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Ministry of Health of the Russian Federation

Department of Biological Chemistry

DISORDERS OF CARBOHYDRATE METABOLISM. DIABETES. METABOLIC COMPLICATIONS IN DIABETES MELLITUS

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Educational and methodological recommendations

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Classification of sugars.

Carbohydrates are compounds up of carbon, hydrogen and oxygen. Complex carbohydrates can include nitrogen and sulfur. Carbohydrates make up the bulk of human nutrition and are the body's main source of energy. Complete oxidation of carbohydrates is accompanied by the formation of carbon dioxide, water and energy in the form of ATP.

Carbohydrates are divided into three main classes:

I. Monosaccharides contain from 3 to 9 carbon atoms. For diagnostics, they matter

Trioses (lactate, pyruvate);

Pentose (ribose, deoxyribose);

Hexose (D-glucose, D-galactose, D-fructose, D-mannose).

Lactate (lactic acid) and pyruvate (pyruvic acid) contain 3 carbon atoms and are intermediate products of carbohydrate metabolism.

Ribose and deoxyribose contain 5 carbon atoms, are part of nucleic acids, high-energy phosphates (ATP, GTP, etc.), coenzymes (NAD, NADP, FAD, CoA, etc.).

Hexoses or simple sugars contain 6 carbon atoms and are the main source of energy in the body. Simple sugars are optically active and rotate the plane of polarized light to the right and left. In the human body, only dextrorotatory isomers (D-isomers) are included in the metabolism. The optical activity of sugars is used to determine their content in urine using a polarimeter.

II. Oligosaccharides contain a small number (2-9) of monosaccharides. For diagnostics, the most important are disaccharides - oligosaccharides, including 2 sugars:

- sucrose or beet sugar, consists of glucose and fructose linked by a-glycosidic bonds,
- lactose or milk sugar, consists of glucose and galactose linked by b-glycosidic bonds:
- maltose or malt sugar, consists of 2 glucose molecules linked by a-glycosidic bonds,

III. Polysaccharides make up the majority of sugars in nature. Distinguish

1) homopolysaccharides, consisting of one type of sugar. These include:

- starch - the main polysaccharide of plant origin, consisting of repeating residues of maltose. The carbohydrate is a mixture of amylose, a long, unbranched sugar, and amylopectin, a shorter branched chain sugar.

B-cellulose is a high molecular weight unbranched polysaccharide consisting of glucose residues linked by β-glycosidic bonds, which prevents its degradation by digestive juices containing α-amylase.

-Glycogen is a highly branched polysaccharide of animal origin with branching through 8-12 glucose residues, which ensures its good solubility.

2) heteropolysaccharides, in addition to simple sugars, contain their derivatives - hexuronic acids, hexosamines, deoxysaccharides. Examples of heteropolysaccharides include heparin, sialic acids, hyaluronic acid, etc.

The metabolism of carbohydrates is normal.

Carbohydrates in food products enter the oral cavity, where in a slightly alkaline medium, under the action of the saliva a-amylase enzyme, they begin to break down to oligosaccharides. Further digestion of carbohydrates continues in the alkaline medium of the small intestine under the action of pancreatic a-amylase. The final cleavage product is disaccharides, which are converted into monosaccharides with the participation of special enzymes associated with the membranes of intestinal epithelium cells: sucrase, which breaks down sucrose, lactase, which breaks down lactose, and maltase and isomaltase, which break down maltose and isomaltose, respectively.

In the form of monosaccharides, carbohydrates are absorbed by the cells of the intestinal epithelium by facilitated diffusion, and glucose and galactose are also absorbed by active transport (with the expenditure of energy). For glucose to enter the cell, insulin is required, a peptide hormone produced by the b-cells of the islet apparatus of the pancreas.

Glucose that has entered the cell for inclusion in this or that type of metabolism turns into an active form - it is phosphorylated:

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{Hexokinase}} \text{glucose-6-phosphate} + \text{ADP}$$

Phosphorylation of glucose in all tissues occurs with the participation of the enzyme hexokinase (HK), and in the liver - glucokinase. In the activated form, glucose undergoes the following main transformations:

1. Oxidation with the formation of energy.

$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + 38\ ATP$

Oxidation is carried out in 3 stages:

1.1. Anaerobic breakdown of glucose (glycolysis) includes the 1st stage of oxidation in the absence of oxygen. The end products of oxidation are pyruvate, which is converted to acetyl-CoA, lactate and 2 ATP molecules are synthesized. The formation of lactate is characteristic when there is a lack of oxygen, for example, in muscles during active work, and in erythrocytes, since the conversion of pyruvate occurs in mitochondria, and they are absent in erythrocytes.

1.2. Formation of acetyl-CoA, a key product that allows mutual conversions of proteins, fats and carbohydrates.

1.3. Aerobic oxidation of acetyl-CoA in the reactions of the Krebs cycle and associated oxidative phosphorylation. The biological meaning of oxidation is the formation of energy in the form of ATP (36 molecules in total).

2. The pentose pathway is typical for most tissues. The biological meaning of the path lies in the formation of pentoses for the synthesis of DNA, RNA and coenzymes, and in the formation of the reduced form of NADP - NADPH, which is necessary as a hydrogen donor in synthesis reactions.

3. Glycogenesis - the synthesis of glycogen, is carried out in most organs and tissues: liver, muscles and other tissues, except for the nervous one. Glycogen is the deposited form of glucose.
4. The formation of triglycerides in the liver and adipose tissue with excessive consumption of carbohydrates.
Glucose entered the cells of the intestine remains in the required amount for the needs of the cell, and the rest of it is transported through the intercellular fluid into the blood and provides the needs of other organs. However, when glucose enters the cell, it is immediately phosphorylated. The mechanism of immediate phosphorylation of glucose is required to maintain glucose in the cell. It is known that phosphorylated compounds cannot enter either the cell or from the cell. For the release of glucose from the cell, an enzyme is needed that allows the cleavage of the phosphate group. Such an enzyme is glucose-6-phosphatase, which is contained only in three tissues of the body: the epithelium of the intestine, liver and kidneys, and only these organs are able to secrete glucose from the cell and maintain its level in the blood. The presence of the enzyme in the above tissues is functionally determined, since the intestine provides blood glycemia after a meal, the kidneys return reabsorbed glucose to the body, and the liver maintains an optimal stable level of glycemia: after a meal, converting excess glucose into glycogen, and converting glycogen into glucose (glycogenolysis), in between meals. The rest of the tissues do not contain this enzyme.

There are 3 levels of glycemic regulation: nervous, hormonal and organ. Nervous regulation is carried out through the central nervous system ("sugar" center - at the bottom of the 4th ventricle), hormonal - through the endocrine system. The main hormones that affect blood glucose levels are:

1. **Insulin** is a peptide hormone produced by b-cells of the islet apparatus of the pancreas. A polypeptide consisting of 2 polypeptide chains. Chain A contains 21 AK, chain B contains 30 AK. Both chains are interconnected by 2 disulfide bridges. Insulin can exist in several forms: monomer, dimer, and hexamer. The hexameric structure of insulin is stabilized by zinc ions, which binds to His residues at position 10 of the B-chain of all 6 subunits. The molecule contains an intramolecular disulfide bridge connecting the sixth and eleventh residues in the A-chain.

Biosynthesis includes the formation of 2 inactive precursors, preproinsulin and proinsulin, which are converted into an active hormone as a result of sequential proteolysis. The synthesis of preproinsulin begins with the formation of a signal peptide on polyribosomes associated with ER. The signal peptide penetrates into the ER lumen and directs the entry of the growing polypeptide chain into the ER lumen. After the end of preproinsulin synthesis, the signal peptide is cleaved off.

Proinsulin, which enters the Golgi apparatus, is cleaved by specific proteases to form insulin and C-peptide. They are included in the secretory granules in equimolar amounts. The latter merge with the cytoplasmic membrane and are secreted into the extracellular fluid as a result of exocytosis. After secretion into the blood, oligomers break down. However, about 3% of proinsulin does not undergo proteolysis and also enters the blood unchanged. In pancreatic β-cell pathology and obesity, the proportion of proinsulin increases.

Stages of realization of functional activity of GLUT 4 and insulin receptors on the membrane of insulin-dependent cells

• The stages of implementation take place with the participation of special carrier proteins regulated by insulin — GLUT-4, which is contained only in muscles and adipose tissue (insulin-dependent tissues). In the absence of insulin, GLUT-4 is found in cytosolic vesicles. Under the influence of insulin, vesicles are translocated into the plasma membrane, with a decrease in the concentration of the hormone, glucotransporters return to the cytosol, and glucose transport stops. In liver cells, insulin induces the synthesis of glucokinase. As a result of phosphorylation, the concentration of free glucose in cells is maintained at a low level, which contributes to its transport from the blood along the concentration gradient.

• The insulin receptor is a tyrosine protein kinase that phosphorylates proteins at the OH group of tyrosine. It is a glycoprotein built from 2 alpha and 2 beta subunits. The former are located outside the cell, while the others penetrate the plasma membrane. The insulin binding site is formed by the N-terminal domains of the alpha subunits, the catalytic tyrosine-protein kinase center is located on the beta subunits. The attachment of insulin to the binding site activates the enzyme, and this enzyme itself serves as a substrate, i.e. autophosphorylation occurs: the beta subunits of the insulin receptor are phosphorylated at several tyrosine residues. It is the beta subunit that has tyrosine kinase activity. Tyrosine kinase is an indispensable mediator of all pleiotropic actions of insulin, since mutations in the ATP binding region lead to the loss of the ability of IR to autophosphorylation and the ability of the cell to respond to insulin. Stimulated autophosphorylation of the beta subunit of IR at tyrosine residues leads to phosphorylation of other intracellular substrate proteins of the insulin receptor, IRS-1, IRS-2.

• IRS-1 plays the main role in the formation of the cell's response to the insulin signal. When stimulated with insulin, the degree of phosphorylation of IRS-1 increases and gives it the ability to bind to other proteins. This leads to the activation of several pathways, representing a cascade of reactions for the activation of specific protein kinases. As a result of the activation of protein kinases, phosphorylation of enzymes and transcription factors occurs, which is the basis for the many effects of insulin.

Effects of insulin on carbohydrate, protein, and lipid metabolism

Carbohydrate	Lipid	Protein
1. Increased glucose utilization by muscles and adipose tissue	1.Increased lipogenesis	1. Increasing protein anabolism
2. Increased glycogen synthesis by the liver and muscles	2. Increased lipoprotein lipase activity	2. Increase in amino acid uptake
3. Increased glucose phosphorylation	3. Increased fatty acid synthesis	3.Increase in protein synthesis
4. Increased glycolysis	4.Increased esterification of fatty acids into triglycerides	4. Decrease in protein catabolism
5.Reduction of gluconeogenesis	5. Decreased lipolysis	5. Increase in nucleic acid uptake Increase in RNA synthesis
6. Reduction of glycogenolysis	6. Decreased ketogenesis Increased glycerine phosphate formation	6. Increase in DNA synthesis

However, not all tissues require insulin to be supplied with glucose. In some tissues, glucose can penetrate even in the absence of insulin. These are the so-called non-insulin dependent tissues. These include the intestinal epithelium, nerve tissue, red blood cells, and testes.

2. **Glucagon** is a peptide hormone produced by a-cells of the islet apparatus of the pancreas. The hormone increases blood glucose by activating glycogenolysis, gluconeogenesis, proteolysis, as well as inhibiting glycogenesis, proteogenesis.

3. **Glucocorticoids** increase blood glucose levels by accelerating gluconeogenesis and glycogenolysis.

4. **Epinephrine** raises glucose levels by activating glycogenolysis.

5. **Growth hormone** (STH) stimulates the secretion of glucagon and insulin, which leads to acceleration of glucose metabolism.

6. **Somatostatin** is a peptide hormone produced by D-cells of the islet apparatus of the pancreas. It reduces the production of growth hormone, which inhibits the formation of insulin and glucagon.

Thus, all of these hormones are insulin antagonists and cause an increase in the level of glucose in the blood, and only one insulin lowers this level using different mechanisms, but the leading one is to ensure the flow of glucose into the cells.

At the organ level, glycemia is supported by the intestines, kidneys and liver. However, the main organ that maintains the required blood glucose level is the liver, and its function to maintain this level is one of the most stable functions of

the liver. Maintaining physiological glycemia is necessary, first of all, for the functioning of cells of the nervous system and, in particular, the brain, since the main energy substrate of these cells is glucose, and brain cells are not capable of accumulating substrates, but use glucose from the surrounding space constantly as needed ... A decrease in glucose below the level that ensures diffusion into brain cells leads to the cessation of energy production with subsequent loss of consciousness, i.e. develop hypoglycemic coma.

Violation of carbohydrate metabolism.

All monosaccharides absorbed in the intestine are converted into glucose or its derivatives at some stage of metabolism. So, galactose after a series of chemical reactions is transformed into glucose-1-phosphate, and fructose is converted into fructose-1-phosphate, an intermediate product of glycolysis. Thus, the metabolism of carbohydrates to one degree or another is reduced to the exchange of glucose.
The level of glucose in the blood is determined by its absorption from the intestine, consumption by tissues, reabsorption from primary urine, intake from the liver glycogen depot, and gluconeogenesis processes. Disruption of any of these processes can lead to changes in blood glucose levels. The normal concentration of glucose in the blood is 3.5-5.7 mmol / l (normoglycemia). There may be slight fluctuations when using different methods, as well as when determining glucose in serum and whole blood: in whole blood, the glucose content is 8-10% lower than in serum, which is associated with the distribution of water between plasma and erythrocytes. In newborns, the concentration of glucose in the blood corresponds to the level of the mother, after 3-6 hours the sugar level drops by about 2 times (physiological hypoglycemia), but by 5-6 days the concentration rises to about 3/4 of the content of an adult and stabilizes by 15 years. In a healthy person, the fasting glucose level is a fairly stable indicator and is determined by the characteristics of the metabolism of a particular individual.

IMPAIRMENT OF CARBOHYDRATE EXCHANGE EXPRESSED HYPERGLYCEMIA AND HYPOGLYCEMIA.

I. Hyperglycemia is characterized by an increase in blood glucose. There are 2 main types of hyperglycemia: pancreatic hyperglycemia, extrapancreatic hyperglycemia.
1.1 Pancreatic hyperglycemia is observed in diabetes mellitus, bronze diabetes, pancreatitis and pancreaticirrhosis.
1.1.1. Diabetes mellitus (diabetes) is an metabolic disease characterized by a violation of all types of metabolism with a predominant violation of carbohydrate metabolism. Distinguish between insulin-dependent diabetes mellitus, for the treatment of which insulin is required (IDDM, synonyms: juvenile diabetes

mellitus, young diabetes mellitus, and non-insulin dependent diabetes mellitus, for the treatment of which diet and sugar-reducing drugs (NIDDM, elderly diabetes) are used. diabetes has a hereditary predisposition. The manifesting factors can be stress, intercurrent illness, intake of certain medicinal and chemical substances, malnutrition, pregnancy, excess carbohydrate food, other metabolic disorders (obesity), etc. Diabetes can be caused by insufficient insulin secretion, abnormalities of insulin or its receptors, impaired insulin activation.

1.1.2. Bronze diabetes is observed in hemochromatosis and is caused by the deposition of iron in the cells of the islet apparatus of the pancreas.

1.1.3. Hyperglycemia in pancreatitis and pancreatic necrosis is associated with damage to the pancreas and disruption of its endocrine function.

Diabetes.

A group of endocrine diseases associated with impaired glucose uptake and developing as a result of absolute and relative insufficiency of the hormone insulin - hyperglycemia - a persistent increase in blood glucose. All types of metabolism are violated:

• Carbohydrate metabolism
• Fat metabolism
• Protein metabolism
• Mineral exchange
• Water-salt exchange

Types of diabetes mellitus:

• Insulin resistance syndrome
• Diabetes mellitus type I (insulin dependent)
• Diabetes mellitus type II (non-insulin dependent)

Risk factors for diabetes I:

✓ Viral infections that cause inflammation of the islets of Langerhans (insulitis) and damage (β-cells).

✓ Heredity burdened by diabetes mellitus;

✓ Arterial hypertension, leading to impaired microcirculation in the pancreas.

✓ Autoimmune diseases, primarily endocrine diseases (autoimmune thyroiditis, chronic adrenal cortex insufficiency);

✓ Chemical agents and toxins that destroy β-cells (nitrosamines contained in some foods, streptozotocin, etc.);

✓ Nutritional factor (early intake of cow's milk);

✓ Some other factors, such as stress.

Violation of carbohydrate metabolism is associated, first of all, with a decrease in the flow of glucose into cells, caused by a lack of active insulin. Since cells need glucose, the body, through humoral regulation, tries to satisfy the needs of cells in the only way possible for it: by increasing the concentration of glucose in the blood by activating glycogenolysis, gluconeogenesis, and increasing the absorption of glucose in the intestine. Despite the adaptive mechanisms, the body is not able to compensate for the situation on its own, since all its activity is aimed at increasing the level of glucose in the blood, and it does not have a mechanism for lowering the level of insulin, therefore, there is a violation of all types of metabolism.

Biochemical parameters and clinical symptoms in diabetes mellitus.

Biochemical Changes	Laboratory Parameters	Clinical signs
Carbohydrate metabolism: •impaired glucose utilization; •activation of glycogenolysis and glyconeogenesis	hyperglycemia, glucosuria	polyuria, polyphagia, polydipsia, exicosis, genital itching
Lipid metabolism: •inhibition of lipogenesis; • activation of lipolysis; •increased formation of ketone bodies	hyperlipidemia, hyperketonemia, ketonuria, ketoacidosis	acetone odor from the mouth nausea, vomiting weight loss
Protein metabolism:•activation of proteolysis; •gluconeogenesis; •decrease in protein mass	hyperglycemia, glucosuria, aminoaciduria, uricemia	asthenization decreased immunity

An increase in the concentration of glucose in the blood causes its appearance in the urine (glucosuria). Glucose enters the urine by filtration of blood plasma in the renal glomeruli and then is almost completely reabsorbed in the renal tubules. Glucosuria is observed when glycemia exceeds the renal threshold - that concentration of glucose in the blood at which the kidneys are able to almost completely reabsorb glucose filtered through the glomeruli. The value of the renal threshold for each patient is individual, on average it is 9-10 mmol / L. Glucose in the urine acts as an osmotic diuretic and increases urine output (polyuria), which in turn is accompanied by the appearance of thirst (polydipsia). If the patient's water needs are not met, tissue dehydration (exicosis) occurs. Urine glucose causes genital itching and is a good breeding ground for bacteria.

The pathology of lipid metabolism includes inhibition of lipid synthesis and activation of lipolysis, which leads to the excessive formation of ketone bodies: acetoacetic, b-hydroxybutyric acids and acetone. Lipolysis leads to hyperlipidemia,

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hyperketonemia, ketoacidosis. Clinically, lipid metabolism disorder is accompanied by intoxication (nausea, vomiting).
Violation of protein metabolism includes proteolysis and loss of protein, which leads to excessive formation of amino acids (aminoaciduria), urates, exacerbates hyperglycemia with glucosuria and leads to a decrease in immunity.
All types of metabolism in the body are closely interconnected. There are mechanisms to transform proteins into fats and carbohydrates, carbohydrates into proteins and fats, etc. The key metabolite of such transformations is acetyl coenzyme A (acetyl-CoA). Some of the metabolic pathways work all the time, some are turned on when needed. In diabetes mellitus, metabolism is rebuilt to increase blood glucose both due to carbohydrates and due to lipids and proteins.
Diabetes mellitus is dangerous for its complications, they are the cause of the death of the patient. Complications are manifested by the development of coma (coma - loss of consciousness), and angiopathies.
In diabetes mellitus, the development of 4 types of comas is possible: hypoglycemic, hyperglycemic, hyperosmolar, lactatacidemic.

DIFFERENTIAL DIAGNOSTICS COM.

	Diabetic	Hyperosmolar	Hypoglycemic	Lactic acid
Glycemia mM	(14-35)	(30-100)	N or -	N or
Glucosuria	significant	significant	No	N or
Ketonemia		N	N	N
Ketonuria		-	-	-
Osmolarity mosmM		(>400)	N	N
pH	-(acidosis)	N	N	-(acidosis)
Blood Lactate	N	N	N	

Note: - increase, ¯ - decrease, N - norm.

A hypoglycemic coma occurs when blood glucose levels fall below the level at which brain cells are able to receive glucose. The brain is left without a source of energy and the patient loses consciousness. A hypoglycemic coma develops rapidly. Treatment is glucose administration.
Hyperglycemic coma is observed with an increased glucose content against the background of lipid metabolism disorders. It is caused, on the one hand, by an excess of keto acids and the associated intoxication, on the other hand, by an increase in the osmolarity of blood plasma and dehydration of brain cells. Patients gradually fall into a coma.
Hyperosmolar coma also develops gradually, its appearance is associated with pronounced hyperosmolarity of the blood plasma without identified disorders of lipid metabolism. Osmolarity is due to the pressure of the solute in solution, it is determined by the number of particles and does not depend on their nature. There is a special device for measuring osmolarity - an osmometer. Its action is based on a change in the freezing point of a liquid, depending on the amount of particles

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dissolved in it. In the absence of a device, osmolarity can be approximately calculated using one of the formulas. The simplest one is:

osmolarity = 2 [Na +] + [glucose] + [urea]
concentrations of substances are indicated in brackets.
... Normal blood osmolarity is 285-310 mosmol / l.
Lactic acid-lowering coma is not common in diabetes mellitus. However, due to impaired blood circulation and oxygenation in diabetes mellitus, as in other diseases, there is a relationship between the indicators of lactatemia and the condition of patients.

Lactatemia and the severity of the patient's condition.

Lactatemia(mmol/l)	The outcome
<5	favorable
5-10	50% of patients survive
>10	mortality is 90%

Complications of diabetes mellitus are also angiopathies: microangiopathies caused by damage to small vessels of the retina and kidneys, and macroangiopathies associated with damage to the vessels of the heart, brain, and extremities. The causes of angiopathies have not been definitively established. It is believed that vascular lesions are associated with excessive formation of sorbitol and some other compounds, increased osmotic pressure and excessive formation of glycosylated proteins, in particular, glycosylated hemoglobin (glycohemoglobin), the oxygen dissociation curve of which differs from the normal curve and is shifted to the left as in fetal hemoglobin and myoglobin , which complicates the return of oxygen to the tissues of the vascular endothelium. Glycosylation of proteins is a normal process for the body. It consists in the non-enzymatic formation of glycosidic bonds between glucose and protein molecules. Glycosylated proteins differ from ordinary ones in structure and, as a rule, cannot perform their function in full. The level of glycosylated proteins depends on the average blood glucose, therefore, in healthy people, it is low and does not cause dysfunction.
Diabetes mellitus is quite common. In developed countries, about 3% of the population suffers from it, so it is important to identify the disease as early as possible. The risk group for diabetes mellitus includes patients with at least one symptom from those indicated in Table 2, persons with vascular pathology, patients over 40 years old, women who have given birth to a child weighing more than 4.5 kg, as well as persons by gender forced to be in a stressful situation (pilots, etc.).
1.2. Extrapancreatic hyperglycemia.
1.2.1. Alimentary hyperglycemia is associated with the simultaneous intake of large doses of sugar. Tolerance of gdukoza is 100 g.

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1.2.2. Neuro-emotional hyperglycemia is caused by excitation of the central nervous system (stress, stroke).
1.2.3. Hormonal hyperglycemia occurs when there is an excess of hormones that increase blood glucose levels. For example, adrenaline with pheochromocytoma, glucocorticoids with Itsenko-Cushing's syndrome, with treatment with prednisone or its analogues.
1.2.4. Hyperglycemia in diffuse liver diseases.
1.2.5. Hyperglycemia with the introduction of pharmacological drugs (caffeine, diuretics, large doses of niacin, salicylic acid derivatives, etc.).
2. Hypoglycemia - a condition in which the concentration of glucose in the blood falls below 2.8 mmol / l.
2.1. Hypoglycemia due to hyperinsulinism.
2.1.1. Overdose of insulin or sugar-reducing drugs.
2.1.2. Insuloma, a tumor of the islet cells of the pancreas. If a tumor is suspected, it is recommended to measure the level of insulin and C-peptide in the blood.
2.1.3. Other tumor processes that cause hypoglycemia (pancreatic adenoma, primary liver cancer, etc.).
2.2. Hypoglycemia without an increase in insulin content.
2.2.1. Impaired absorption of sugars.
2.2.2. Liver diseases with a decrease in glycogenesis processes.
2.2.3. Insufficiency of the adrenal glands and other endocrine diseases, leading to a decrease in the secretion of hormones.
2.2.4. Prolonged fasting.

2.2.5. Hypoglycemia associated with kidney disease (primary and secondary renal diabetes). Hypoglycemia is caused by a decrease in glucose reabsorption (sugar threshold).
2.2.6. Hereditary disorders of carbohydrate metabolism.
Impaired absorption of glucose and galactose.
Lactose intolerance.
Lack of enzymes that break down disaccharides.
Glycogenoses are hereditary diseases associated with impaired synthesis or breakdown of glycogen. Different authors distinguish from 6 to 8 diseases. Type I glycogenosis or Gierke's disease is associated with a deficiency of glucose-6-phosphatase and the accumulation of glycogen in the liver, kidneys and intestinal epithelium.
Aglycogenosis is a hereditary disease caused by a lack of glycogen synthetase. It is characterized by severe hypoglycemia (0.4-0.7 mmol / l), lack of glycogen in the liver, cramps in the morning due to lack of glycogen in the muscles.

Biochemical diagnosis of diabetes mellitus.
1. Determination of fasting blood glucose concentration.
According to the World Health Organization (WHO), the normal blood glucose level is 3.5 - 5.7 mmol / L, and in people over 50 years old - 4.4 - 6.2 mmol / L. In serum and plasma, the glucose content is higher than in whole blood and is 6 mmol

/ l and 6.6 mmol / l, respectively. An increase in blood glucose in young people over 7 mmol / L, and over 50 years old, 7.2 mmol / L, or in blood plasma 7.2 mmol / L or 7.8 mmol / L, respectively, is a reliable sign of diabetes mellitus (Table. 4). Obtaining intermediate values indicates impaired glucose tolerance and requires a glucose tolerance test (TSH).
With different methods, there may be slight fluctuations in glucose.

Whole blood and plasma glucose levels.

	Blood	Blood	Plasma	Plasma
	up to 50 years	> 50 years	up to 50 years	> 50 years
Norm	3,5-5,7	4,4-6,2	3,5-6,0	4,4-6,6
Violation of tolerance	5,7-7	6,2-7,2	6,0-7,2	6,6-7,8
Diabetes	>7	>7,2	>7,2	>7,8

Usually, when pathological results are obtained, the analysis is repeated to exclude random fluctuations.
2. Determination of the concentration of glucose in urine.
In a healthy person, the glucose content in urine is so small that it cannot be determined by conventional laboratory methods. Glucose in urine is defined as positive at a concentration of 0.3-0.5 mmol / L. The appearance of detectable glucose in the urine is called glucosuria and depends on the renal threshold. Normally, the renal threshold is about 10 mmol / L, it rises with age and in persons over 50 years old is about 12 mmol / L. In patients with a reduced threshold (congenital or acquired disorders of glucose reabsorption in the tubules), glucose in the urine may appear at normal or low blood levels; in some cases, glucosuria may be the cause of hypoglycemia. Thus, glucosuria occurs in hyperglycemia, normoglycemia and hypoglycemia and cannot be a criterion for the disease, just as its absence does not mean the absence of diabetes mellitus. However, high blood glucose (more than 10 mmol / l) together with glucosuria is considered a clear sign of diabetes mellitus, and the determination of glucose in urine is regarded as an additional criterion for diagnosing diabetes mellitus.
3. Oral glucose tolerance test (TSH).
When questionable results are obtained, as well as in the presence of symptoms of diabetes, glucosuria and in patients at risk for diabetes, TSH is performed with oral glucose.
The test is carried out in the morning after a 10-14 hour fast. Fasting glucose is monitored by a physician. Glucose is administered in a glass of warm water or tea at a dose of 75 g for adults or 1.75 g / kg of body weight in children, but not more than 75 g. According to the WHO recommendation, glucose testing is carried out in capillary blood on an empty stomach and 2 hours after taking glucose. which is convenient for mass examinations. In a healthy person, the fasting blood glucose

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level is within the normal range, and after 2 hours it does not exceed the limits of the DM criterion. However, many domestic researchers consider the level of glycemia to be the most informative in an hour, therefore the test includes 3 analyzes: on an empty stomach, after 1 hour and after 2 hours.

TSH characteristics in healthy and diabetic patients.

	up to 50 years	up to 50 years	> 50 years	> 50 years
	After 60 minutes	After 120 minutes	After 60 minutes	After 120 minutes
Norm	Up to 8,8	Up to 6,6	Up to 9,8	Up to 7,7
Doubtful	8,8-9,9	6,6-7,7	<11	<8,8
Diabetic	>9,9	>7,7	>11	8,8-11,0

For more information, the following coefficients are calculated:
one). Hypoglycemic coefficient = glucose after 2 hours / fasting glucose,
in healthy people it is less than 1.3.
2). Hyperglycemic coefficient = glucose after 1 hour / fasting glucose,
in healthy people it is less than 1.7.
With an increase in the coefficients, even in the absence of pathology in the glucose content, the test is interpreted as doubtful or as a violation of glucose tolerance.
Reduced glucose tolerance can occur with increased absorption of glucose from the intestine (excessive intake of glucose, hyperthyroidism, gastrectomy, etc.), increased glycogenolysis and gluconeogenesis (pheochromocytoma, Itsenko-Cushing's disease, infection, etc.), with the impossibility of glycogen formation (glycogenosis , liver damage), or the inability of tissues to utilize glucose (diabetes, steroid diabetes, hypothalamic damage). In some cases, the initial glucose level may be below normal (glycogenosis, thyrotoxicosis).
Increased glucose tolerance (hypoglycemia, flattened peak) occurs with a low rate of absorption from the intestine (bowel disease, hypothyroidism, adrenal hypofunction) or with excessive insulin secretion (insuloma, pancreatic tumor).
In some cases, when an oral test is not possible, an intravenous glucose tolerance test is used.

Monitoring of patients with diabetes mellitus.
Determination of glucose in blood is used not only for diagnostics, but also for assessing the effectiveness of treatment and compensation for diabetes. Patients with an established diagnosis of diabetes are sent to the hospital, where they are assigned glycemic and glucosuric profiles. The glycemic profile involves multiple measurements of blood glucose concentration, usually on an empty stomach and

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approximately 2 hours after each meal. Often glucose is prescribed at the peak of hypoglycemia - at 4-5 am. The glucosuric profile usually matches the glycemic profile over time. It is prescribed for the purpose of detecting the renal threshold for glucose, elucidating the loss of glucose in the urine and assessing the violation of lipid metabolism (ketone bodies). Based on the results of the profiles, patients are prescribed therapy in order to achieve compensation for diabetes mellitus.

Criteria for compensation of diabetes mellitus.
one). Blood glucose.
Type I diabetes mellitus (IDDM) is considered compensated if fasting blood glucose and in daily fluctuations does not exceed 10 mmol / L. For type II diabetes (NIDDM), compensation is considered to be a decrease in fasting glucose to 6 mmol / L, and in daytime fluctuations - to 8.25 mmol / L.
2). Glucose in the urine. Normally, glucose, as a nonthreshold substance, is filtered in the glomeruli of the kidneys, but then it is almost completely reabsorbed in the proximal tubules. Transport proteins and hexokinase, which phosphorylate glucose to keep it in the epithelial cells of the tubules, take part in reabsorption. The value of tubular reabsorption is relatively constant, but with age, there is a tendency to decrease it. The maximum number of glucose molecules reabsorbed from primary urine into the blood depends on the number of volatile glucose transporters and the rate of their turnover in the membrane. The amount of glucose reabsorbed at the maximum loading of its carriers serves as an important indicator of the functional state of the proximal nephron. If the blood glucose exceeds the amount that can be reabsorbed in the tubules, glucose appears in the urine. When the level of 8.8-8.9 mmol / l in the blood is exceeded, glucose is excreted in the urine. The glycemic index at which glucosuria appears is called the renal threshold. With age, the renal threshold for glucose decreases. The renal threshold also decreases in chronic kidney disease, hypertension, diabetic nephropathy. In these diseases, glucosuria may appear when the blood glucose concentration is below the threshold (<8.8 mmol / l). The excretion of glucose in the urine is affected by the glomerular filtration rate, which is normally 130 ml / min. Patients with renal insufficiency or those suffering from a decrease in blood supply to the kidneys will not have glucosuria even with a high concentration of glucose in the blood. Due to the low urine blood flow rate in the glomeruli, less glucose is filtered and all of it has time to be reabsorbed in the proximal nephron, therefore, the diagnosis of diabetes mellitus cannot be made based on the level of glucose in the urine. The criterion for compensation of type II diabetes mellitus is the achievement of aglucosuria, and with type I diabetes, a loss of 2-3 g of glucose per day with urine is allowed. Pancreatic or insular glucosuria appears with a decrease in the production of insulin by the pancreas. The most common cause of glucosuria is diabetes mellitus. The amount of glucose in the urine in diabetic patients can reach 100-120 g / l. The total loss of glucose in the urine depends on the degree of polyuria, and there is usually a parallelism between the amount of glucose excreted and the degree of

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polyuria. For diabetic glucosuria, the excretion of glucose in the urine on an empty stomach and an increased concentration of fasting blood glucose are characteristic.
3). Ketone bodies in moche.
In the human body, three ketone bodies have diagnostic value: acetoacetic acid, acetone, b-hydroxybutyric acid. In healthy people, in the process of lipolysis in adipose tissue, fatty acids and glycerol are formed, fatty acids, in turn, through acetyl-CoA are converted in the liver into acetoacetate. The main part of acetoacetate with the participation of enzymes is converted into b-hydroxybutyrate, a small part is spontaneously decarboxylated into acetone. Ketones are actively consumed as energy substrates for the central nervous system, heart and other organs. Ketone bodies are formed in the liver from lipolysis products and ketogenic amino acids (leucine, isoleucine, valine), enter the bloodstream and are filtered into urine through the kidneys.
Ketone bodies can be detected in blood and urine. The proportions in the blood between them are individual and depend on the severity of ketogenesis. With mild ketosis, the main component of ketones is acetoacetic acid. When it is converted to b-hydroxybutyric acid, the relatively strong acetoacetic acid is replaced by the weaker b-hydroxybutyric acid. Acetone in a significant amount can be removed from the blood by the respiratory system, therefore, its level among the three components of ketones is the lowest. Ketones are excreted in the urine in the following ratio: b-hydroxybutyric acid 60-70%, acetoacetic acid 27-36%, acetone 3-4%.
In a healthy person, 20-50 mg of ketones per day are excreted in the urine. The excretion of large amounts of ketones in the urine is called ketonuria. Ketonuria is a consequence of a violation of carbohydrate, fat, protein metabolism and is of great clinical importance. As a rule, their appearance is associated with a violation or activation of lipid metabolism. An increase in ketone bodies is observed with prolonged fasting and restriction of carbohydrates in food (diet), as a result of eating foods rich in ketogenic substances (fats, proteins containing a large amount of ketogenic amino acids), is associated with enhanced ketogenesis, as a result of which transient ketonuria occurs, ketonuria is observed in young children with starvation against the background of exhaustion, as well as with fever, alcohol intoxication, poisoning, infectious diseases (secondary ketonuria). Ketonuria after surgery, with extensive mechanical muscle injuries, when protein breakdown occurs with subsequent partial proteolysis, caused by the hubbub of stress, and the simultaneous limitation of processes in the Krebs cycle leads to the accumulation of bicarbon compounds in the tissues, including acetyl Co-A. Central ketonuria develops after surgery on the meninges, strong excitement and irritation of the central nervous system. Ketosis can develop with glycogenous disease (lack of glucose-6-phosphatase - the first type, the third type - lack of amino-1,6-glucosidase and the sixth type - lack of hepatic phosphorylase) due to impaired formation of glycogen in the liver, accumulation of acetyl CoA and its enhancement conversion to acetoacetic acid.

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With diabetes, an increase in the level of ketone bodies is associated with a lack of insulin, while gluconeogenesis from the substrates of anaerobic metabolism (lactate) and proteolysis (alanine and other amino acids) is stimulated. Decreased utilization of glucose as an energy source in insulin-dependent tissues leads to increased lipolysis and hydrolysis of triglycerides in adipose tissue and proteolysis in muscle tissue. Free fatty acids in the liver are broken down by a beta-oxidation process to acetyl-CoA, which is then oxidized in the Krebs cycle. In diabetes, increased lipid and protein metabolism leads to the accumulation of acetyl-CoA, which in the course of successive reactions is converted into acetoacetic acid. Cells of the central nervous system and some other tissues consume glucose in an insulin-independent mechanism. Under normal conditions, in addition to glucose, they use ketone bodies as a substrate. With hyperglycemia, the supply of glucose to non-insulin dependent tissues increases, and they practically cease to utilize ketone bodies, therefore ketones are not completely utilized by tissues, accumulating in the blood and filtered through a renal filter into urine. The greatest increase in ketone bodies is observed in diabetic coma. The appearance of ketone bodies in the urine of diabetic patients indicates decompensation of the disease.

A patient with diabetes mellitus can excrete from 10 to 50 g of ketones with urine during the day. The combination of ketonuria with glucosuria is evidence of diabetes mellitus, however, the absence of glucosuria in ketonuria allows the diagnosis of diabetes to be excluded with confidence.

Determination of glycated hemoglobin

Glycosylated hemoglobin - represents the hemoglobin of erythrocytes, irreversibly linked by glycosidic bonds to glucose. In healthy people, glycosylated proteins are present in small amounts; in patients with diabetes mellitus, their amount increases in accordance with the average blood glucose level during the life of erythrocytes - 90-120 days. Glycohemoglobin provides delayed control of compensation for patients with diabetes mellitus. Its level increases from 4-5% in healthy people to 18-20% in patients with diabetes mellitus. Recently, it has been shown that an increase in the level of glycohemoglobin is one of the causes of microangiopathies in diabetes mellitus, since glycosylated proteins cannot perform their functions in full. Glycated hemoglobin is a biochemical blood index reflecting the average blood sugar level over a long period of up to 3 months. This indicator reflects the percentage of blood hemoglobin that is irreversibly combined with glucose molecules. Glycated hemoglobin is formed as a result of the Maillard reaction between hemoglobin and glucose (attachment of the terminal valine to the β-chain)

Glycated hemoglobin is an integral indicator of glycemia for three months. The higher the level of glycated hemoglobin, the higher the glycemia has been over the past three months and, accordingly, the greater the risk of developing complications of diabetes

The test is used to monitor patients with diabetes mellitus and assess the degree of their compensation for a period of 2-3 months, to assess the degree of

compensation in patients with dysproteinemia in order to avoid errors associated with the determination of fructosamine.
The method can be used in the bureau of forensic medical examination to establish or confirm the cause of death of a patient, since blood glucose after death is destroyed quickly, and glycohemoglobin remains unchanged for a long time
5). Serum fructosamines.
Fructosamine is a shorthand for a group of glycosylated plasma proteins. Their appearance is associated with a non-enzymatic reaction of the interaction of glucose with proteins and the formation of a glycosidic bond. The level of glycosylated proteins depends on the average level of glucose in the blood and persists for the entire life of the given protein. The fructosamine content is associated primarily with albumin. The life span of fructosamine is determined by the life span of albumin, i.e. 20 days. Compared to glycosylated hemoglobin, which has a life span of 2-3 months, fructosamine is a shorter-term glycemic index. It is advisable to determine fructosamine when assessing the compensation of patients with diabetes mellitus for a period of 2-3 weeks, when assessing the compensation of pregnant women, when monitoring diabetes in patients with red blood pathology and after blood transfusions, in children under 2 years of age with hemoglobinopathies.
The normal fructosamine content is 2-2.8 mmol / L. When the content of frkutosamine in patients with diabetes is 2.8-3.2 mmol / l, the compensation is satisfactory, above 3.2 mmol / l - decompensation.
6. Blood lactate.

At rest, erythrocytes are the source of lactate in the blood plasma. During physical exertion, due to insufficient oxygenation of the muscles, an increased formation of lactate is observed, which is released into the blood from the muscles and is further metabolized mainly in the liver. Lack of oxygen (hypoxia) leads to a decrease in the production of energy in the tissues and the accumulation of lactate in the blood, which is accompanied by a decrease in blood pH (acidosis) and can cause lacticidemic coma. Thus, the blood lactate level reflects the degree of tissue hypoxia.
Practically the only direct and accessible method for assessing tissue hypoxia is the measurement of lactate content. The following experimental and clinical observations support the determination of lactic acid:
an increase in blood lactate levels correlates with the severity of the disease and the likelihood of death;
measurement of lactate content can be a criterion for the effectiveness of therapy;
lactate content can confirm or remove the diagnosis of lactic acidosis in the presence of metabolic acidosis;
a significant increase in lactic acid content often precedes the appearance of clinical signs of circulatory dysfunction, therefore, its determination will help timely therapy and prevent an unfavorable outcome.
The normal content of lactate in venous blood is 0.5-2.2 mmol / l, in arterial blood - 0.5-1.6 mmol / l. The concentration of lactate in the blood increases significantly

in diabetic coma, which develops in patients with diabetes in combination with pathology of the cardiovascular and respiratory systems.
7). Microalbumiuria.
Protein filtered in the renal glomeruli from blood plasma into urine consists mainly of low-molecular-weight albumin and is almost completely (90%) reabsorbed in the renal tubules. The concentration of protein in urine is negligible and cannot be determined by conventional chemical methods. Protein can be reliably determined in urine at a concentration of about 150 mg / l.
Microalbuminuria is understood as excretion of aalbumin in the range of 30-200 mg / day, which is not available for conventional laboratory methods, but extremely important for patients with diabetes mellitus. In insulin-dependent diabetes mellitus, accompanied by microangiopathies, renal microvessels are primarily affected, which leads to renal failure, which is the main cause of death in this category of patients. The first sign of kidney damage is the appearance of microalbuminuria. Filtration of albumin in healthy people is limited by the level of blood pressure, the size and number of pores of the basement membrane, the presence of a negative charge on it, which prevents the penetration of negatively charged albumin molecules into the urine through the renal filter. A decrease in the charge on the membrane increases the excretion of protein and, first of all, albumin. It has been shown that an increase in the content of albumin in urine up to 200 mg / l is reversible, and more than 200 mg / l is irreversible. Early detection of albumin in urine and measurement of its content makes it possible to diagnose renal pathology in the initial period of the disease and to take appropriate therapeutic measures.

To identify early signs of kidney damage, it is recommended for patients with insulin-dependent diabetes mellitus to test urine for microalbumin once every 3-4 months and non-insulin-dependent diabetes mellitus 1-2 times a year.

Methods for determining indicators of carbohydrate metabolism.
To determine the indicators of carbohydrate metabolism, blood, blood serum, blood plasma, and urine are used.
Determination of glucose in the blood.
Capillary blood is drawn from a fingertip into a deproteinizing solution or an anticoagulant solution. A blood sample taken in a deproteinizing solution can be stored for 24 hours, since proteins, including glycolysis enzymes, coagulate, glycolysis stops and the glucose content does not change during storage. When taking blood in an anticoagulant, you must pay attention to the composition of the anticoagulant. If it contains NaF or KF (fluorides), blood can be stored for 2-3 hours, since fluorides inhibit glycolysis.
When using serum or blood plasma without added fluorides, serum and plasma should be immediately drained from erythrocytes and glucose determined in them. Storage of serum over erythrocytes has a hemolysis effect with solubilization of erythrocyte enzymes, including glycolysis enzymes that destroy glucose. The biological material used must comply with the determination method.

The main methods for determining glucose concentration are:
1. The glucose oxidase method is accurate and accessible to any laboratory.
2. The hexokinase method is the fastest, most accurate and most sensitive.
The determination is carried out at the end point and is available for all photometers and biochemical analyzers. Attention should be paid to the linearity limit of the method and the period of color stability. If the linearity limits are exceeded, the sample must be diluted with water or saline solution, the analysis should be repeated and the result multiplied by the dilution, or the sample volume must be reduced and the result multiplied by the degree of reduction. Color stability is defined as the period of time during which the color is stable and the samples must be measured. Methods with high linearity and long color stability times are preferred.
Determination of glucose in urine.
In all portions of urine, where it is necessary to determine glucose, it is firstly determined qualitatively or semi-quantitatively using diagnostic strips. The sensitivity of the test strips is sufficient to detect low glucose concentrations. Some compounds, the so-called reducing substances, including vitamin C, can underestimate the results of the analysis. On some strips there is a special test for the determination of reducing substances, which must be paid attention to.
If you need to know the exact concentration of glucose in the urine, its measurement is carried out by the same method as in the blood, with a preliminary dilution of the urine sample by 10-20 times and then multiplying the results by the dilution.

The loss of glucose in urine can be assessed by collection of daily urine or by conducting a glucosuric profile.
The loss of glucose in urine is calculated as follows:
glucose (g) = concentration (g / l) x volume (l).
In the presence of multiple urine samples, the glucose loss is determined in each urine sample and then all the results are added.
Conversion of glucose concentration (C) from mmol / l to g / 100 ml (%).
C glucose g / 100 ml (%) = C glucose mmol / l x 0.018
Conversion of glucose concentration (C) from mmol / l to g / l.
C glucose g / l = C glucose mmol / l x 0.18
Urine for glucose measurements should be stored in a cool, dark place. When storing urine containing bacteria, the glucose level will be lower than the original level, as the bacteria will utilize it.
Determination of ketone bodies in urine.
The determination of ketone bodies is usually carried out by the Legal test with sodium nitroprusside. Acetone and acetoacetic acid enter into the reaction. The sample is evaluated semi-quantitatively (from + to ++++).
Determination of lactate is carried out by the method of final points using commercial sets. Fluoride is added to the sample to stop glycolysis. The research should be carried out as soon as possible.

When conducting analyzes, it must be remembered that the norm represents the average value, and some patients may have parameter values that differ from the norm, but without metabolic pathology. The data obtained by different methods do not always coincide, so you should not try the new mastered method according to the previous one. For quality control, there are commercial control sera with a designated measurement method.

In large laboratories, glucose is often determined in the biochemical, clinical, duty department of the CDL and sometimes by different methods. To obtain stable, reproducible and comparable results, it is desirable to standardize all stages of sample processing and analysis methodology.

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