the hormones produced by the epiphysis and their effects?

Question 6.6.46.
Describe the action of aldosterone and how its secretion is regulated.

Question 6.6.47.
What stimulates parathyroid hormone secretion, and how does this hormone influence blood Ca^{2+} levels?

Question 6.6.48.
Describe the TRH-TSH-T_3,T_4 system.

Question 6.6.49.
Describe the steps leading to the production of T_3 and T_4 in the thyroid follicular cells.

Question 6.6.50.
Describe the physiological functions of cortisol.

Question 6.6.51.
Which of the following hormones is a mineralocorticoid: cortisol, androstenedione, aldosterone, or hydrocortisone?

Question 6.6.52.
How is the control of hormone secretion from the anterior pituitary by the central nervous system carried out?

Question 6.6.53.
How is the control of neurohypophysis hormone secretion by the central nervous system carried out?

Question 6.6.54.
What are the differences between the anterior and posterior lobes of the pituitary?

Question 6.6.55.
What is the function of the hypothalamic-pituitary portal vessel?

Question 6.6.56.
Is pregnenolone a precursor of aldosterone, cortisol, β-oestradiol, progesterone and/or vitamin D_3?

Question 6.6.57.
Complete the following statement: All the following organs are associated with vitamin D metabolism or the effects of vitamin D on calcium metabolism, except:
a) Bone.
b) Erythrocytes.
c) Intestine.
d) Kidney.
e) Liver.

Question 6.6.58.
State, justifying, which of the following options may constitute a manifestation of the syndrome of inappropriate antidiuretic hormone secretion:
a) Hypernatremia with high urinary osmolality.
b) Hyponatremia with high urinary osmolality.
c) Hypophosphatemia with low urinary osmolality.
d) Hyponatremia with low urinary osmolality.

Question 6.6.59.
Complete the following sentence: Oxytocin…
a) Is released by the adenohypophysis during childbirth.
b) Is produced in the neurohypophysis and released during childbirth.
c) Is produced in the hypothalamus and released from the neurohypophysis during childbirth.
d) Is produced in the hypothalamus and released by the adenohypophysis during childbirth.

Question 6.6.60.

Laceration of the pituitary pedicle during an automobile accident can be expected to result in all the following changes, except:

a) Decreased prolactin release.

b) Decreased TSH release.

c) Decreased release of LH and FSH.

d) Decreased GH release.

Question 6.6.61.

One patient has been taking pharmacological doses of a steroidal anti-inflammatory drug for a prolonged period for the treatment of asthma attacks. State, justifying, whether, if the patient is submitted to laboratory tests, obtained results are compatible with:

a) Low CRH and high cortisol levels.

b) Elevated levels of CRH and cortisol.

c) Low levels of CRH and cortisol.

d) High levels of CRH and low levels of cortisol.

Question 6.6.62.

Complete the following sentence: The physiological effects of cortisol include…

a) Hypoglycaemia, increased mobilization of fatty acids and decreased fat deposition.

b) Increase in amino acid-derived gluconeogenesis, increased glucose utilization and hypoglycaemia.

c) Hyperglycaemia, decreased mobilization of fatty acids and decreased fat deposition.

d) Decreased glucose utilization, hyperglycaemia and lipolysis.

Question 6.6.63.

Regarding the production and release of aldosterone from the adrenals, indicate whether the following statements are true or false, justifying:

a) Aldosterone production in the zona glomerulosa is mainly controlled by ACTH.

b) Aldosterone production in the zona glomerulosa is mainly controlled by angiotensin II.

c) The production of aldosterone in the adrenal medulla is mainly controlled by angiotensin II.

d) Aldosterone production in the zona glomerulosa is mainly controlled by K^+.

e) Stimulates the excretion of more Na^+ and water in the urine.

f) Exerts negative feedback inhibition on ACTH secretion.

g) Stimulates the excretion of more K^+ in the urine.

h) Its secretion is stimulated by ACTH.

Question 6.6.64.

Complete the following sentence: Physiological responses to insulin include…

a) Stimulation of glucose transport in skeletal muscle, erythrocytes and brain.

b) Inhibition of triacylglycerol synthesis in adipose tissue.

c) Stimulation of amino acid uptake in skeletal muscle.

d) Stimulation of glucose reabsorption in the kidney.

Question 6.6.65.
State which of the following neuro-endocrine responses contributes to meeting the increased energy needs during exercise:
a) Stimulation of hepatic synthesis of glycogen by glucagon.
b) Stimulation of hepatic glycogenolysis by epinephrine.
c) Stimulation of norepinephrine-induced insulin release.
d) Inhibition of gluconeogenesis by cortisol.

Question 6.6.66.
State, with justification, which of the following processes occurs immediately after a balanced meal:
a) Suppression of pancreatic insulin.
b) Increased glucose uptake by muscle and adipose tissue.
c) Increased hepatic glycogenolysis.
d) Increased lipolysis.

Question 6.6.67.
State whether the following statements are true or false, and if they are false, write the correct statement. Regarding the adrenaline hormone:
a) It is produced in the pancreas.
b) It acts mainly on muscle tissue.
c) Causes an increase in the rate of glycogen synthesis.
d) It is an allosteric modulator of the AMP-cyclase enzyme.
e) It is a specific hormone for carbohydrate metabolism, not interfering, for example, with lipid metabolism.

Question 6.6.68.
Does insulin increase glucose uptake by muscle, glucose uptake by adipocytes, fat synthesis in adipocytes, and/or glycolysis in hepatocytes? Justify.

Question 6.6.69.
In a fed state, adipose tissue primarily responds to (hormone) to (process):
a) Epinephrine ..... to degrade plasma lipoproteins.
b) Epinephrine ..... transport glucose into cells.
c) Insulin ..... hydrolyse triacylglycerols.
d) Insulin ..... synthesize fatty acids.

Question 6.6.70.
Does high plasma calcium level cause an increase in 1,25-(OH)2D, an increase in PTH, an increase in TC or bone demineralization? Justify.

Question 6.6.71.
Indicate the effect of vitamin D on calcium metabolism, referring to situations of low vitamin D or excess vitamin D.

Question 6.6.72.
State whether the following sentences about steroid hormones are true or false. Explain:
a) Steroid hormones are originally derived from cholesterol.
b) Steroid hormones are secreted by the gonads.
c) Steroid hormones are secreted by the adrenal medulla.
d) Oestradiol is derived from testosterone.

Question 6.6.73.
State, with justification, whether the following sentences about thyroid hormones are true or false:
a) They are non-polar hormones.
b) They are derived from progesterone.
c) Contain iodine.
d) They can be taken orally, in tablets.

Question 6.6.74.
If a person takes hydrocortisone tablets, which of the following will occur? Justify.
a) The secretion of CRH will increase.
b) ACTH secretion will decrease.
c) Cortisol secretion from the adrenal cortex will increase.
d) All of these will occur.

Question 6.6.75.
If a person takes a high dose of thyroxine pills for an extended period, which of the following statements will be true? Justify.
a) Thyroxine levels in the blood will be high.
b) TSH secretion will be low.
c) Thyroxine secretion by the thyroid will be reduced.

Question 6.6.76.
The formation of glycogen from glucose is an example of what type of metabolism, and is it stimulated by which hormone? Justify.

Question 6.6.77.
Which of the following stimulates insulin secretion? Justify.
a) A decrease in glucagon secretion.
b) An increase in blood levels of thyroxine.
c) An increase in blood glucose levels.
d) A decrease in blood glucose levels.

Question 6.6.78.
Which of the following statements describes what happens during fasting? Justify.
a) The secretion of insulin and glucagon decreases.
b) Insulin and glucagon secretion increases.
c) Insulin secretion increases and glucagon secretion decreases.
d) Insulin secretion decreases and glucagon secretion increases.

Question 6.6.79.
Glycogenolysis, gluconeogenesis, lipolysis, and ketogenesis occur in response to what hormone and during what state? Justify.

Question 6.6.80.
Complete the following sentence: Plasma calcium concentration is regulated by various hormones being…
a) Increased by PTH, which increases bone resorption.
b) Decreased by calcitonin which increases bone resorption.
c) Decreased by calcitriol by decreasing intestinal calcium absorption.
d) Decreased by PTH due to decreased tubular reabsorption of calcium.

Question 6.6.81.
Physical or psychological stress, increase the release of catecholamines in the adrenal medulla and neurons of the sympathetic system. Clarify the role of adrenaline and noradrenaline in mobilizing energy stores in adipose tissue, muscle, and liver.

Question 6.6.82.
It is part of the actions of insulin to promote the entry of glucose into the cells. However, some people have high plasma levels of insulin and glucose at the same time. Explain why.

Question 6.6.83.
Excess cortisol is often due to ACTH stimulation of the adrenal cortex. Explain indicating the metabolic relationship between cortisol and ACTH.

Question 6.6.84.
One patient has high levels of ACTH. What metabolic processes are affected? How does this interfere with glucose plasma levels (increase or decrease)?

Question 6.6.85.
Which enzyme(s) biosynthesis is induced by insulin action?

Question 6.6.86.
Develop the following topic: "Hormonal regulation of gluconeogenesis and liver glycogen metabolism".

Question 6.6.87.
An uncontrolled insulin-dependent individual has hyperglycaemia, hypertriglyceridemia, and metabolic ketoacidosis. Explain why.

Question 6.6.88.
What are the possible advantages for the synthesis of hormones in the form of prohormones or preprohormones?

Question 6.6.89.
Thyroid hormones are closely involved in the regulation of basal metabolic rate. The supply of excess thyroxine to the liver of animals causes an increased rate of O_2 consumption and increased heat release (thermogenesis), but the concentration of ATP in the tissue is normal. Different explanations have been given for the thermogenesis effect of thyroxine. One of them says that excess thyroxine leads to the uncoupling of oxidative phosphorylation in mitochondria. How can this effect lead to thermogenesis? Another explanation suggests that thermogenesis results from an increased rate of ATP utilization by the thyroid. Is this a reasonable explanation? Why?

Question 6.6.90.
Describe the cellular mechanism of action of hydrophilic hormones by giving 2 examples of receptors.

Question 6.6.91.
Describe the cellular mechanism of action of hydrophobic hormones.

Question 6.6.92.
Explain the possible causes of Diabetes Insipidus and associated symptoms.

Question 6.6.93.
Explain the effects of GH on metabolism.

Question 6.6.94.
Explain the regulation of secretion and the effects caused by androgens.

Question 6.6.95.
Explain the systems of counter-regulation of insulin secretion.

Question 6.6.96.
Predict the effect of an insulin overdose on brain function in a normal person.

Question 6.6.97.
Explain why insulin is needed for adipocytes to produce triacylglycerols from fatty acids.

Question 6.6.98.
Many diabetics do not respond to insulin due to a deficiency in the cells' insulin receptors. How does this affect:
a) Circulating glucose levels after a meal.
b) The rate of glycogen synthesis in the muscle.

Question 6.6.99.
Caffeine inhibits phosphodiesterase (the enzyme responsible for the degradation of cAMP). How does this affect metabolic responses to epinephrine?

Question 6.6.100.
Based on their physical properties, hormones fall into two groups: those that are very water-soluble but lipid-insoluble and those that are relatively water-insoluble but highly lipid-soluble. In their role as regulators of cellular activity, most water-soluble hormones do not penetrate target cells. On the other hand, fat-soluble hormones enter target cells and act on the nucleus. What is the correlation between solubility, receptor location and mode of action of the two classes of hormones?

Question 6.6.101.
The half-life of most hormones in the blood is relatively short. For example, if radioactively labelled insulin is injected into an animal, within 30 minutes half of the hormone has disappeared from the blood.
a) What is the importance of the relatively rapid inactivation of circulating hormones.
b) In view of this rapid inactivation, how can the circulating hormone level be maintained under normal conditions?
c) In what ways can an organism make rapid changes in the levels of circulating hormones?

Question 6.6.102.
Name three different classes of hormones and give an example of each.

Question 6.6.103.
What distinguishes eicosanoids from other signalling molecules such as epinephrine?

Question 6.6.104.
Describe the sequence of biochemical events that occur between the release of epinephrine into the bloodstream and the activation of the enzyme glycogen phosphorylase.

Question 6.6.105.
The hormone cortisol, due to its strong anti-inflammatory action, is widely used as a medicine. Observe the following hormone therapy procedure prescribed for a patient:
- Administration of high doses of cortisol daily for thirty days;
- Progressive decrease in doses, after this period, until the end of the treatment.
a) Describe the change in the rate of cortisol production during the first thirty days.
b) Explain why, at the end of the treatment, cortisol doses should be progressively reduced.

Question 6.6.106.
According to a new study, women who have a "lazy" thyroid gland have a lower risk of getting breast cancer. The thyroid is a gland located at the front of the neck and helps regulate, among other things, heart rate, metabolism and mood. The study suggests that the low functioning of this gland, known as hypothyroidism, delays the development of cancer when it already exists. However, for other researchers this subject is still controversial.
a) What relationship can be found between hypothyroidism and the rate at which a cancer spreads?
b) What hormones are secreted by a normal thyroid gland? How is this secretion regulated?
c) For the production of its hormones, the thyroid uses an ion. What element is it?

Question 6.6.107.
An Exam causes in candidates a mixture of sensations such as pleasure for being close to the long-awaited approval; emotion of experiencing a big choice, and fear of making a mistake in answering the questions. These sensations stimulate the nervous system, causing tachycardia and increased respiratory rate. Based on the above sensations, refer which gland was stimulated and the produced hormone.

Question 6.6.108.
State, explaining why, whether the following sentences concerning pancreatic hormones that act on blood glucose are true or false:
a) The pancreas produces insulin and another glucocorticoid, glucagon, which has an antagonistic action.
b) Insulin binds to receptors present on the cell membrane, allowing glucose to cross the membrane.
c) Glucagon has the opposite effect of insulin, increasing blood glucose, as it stimulates the transformation of glycogen into glucose.
d) Insulin has an enzymatic function, since it is a protein, and glucagon promotes the entry of glycogen into hepatocytes.

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ACRONYMS AND ABBREVIATIONS

ABCA1 – ATP binding cassette subfamily A member 1
Acetyl-CoA – Acetyl-coenzyme A
ACTH – Adrenocorticotrophic hormone
ADH – Antidiuretic hormone
ADP – Adenosine diphosphate
ALA – Aminolaevulinic acid
ALT – Alanine transaminase
AMP – Adenosine monophosphate
apoA-I – Apolipoprotein A-I
apoC-II – Apolipoprotein C-II
apoE – Apolipoprotein E
APRTase – Adenine phosphoribosyl transferase
AST – Aspartate transaminase
ATP – Adenosine triphosphate
BPG – 2,3-Bisphosphoglycerate
cAMP – Cyclic adenosine monophosphate
CCK – Cholecystokinin
CDP – Cytidine biphosphate
CERP – Cholesterol efflux regulatory protein
cGMP – Cyclic guanosine monophosphate
CK – Creatine kinase
CMP – Cytidine monophosphate
CNS – Central nervous system
CoA or **CoASH** – Coenzyme A
CoQ – Coenzyme Q (oxidised form)
$CoQH_2$ – Coenzyme Q (reduced form)
CoQ_{10} – Coenzyme Q_{10}
CO_2 – Carbon dioxide
CP – Creatine phosphate or phosphocreatine
CRH – Corticotropin-releasing hormone
CTP – Cytidine triphosphate
DA – Dopamine
DAG – Diacylglycerol
DHAP – Dihydroxyacetone phosphate
DMSO – Dimethyl sulfoxide
DNA – Deoxyribonucleic acid
DTT – Dithiothreitol
ER – Endoplasmic reticulum
ETC – Electron transport chain
F^- – Fluoride ion
FAD – Flavin adenine dinucleotide (oxidised form)
$FADH_2$ – Flavin adenine dinucleotide (reduced form)
FMN – Flavin mononucleotide (oxidised form)
$FMNH_2$ – Flavin mononucleotide (reduced form)
FSH – Follicle stimulating hormone
GABA – γ-Aminobutyric acid
GAGS – Glycosaminoglycans

GAPDH – Glyceraldehyde-3-phosphate dehydrogenase
GDP – Guanosine biphosphate
GIP – Gastric inhibitory peptide
GH – Growth hormone
GHIH – Growth hormone-inhibiting hormone
GHRH – Growth hormone releasing hormone
GLP-1 – Glucagon-like peptide-1
GMP – Guanosine monophosphate
GnRH – Gonadotropin-releasing hormone
GOT – Glutamic-oxaloacetic transaminase
GPT – Glutamic-pyruvic transaminase
GSDVI – Glycogen storage disease VI
γ-GT – γ-Glutamyl transferase
GTP – Guanosine triphosphate
H^+ – Hydrogen ion
Hb – Haemoglobin
HbA1c – Glycosylated haemoglobin
HbCM – Corpuscular haemoglobin
HCM – Haemoglobin corpuscular media
HDL – High-density lipoproteins
Hex A – Hexosaminidase A
HGPRTase – Hypoxantine-guanine phosphoribosyl transferase
HHb – Deoxyhaemoglobin
HIV – Human immunodeficiency virus
HMG-CoA – β-Hydroxy-β-methylglutaryl-coenzyme A
HTLV-1 – Human T-lymphotropic virus type 1
IDE – Insulin degrading enzyme
IGF – Insulin growth factor
IgG – Immunoglobulin G
IMP – Inosine-5'-monophosphate
IP_3 – Inositol triphosphate
IRS – Insulin receptor substrates
K^+ – Potassium ion
KCl – Potassium chloride
LCAT – Lecithin cholesterol acyltransferase
LDH – Lactate dehydrogenase
LDL – Low-density lipoproteins
LDLR – Low-density lipoproteins receptor
L-DOPA – 3,4-Dihydroxyphenylalanine
LH – Luteinizing hormone
LPL – Lipoprotein lipase
MCV – Medium corpuscular volume
MPS – Mucopolysaccharidosis
MRI – Magnetic resonance imaging
NAD^+ – Nicotinamide adenine dinucleotide (oxidised form)
NADH – Nicotinamide adenine dinucleotide (reduced form)
$NADP^+$ – Nicotinamide adenine dinucleotide phosphate (oxidised form)

NADPH – Nicotinamide adenine dinucleotide phosphate (reduced form)
NH_4^+ – Ammonium ion
OAA – Oxaloacetate
O_2 – Molecular oxygen
OGTT – Oral glucose tolerance test
OMP – Orotidine-5´-monophosphate
OPRTase – Orotate phosphoribosyl transferase
PAH – Phenylalanine hydroxylase
PAPS – 3'-Phosphoadenosine-5'-phosphosulphate
PEP – Phosphoenolpyruvate
PFK – Phosphofructokinase
P_i – Inorganic phosphate
PIF – Prolactin inhibiting factor
PLP – Pyridoxal phosphate
PPi – Pyrophosphate
PRF – Prolactin releasing factor
PRPP – 5-Phospho-α-D-ribosyl-1-pirophosphate
PTH – Parathyroid hormone or parathormone
PVN – Paraventricular nuclei
RAMPs – Receptor activity modifying proteins
RER – Rough endoplasmic reticulum
ROS – Reactive oxygen species
SAM – S-Adenosylmethionine
SER – Smooth endoplasmic reticulum
SLC22A5 – Solute Carrier Family 22 Member 5 gene
SON – Supraoptic nuclei
StAR – Steroidogenic acute regulatory protein
TBG – Thyroxine-binding globulin
Tg – Thyroglobulin
TPO – Thyroid peroxidase
TPP – Thiamine pyrophosphate
TRAb – Thyrotropin receptor antibodies
TRH – Thyroid regulatory hormone
TSH – Thyroid stimulating hormone
T_4 – Thyroxine
T_3 – Triiodothyronine
UDP – Uridine biphosphate
UDPGA – UDP-glucuronic acid
UMP – Uridine monophosphate
UMPS – UMP synthase
UTP – Uridine triphosphate
VDRL test – Laboratory test for investigation of venereal diseases
VIP – Vasoactive intestinal peptide
VLDL – Very low-density lipoproteins
XMP – Xanthosine monophosphate

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