9. PATHOPHYSIOLOGY OF THE INTERMEDIARY METABOLISM

The utilization of various nutrients throughout the tissue network is a complicated, multilevel process. While the utilization of individual nutrients is in interaction among one another, a continuous and balanced supply of every nutrient variety is also necessary in support of the tissues. In this sense, with regards to the specific details of the intermediary metabolism the protein-, nucleic acid-, carbohydrate- and lipid-metabolism can be described, comprehensively, among their various interactions and pathological changes. Disorders of the intermediary metabolism may lead to severe short-term or long-term consequences, including e.g. hypoglycemic coma, late complications of diabetes, gout and atherosclerosis.

9.1. PROTEIN, AMINO ACID, NUCLEIC ACID METABOLISM AND THEIR DISORDERS

9.1.1. DISORDERS OF PROTEIN NEED AND PROTEIN SUPPLY

Proteins are indispensable elements of the organism, and they have tremendous specific and non-specific functions. In protein deficiency, these functions are disordered.

The most important protein functions include the followings:

1. Structural proteins (growth, wound-healing, cell production)
2. Specific protein functions (enzymes, peptide hormones, inflammatory proteins, immunoglobulins, transport-proteins, Hb, coagulation factors, etc.)
3. Less specific functions: pH-regulation, role of se-proteins in maintenance of colloid-osmotic pressure
4. Proteins (non-specifically) are important as calorie-sources (gluconeogenesis)

In the human body, proteins can be produced exclusively from the absorbed amino acids, and no other nitrogen source can be used for this purpose. A certain amount of proteins is unavoidably lost, due to continuous protein breakdown, therefore, proteins must be replaced from exogenous sources. The concept of nitrogen balance epitomizes the balance between the intake and loss of N-containing protein products.

Normally, the overall protein content of the human body is nearly 12-14 kg (ca. 20% of the total body weight), with a daily exchange (breakdown vs. new production) of 300-500 g (Fig. 9.1.). A part of the amino acids among metabolized proteins can be re-utilized for producing new proteins, therefore, the need of intake from exogenous sources is much smaller. A great portion of the protein content of intestinal digestive enzymes is absorbed, thus, more protein is absorbed than the protein content

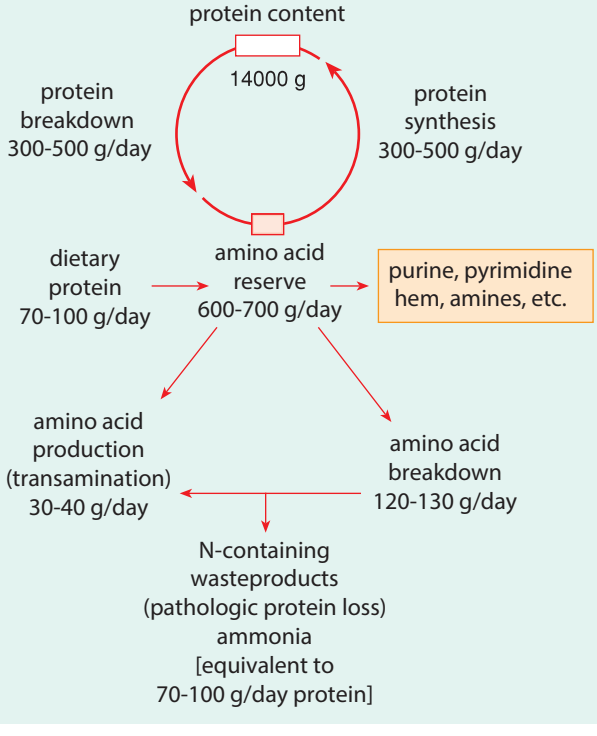

Fig. 9.1.: Steps of metabolism and exchange of proteins and amino acids.

of the food, and this also explains that in disorders of absorption, the protein loss may be very pronounced (ch. 7.4.1.). The extent of obligatory protein loss (absolute protein-minimum or quota of wearing): using food, which covers the calorie need, but is free of proteins, the protein loss is about 0.2-0.3 g/kg/day (minimal N-excretion x 6.25). The *physiological protein minimum* implies that the nitrogen balance can be stabilized by 0.5 g/kg/day complete protein (complete = contains all essential amino acids in appropriate amount and appropriate ratio). However, in practice, in order to fully ensure the sufficient food intake, the increased levels of *hygienic protein minimum* is needed, i.e. in adults, nearly 0.8-1.0 g/kg/day of protein (60-70 g/day), provided half of this is complete protein. These are primarily proteins of animal origin. In contrast to non-essential amino acids, the essential versions cannot be produced by the transformation from other amino acids.

The nitrogen balance should be positive regarding the states, in which synthesis of protein-containing structures dominates, as seen among babies and children, throughout puberty, pregnancy, lactation and after enduring a lasting disease or starvation. In these situations, the protein need is considerably higher (amongst infants, it may be 2.0-2.5 g/kg/day, and in premature infants even 3.0 g/kg/day). Apart from these situations, the positive nitrogen balance is rather harmful, it may induce pathological consequences, hyperproteinemia, gout, etc. (ch. 9.1.1.2.). The negative nitrogen balance is always a pathological state, and apart from the special protein-deficient menu and severe protein loss, it is characteristic in lasting starvation and among the elderly.

9.1.1.1. STATES OF PROTEIN DEFICIENCY

Protein deficiency, in fact, is defined as a special form of partial starvation (ch. 8.4.2.), yet all forms of calorie deficit tend to elicit a protein-deficient state (e.g., malabsorption syndromes; ch. 7.4.1.)

Causes of protein deficiency

- Regarding either the quantity or the quality of protein, the insufficient intake of either exogenous or endogenous origin (e.g. impoverished, the elderly, alcoholics, vegetarian/vegan diet, versus disorders of digestion or absorption)
- Increased protein breakdown (starvation-induced insufficiency of energy intake /gluconeogenesis from glucoplastic amino acids of proteins/diabetes mellitus, Cushing's syndrome, chronic inflammations, tumors, etc.)
- Loss of full proteins (proteinuria – nephrotic syndrome, ascites, enteral protein loss by diarrhea, protein-losing enteropathy, fistulas, suppurations, tissue injuries, burns, etc.)

Consequences of protein deficiency (according to the groups of functions mentioned above)

ad 1) The shortage of structural protein production during childhood leads to the retardation in growth. In adults, the replacement of destroyed cellular elements is disordered, and wound healing is protracted. Particularly pronounced is the loss of muscle mass (including the myocardium) and a slowdown of the synthesis of quickly exchanged cells (e.g. gastrointestinal epithelial cells and red blood cells). All these aspects lead to weakness, decreased absorption and the development of normocytic normochromic anemia. The development of osteoporosis becomes increasingly faster. Due to the decreased amount of active cell mass, the BMR may be lower.

ad 2) The deficient function of intracellular enzymes may interfere with the function of various cells/tissues, may hamper the synthesis, catabolism, and the transformation of a number of substances. Insufficiency of extracellular enzyme effects (digestive enzymes, enzymes in the circulation, etc.) also results in clinical symptoms (e.g. disorders of digestion, or absorption, and the slow removal of lipids from the plasma due to lipoprotein lipase deficiency. Defects of peptide- and amine-type hormones cause delay of development in protein deficient children, yet a complex abnormality of endocrine regulations is characteristic, also in adults (e.g. gonad-hormones are affected through their peptide regulators, the FSH and LH). Production of inflammatory acute-phase proteins is defective. Albumin-production in the liver is enhanced (still insufficient) in order to prevent the development of hypalbuminemia. The liver produces more (yet structurally defective) apoproteins. One consequence is an increase in VLDL amount forwarded to the circulation (ch. 7.6.1.1.), however, due to its defective apoprotein, it cannot be normally transformed and together with the decreased lipoprotein lipase activity, leads to increasingly high plasma lipoprotein levels (secondary hyperlipoproteinemia). The second con-

sequence is, due to the abnormal apoproteins within the liver and the enhanced lipid uptake by the liver (increased fat mobilization from fat tissue to satisfy the calorie need). The lipid content of the liver increases, steatosis develops and the result is an increase in sensitivity regarding the liver (e.g. for toxins), therefore the liver function is now damaged. Due to a decrease in immunoglobulins, the defense against infections is diminished. The deficiency regarding transport proteins may be severe, including Hb (deficiency anemia), transferrin, albumins, while the coagulation factors are scarcely affected, even in severe protein deficiency.

ad 3) A decreased colloid-osmotic pressure of the plasma results in the formation of hypoproteinemic edema. Ascites is produced considerably easier, particularly since the liver is also damaged. The buffer-capacity of pH-regulation decreases.

ad 4) Proteins provide an alternative source of calories. This is important mainly in the event if other calorigenic substances are also missing (starvation), since in these cases, the protein catabolism and the protein utilization for gluconeogenesis are higher and the protein deficiency becomes increasingly more severe.

Clinical consequences:

Metabolic/endocrine system: The loss of body weight is common (protein-, fat-stores, and bone mass decrease), and decreased levels of BMR characteristically result in hypothermia. Hypothyroidism and other endocrine dysfunctions are characteristic (gonads, pancreas and pituitary are hypofunctional, while the adrenal cortex function may even increase).

Circulation/blood: The cardiac output and blood pressure decrease, the circulation time is longer, and despite the resting bradycardia, there is a tendency for tachycardia even upon small exertion. In hypoproteinemia, due to the concurrent hyperlipoproteinemia, there is a tendency for atherosclerosis. Anemia frequently occurs (the protein need of the fast red blood cell formation is high), and leukopenia is a possibility. Vascular-type bleedings may appear.

Gastrointestinal system: The normally rapid proliferation of intestinal mucosa cells is slower, and the atrophy of the mucosa causes the lack of digestive enzymes, thereby it leads to maldigestion, malabsorption and, in the very least, to dyspepsia. In the pancreatic ducts precipitates or stone formation may develop, accompanied with chronic pancreatitis (ch. 7.5.2.) and malnutritional diabetes (ch. 9.2.2.3.). Liver cell damage, hepatic steatosis, and in severe forms, even cirrhosis may develop with ascites. The liver damage impairs the synthesis of plasma proteins.

In the event of perinatal protein deficiency, brain development may be disturbed, and the mental development is slowed, this hinders learning ability and may cause problems associated with behavior, adaptation and integration.

Generalized edema is common due to RAAS activity, accompanied by K^+-loss.

Infections may occur easily. Both the cellular and humoral immunities are insufficient.

Protein synthesis is a process of high energy need to build up protein of 1 kcal /= 4 kJ/ nearly 6 kcal /25 kJ/ energy is needed), therefore, in the absence of a sufficient amount of energy, apart from using proteins as sources of calorie, the protein synthesis will also be impaired, and soon protein deficiency develops on the basis of calorie deficiency. There are two basic forms of *protein-calorie malnutrition*: kwashiorkor disease and marasmus (Table 9.1.).

Kwashiorkor typically occurs among small children (following weaning) in the tropical zones, primarily Africa, and other developing countries. Characteristically, kwashiorkor appears in the form of a child who is retarded in growth and development, big-bellied due to ascites, has swollen legs, yet, otherwise is lean, and has a bleached appearance regarding the hair. It was assumed that an extremely low-protein, yet calorically sufficient diet (mainly cheap carbohydrates of vegetable origin) might be the explanation. According to current views, in addition to the deficiency of methionine, antioxidant vitamins and trace elements (e.g. Se) may contribute to its development, particularly in the development of liver damage. Furthermore, the role of the hepatotoxic aflatoxin (also cancerogenic), which originates commonly from fungal infections of badly stored wheat at the humid warm tropical climate, is of considerable importance. Protein deficiency prevents sufficient apoprotein synthesis in support of the VLDL formation in the liver (while visceral fat mobilization and uptake by the liver are enhanced), therefore steatosis develops. Such a liver is particularly sensitive to toxins and aflatoxin soon causes cirrhosis. The mental development is also retarded among these children, and it is often combined with neurological, psychological and social disturbances (repetition of all these from generation to generation causes increasing problems for the entire society).

The name "kwashiorkor" originates from West Africa (Ghana). It means, "red child", referring to character-

Table 9.1.

Differentiation of marasmus and kwashiorkor disease

Main points/ways of differentiation	Marasmus	Kwashiorkor disease
Characteristic occurrence	Poor countries; infants, children	Poor tropical countries; after breastfeeding is finished
Cause	Severe calorie deficit (food composition is acceptable)	Severe protein deficiency, sufficient calorie-intake (cereal/carbohydrate is the main food) + irreversible liver damage
Appearance	Looks skin and bone, growth retardation	Appears well-fed, with distended abdomen, growth retardation
Amount of muscle mass, subcutaneous fat tissue	Muscle wasting, fat tissue decreased	Lower extremities are thick, the muscle and subcutaneous fat are relatively maintained
Insulin-level	Low	Oscillating
Glucocorticoid level	High	Not increased
Albumin level	Normal	Hypoproteinemia
Edema, ascites	None	Extensive

istic changes in the black skin and reddish-orange-yellow colored hair. In consideration of other explanations, the name refers to a "first-second" meaning: it develops in the first child, who is weaned too early due to the arrival of the second child, and the only protein source is lost (carbohydrate is available). Similar states in association with kwashiorkor were reported during the era of post-war famines throughout Europe. Here the disease is named by pediatricians as, "flour-induced damage" (flour-products given instead of milk), what is at least partly explained by the combination of protein-deficient + carbohydrate-rich diet.

Marasmus is caused by a simultaneous decrease in protein- and calorie-intake among children. In adults, its clinical picture characteristically portrays the severe cachexia typically resulting from malignant tumors.

Insulin production and the adrenal cortex behave differently in marasmus and kwashiorkor, likely contributing to the differences in symptoms. In marasmus, the insulin level is low, and the glucocorticoid level is high, therefore, fat mobilization is high, and since the enhanced breakdown of muscle proteins elevate the free amino acid levels in the plasma, the liver can produce acceptable amounts of albumin to maintain the colloid osmotic pressure of the plasma. This may explain the severe muscle atrophy. Due to the known tubular insulin actions, the insulin deficiency allows salt- and water-loss, and this also inhibits the starvation-induced edema. In contrast to this, in kwashiorkor the carbohydrate intake is sufficient, the corticoid levels do not increase, while the insulin level may be high (depending on food intake), therefore, the peripheral fat- and protein-breakdowns are inhibited, the amino acid outflow decreases, the liver cannot synthesize sufficient amounts of albumin, and hypoproteinemia develops with a pronounced tendency for the formation of edema.

9.1.1.2. EXCESSIVE PROTEIN INTAKE, HYPERPROTEINEMIA

Although in great parts of the world protein deficiency is the leading nutritional problem and some form of protein deficiency may be a significant problem in any country, regarding the more developed countries (including Hungary), the average protein intake exceeds the physiologically necessary or reasonable amounts. It is not often considered, however, that protein excess may also evoke pathological consequences.

Throughout the body, protein excess is catabolized or transformed to glucose following deamination, and is stored as fat. High protein intake is still accompanied (either in a temporary or sustained fashion) with moderate hyperproteinemia in the plasma. The amount of N-containing waste-products simultaneously rises, in support of renal excretion.

One consequence of hyperproteinemia affects the kidney. The dietary protein excess induces *hyperfiltration* (ch. 5.1.1.) due to tubulo-glomerular feedback. This injures the glomeruli and induces lasting and progressive renal damage. The age-dependent decrease in GFR is partly explained by the dietary protein excess. A high protein intake has particular importance in chronic renal diseases and diabetes mellitus, the progression of renal damage can be attenuated by limiting protein intake. Distinctly, as witnessed among body builders, in whom,

elevated amounts above 5g/kg protein intake affects the amino acid reabsorption of proximal tubules. This process needs so much energy that it may not be covered during exercise-induced redistribution of cardiac output and even ischemic tubular damage may develop.

In the presence of protein excess, the construction of cellular elements becomes easier, and in locations where the cell proliferation is normally high, may lead to excessive proliferation. A characteristic consequence is *polyglobulia*, accompanied with all of its pathological consequences (ch. 4.2.2.).

A high proliferation is followed by an enhanced breakdown. This implies a burden regarding purine metabolism. Additionally, protein excess is typically coupled with higher intake of purines. Understandably, a disorder of *purine metabolism* (ch. 9.1.3.) occurs more often if and when the protein intake is high.

In hyperproteinemia, the *tendency for allergy* increases. Furthermore, toxic metabolites of protein origin enhance the risk of *colon carcinoma*.

Hyperproteinemia may develop not only as the result of high protein intake. The total protein levels of the plasma may be elevated due to a rise in the amount of a certain type of protein, often occurring in dys- or para-proteinemias.

Dysproteinemia and paraproteinemia

In dysproteinemia, the total amount of plasma proteins typically hovers at the normal level, however, the protein composition is altered. Common forms of this include the relative decrease in albumin levels, or the se-protein composition is pathological, as seen in acute or chronic inflammations. Malignancies, nephrotic syndrome, cirrhosis, autoimmune diseases may also lead to dysproteinemia, typically associated with a decrease in the albumin level and an increase in various globulin fractions. A proper diagnosis of dysproteinemia is possible only by a detailed quantitative and qualitative analysis of plasma proteins, however, a change in RBC sedimentation rate, which is dependent upon the albumin/globulin ratio, may be evaluated as an indirect sign of dysproteinemia.

Paraproteinemia is a special form of dysproteinemia, in which appearance of a normally absent protein explains the shift in the ratio among various protein fractions and often includes the rise in total protein amount. It is often seen as a rise in the amount of immunoglobulins, which may be polyclonal (e.g. hypergammaglobulinemic purpura) or monoclonal. In the latter one, only a certain immunoglobulin exhibits high levels, likely due to the tumor-like proliferation of a certain cell clone (e.g. Waldenström macroglobulinemia, multiple myeloma and amyloidosis).

The often referred to defect-dysproteinemias symbolize the isolated lack of a certain protein from the plasma, mainly due to a hereditary disorder associated with an impairment of protein synthesis.

9.1.2. DISORDERS OF AMINO ACID METABOLISM

One group of the disorders is connected with the transport of amino acids, while in the other group, mostly congenital enzyme deficiencies explain the disorder.

9.1.2.1. DISORDERS OF TRANSPORT PROCESSES

Amino acids enter the cells by an active transport acting against the concentration gradient. Similar transport processes function in the gut, and in the kidney (ca. 95% of the free amino acid content of the normal filtrate is reabsorbed by the tubules). Transport exists regarding six types (Table 9.2.) of amino acids (within each group, there is competition among the amino acids associated within the group):

The amino acid transport systems may be important in reference to the explanation of *amino acid imbalance*. In cases in which the proteins originating from various types of food are just enough and their amino acid composition is optimal, yet if some non-essential amino acid is given in excess, a negative N-balance (imbalance) and deficiency of some essential amino acid develops. If the deficient amino acid is also given in increased amounts, the symptoms are attenuated. The essence of imbalance: the amino acid excess saturates a transport way and other essential amino acids belonging to the same group (and compete for the carrier binding places) cannot be effectively transported

Table 9.2.

Classification of amino acids

I.	Neutral amino acids: Ala, Ser, Tyr, Phe
II.	Neutral amino acids: Met, Leu, Val, Ile
III.	Imino-acids: Gly, Pro, OH-Pro
IV.	Acidic amino acids: Glu, Asp
V.	Basic amino acids: Lys, Arg, Orn
VI.	Cystine

in sufficient amounts. Due to poor transport through the cell membrane, the same mechanism leads to poor absorption in the gut, and to poor reabsorption in the kidney.

Disorders of transport processes

Hartnup-syndrome was first described in a child of the Hartnup family, in 1951. The enteric absorption of tryptophan is inhibited due to a disorder of autosomal recessive inheritance. Tryptophan deficiency leads to decreased tryptophan-niacin transformation and to pellagra-like symptoms (dermatitis, diarrhea and dementia). It is likely that the decreased tryptophan-serotonin transformation explains the symptoms regarding the central nervous system: cerebral ataxia, pyramidal disorders, apathy and depression. In the gut, tryptophan undergoes a bacterial transformation resulting in indican and other indole-derivatives, which are partly absorbed, and thus, the feces and the urine excrete more indoles, while the absorbed indole products cause photosensitization and dermatitis.

In **cystinuria,** (incidence ca. 1/10 000 births) the renal cystine- (and lysine-, arginine-, ornithine-) reabsorption is insufficient. The high cystine concentration in the urine exceeds solubility, thus cystine crystals and cystine stones are formed. The leading symptom is nephrolithiasis with its consequences (hematuria, colicky pain, infections, etc.).

Cystine stones are responsible for ca. 1-2% of nephrolithiasis in adults, while in children this is the most frequent form associated with the development of a kidney stone. In the sediment of acidified, concentrated and cooled urine, characteristic hexagonal crystals can be seen.

Aminoaciduria may appear as a part of Fanconi syndrome (ch. 5.2.1.): the complex disorder of proximal tubular active transports leads to phosphaturia, glucosuria and/or polyuria/polydypsia. Milder forms of the disease cause hepatomegaly and rickets (due to phosphate loss), and among the more severe forms, uremia, hypokalemia and Ca-loss (and Ca-loss induced tetany) may occur.

In **methionine malabsorption,** the intestinal absorption of methionine (and leucine, isoleucine, valine, tyrosine) is disturbed. Bacteria in the gut transform the non-absorbed methionine to α-hydroxy-butyric acid, which is absorbed and then excreted by the urine and exhibits a characteristic odor of fermented marc-brandy (of mashing). Symptoms: diarrhea, mental retardation, light-colored hair (due to the decrease in tyrosine, less DOPA and melanin are produced). A diet including low methionine-content occasionally leads to improvement.

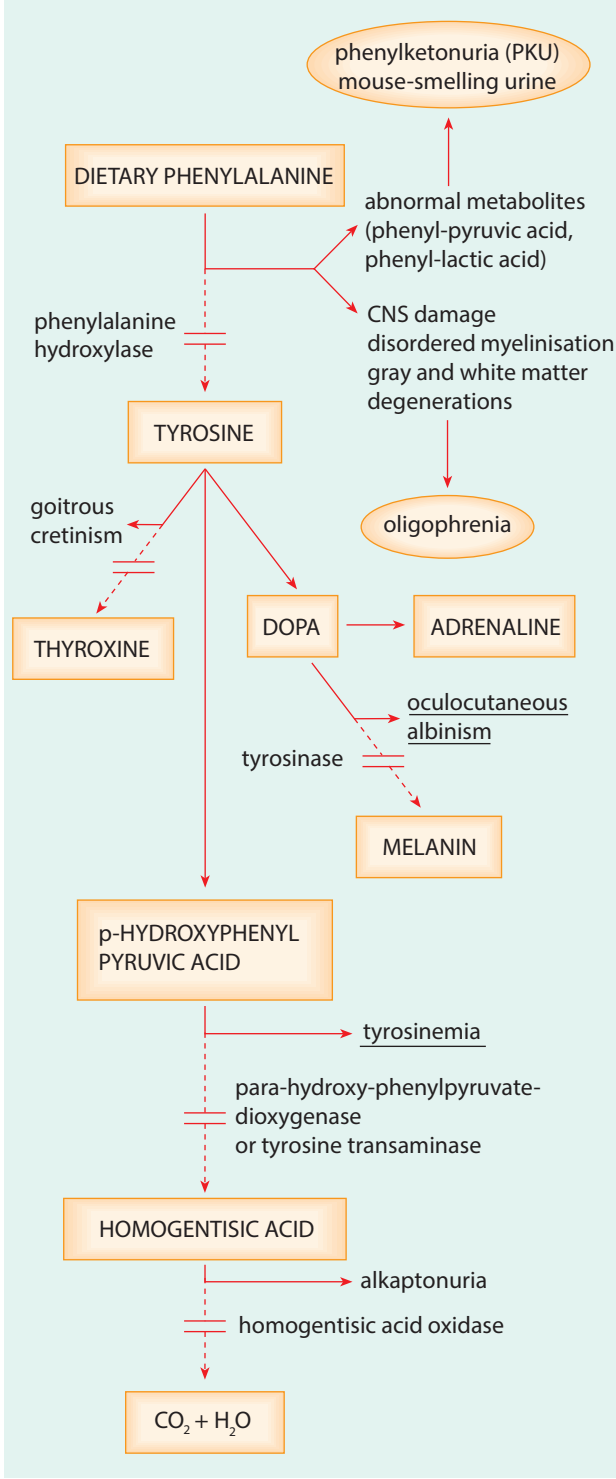

Fig. 9.2.: Enzyme defects of phenylalanine metabolism and their consequences.

9.1.2.2. DISEASES DUE TO DISORDERS OF METABOLISM

An absence or decreased activity of an enzyme participating in the intermediary metabolism and breakdown of amino acids causes accumulation of substances, prior to the blocked step and a decrease of those following the step. Metabolism uses sidetracks and may cause accumulation of substances which are not normally present. The main symptoms: *mental retardation* (due to lack of normal metabolic products and/or accumulation of toxic ones), and *aminoaciduria* (in contrast to transport disorders, only the affected and accumulated amino acid is excreted, since not all the filtered excess can be reabsorbed), occasionally *local deposition of metabolites* (in ochronosis or alkaptonuria: colored deposits in the joints and skin) and a *deficiency of some essential amino acid* (through imbalance and antagonism).

Disorders of phenylalanine and tyrosine transformations include phenylketonuria (PKU), tyrosinosis, alkaptonuria and albinism (Fig. 9.2.).

Phenylketonuria (PKU, oligophrenia phenylpyruvica) is the most frequent of the severe and commonly known disorders regarding amino acid metabolism. It is due to 7-8 mutations (incidence 7-8/100 000). Due to a deficiency of phenylalanine-hydroxylase enzyme (this turns phenylalanine into tyrosine) phenylketoacids (phenyl-acetate, -pyruvate, -lactate) accumulate and impair the central nervous system. They cause mental retardation, oligophrenia, together with the lack of tyrosine and its derivatives DOPA and catecholamines. Since the block prevents its production, tyrosine becomes essential. The excess phenylalanine inhibits the entry of other amino acids to the cells, thus the quantity and quality of amino acids available for the cells are not suitable regarding protein synthesis, resulting in an imbalance. All these aspects are critical for the intensive brain development taking place in the early postnatal weeks. Prevention of an irreversible retardation and other complications is largely dependent upon early diagnosis and on immediately initiated and appropriate phenylalanine-free diet, through which the mental development of the affected children can be greatly improved. To obtain this goal, there is a compulsory screening of the neonates prior to any symptoms which could have developed (e.g. microcephaly, or mouse-smelling urine, due to urinary excretion of phenylalanine-products = phenylketonuria = PKU). In Hungary, the neonates are obligatorily screened prior to age 1-week for PKU and other relatively frequent and serious genetic diseases (hypothyroidism, galactosemia and biotinidase deficiency). In regards to the detection of PKU, the growth of phenylalanine-dependent Bacillus subtilis is examined on a culture-medium, treated with the blood sample: good growth refers to an increase in phenylalanine in the blood (Guthrie-test). A special phenylalanine-poor/free diet should be maintained, at least until 18 years of age, and in women it has to be re-introduced prior to planning conception, to avoid intrauterine injury of the fetus. Orally applied phenylalanine-ammonia-lyase decreases phenylalanine absorption from the gut in adults (with an already normal blood-brain-barrier), however, this cannot be applied in children.

Malignant PKU is a rare disease, which is lethal, despite a phenylalanine-free diet. In the background, a deficiency in tetrahydrobiopterin can be detected, which is a common cofactor of phenylalanine-hydroxylase, tyrosine-hydroxylase and tryptophan-hydroxylase enzymes. The consequences not only affect the synthesis of tyrosine, but also that of dopamine, epinephrine and serotonin. This cofactor may be deficient due to the genetic abnormality of its synthesis (enzyme defect of GTP-cyclohydrolase) or its regeneration (defect of dihydropteridine-reductase). Infants suffering from such PKU may present epileptiform seizures. Presently, there is no methodology to replace the cofactor.

Tyrosinosis was the first described disorder among amino acid metabolism. Its cause is a defect of tyrosine-transaminase or para-hydroxy-phenylpyruvate-oxidase. In addition to mental retardation, liver cirrhosis and multiple impairments of renal tubules, including aminoaciduria, glucosuria, D-vitamin resistant rickets are all characteristic. The therapy is partial, including the reduction of phenylalanine- and tyrosine-intake, and the administration of vitamin C.

In **alkaptonuria** (rate of incidence: only 0.4/100 000), the homogentisic acid cannot be transformed to maleyl-acetic acid, due to a disorder of homogentisic acid-oxidase enzyme, and homogentisic acid accumulates, causes mental retardation, or is excreted by the urine. Upon prolonged contact with air, polymerization products appear, which stain the urine (or the nappies) to brown or black. The progression of the disease results in a brownish-black pigment which is deposited in the joints and bones, sometimes in the skin (ochronosis), leading to early arthrosis, pathological fractures and early disablement.

Albininism (3/100 000) develops in deficiency of tyrosinase enzyme, due to lack of melanin. Total lack of the pigment brings about white hair and red eyes (oculo-cutaneous albinism), which is rare in humans, and more commonly seen among laboratory rodents. Mosaicism is more frequent (in people of dark hair and eyes, light locks are seen, or blue areas in the iris). Melanin has a defensive role regarding the skin, and in its absence, albinos have a higher risk of sunshine-induced skin cancer, while due to the pigment deficiency of the iris and retina, the visual acuity decreases, and blindness occurs more often. Specifically, albinism eventually develops among black African individuals, who, apart from the abovementioned disorders, struggle with the psychological burden of excommunication. At present, there is no known therapy.

Oxalosis (primary hyperoxalemia): Due to the lack of appropriate enzymes, instead of the normal transformation (reduction or condensation with α-keto-glutarate), the glyoxylic acid produced from glycine is transformed to oxalic acid. The hyperoxalemia leads to renal excretion of oxalate (hyperoxaluria), however, oxalate precipitates very easily in the kidney, causing tubular damage and formation of oxalate (mainly Ca-oxalate) stones (ch. 5.2.1.). These appear characteristically as envelop-formed crystals seen in the urine. Oxalate may be deposited at other sites as well, including the myocardium, bone marrow, joints – causing local injury wherever deposited. Secondary hyperoxalemia may be caused by certain substances (dietary oxalate, ethylene-glycol and notably, excess ascorbic acid) and certain states (fat malabsorption) also promote the precipitation of oxalate. In fat malabsorption (Fig. 7.18.), the fatty acids bind increased quantities of Ca within the intestinal lumen (making Ca-soaps), therefore more oxalate can be absorbed.

Maple-syrup disease (incidence: $4/10^6$): It is a disorder of the breakdown of branched-chain amino acids (Val, Leu and Ileu). The α-keto-acids (due to α-keto-acid-dehydrogenase deficiency), as intermediary products, are toxic regarding the central nervous system (they inhibit mainly the glutamic-acid-decarboxylase enzyme), while originating from their excreted products, the odor of urine resembles that of maple syrup. In the pathomechanism, morphological changes of the nerves can be shown: abnormal or missing myelinization of nerve fibers. In mental retardation, the repeated occurrence of hypoglycemia is a contributing factor. From one perspective, the accumulated branched-chain amino acids non-specifically stimulate insulin secretion, while yet another perspective, due to their metabolic disorder, the capacity of gluconeogenesis from alanine decreases. In the clinical picture, anorexia, growth retardation, later seizures and episodes of unconsciousness are hallmarks of the disease.

Homocystinuria: A rather frequent abnormality, that is recently regarded as particularly relevant. Homocysteine (from methionine) is oxidized into homocysteine, its level is high (homocystinemia) and it maintains a continuous homocystinuria. The primary genetic effect may affect the B_6-vitamin-dependent cystathione-β-synthase enzyme, which catalyzes homocysteine, and, cysteine, and cystathione transformation, or alternatively, the recycling "salvage" pathway may be affected: re-transformation of homocysteine to methionine is catalyzed by methylene-tetrahydrofolate-reductase (dependent on folic acid and vitamin-B_{12}). Homocysteine may also accumulate in secondary (acquired) ways, e.g. due to the lack of either of the mentioned cofactor vitamins. Pathological states and drugs may also contribute to homocystinemia, e.g. chronic renal failure, proliferative diseases (tumors), psoriasis, hypothyroidism, diabetes mellitus, rheumatoid arthritis, methotrexate treatment (folic acid antagonist) and SLE. Consequences of homocystinemia include the following:

- enhanced formation of free radicals – this promotes LDL-oxidation (*atherogenic effect*), inhibits glutathione peroxidase, decreases the NO-effect (the so-called endothelium-dependent vasodilation);
- it has a strong prothrombotic effect (inhibits the effects of protein C and antithrombin III) and binds fibrin;
- interferes with collagen polymerization.

The homozygotic form is rare, and due to the disorder of collagen polymerization (insufficiency of Zinner-fibers) lens-luxation, severe atherosclerosis and AMI all potentially appear within an individual's first decade(!) of life. The heterozygotic form is less severe, yet rather frequent (incidence 1:70) and it is coupled with progressive sclerosis of the coronary vessels; the atherosclerosis-induced arterial obliteration in the lower extremities is 60% higher than when compared with controls, while the incidence of stroke is 40% higher. The homocysteine level is normally 5-15 μmol/liter, however, even 15-20 μmol/liter can be tolerated, yet the

suppression of its level was reportedly effective: a decrease in 5 μmol/liter reduced the risk of AMI by 20%. All these add additional emphasis and contribute to the importance of vitamin-supply.

9.1.3. DISORDERS OF NUCLEIC ACID METABOLISM

9.1.3.1. DISORDERS OF PURINE METABOLISM. GOUT.

Purine derived from metabolism of nucleic acids is transformed to hypoxanthine, which is the source of xanthine and the poorly soluble urate. The two latter steps are catalyzed by the xanthine-oxidase enzyme.

With the exception of humans, some primates, birds and several forms of reptiles, all animals transform uric acid with the aid of uricase enzyme to water-soluble allantoin.

Plasma urate is normally below 6.4 mg/dl, at such levels gout occurs in only 0.5%. In levels above 7 mg/dl, the plasma is saturated and the occurrence of gout gradually increases, and at plasma levels above 9 mg/dl, Na-urate crystals (these are sharp as a needle) precipitate from the oversaturated plasma, increasing the risk of gout to 90%. Low temperature and low pH enhance the tendency for precipitation: urate crystals precipitate preferably among tissues of poor circulation and low temperature (cartilage, tendons, and ligaments of the joints of the feet), in which, here they induce an inflammatory process, the *gouty arthritis*.

About 40% of the daily urate gain is exogenous, 60% endogenous (de novo synthesis) in a total amount of 600-1000 mg, which is the same amount excreted mainly by the kidney and (10-30%) by the feces. From the filtrate, 97% of the urate is reabsorbed in the proximal tubules, however, there is a concurrent active tubular secretion, an amount corresponding to ca. 6-7% of the filtered urate, which is emptied. In regards to the tubular secretion, there is competition among other organic anions (e.g. lactate, ketone-bodies and the degradation products of alcohol). At high plasma urate levels, urate secretion also occurs in the distal tubules, and the total renal urate excretion can be significantly enhanced.

The plasma urate level may increase in the event of high production/gain of urate or in states with its defective excretion.

States with enhanced urate production/gain:

Consumed seeds (hazelnut and various nuts), meat, liver (immense quantities of cell nuclei) are synonymous with an exogenous urate load. Gout is often referred to as the ”disease of plenty” – it was common among high-ranking noblemen and hunters, who consumed large quantities of meat and alcohol. Today, gout is more frequent among the obese, hypertensive patients with a tendency for 2DM, and more often seen in men with advancing age.

Causes of enhanced endogenous urate production:

- states of enhanced cell proliferation or destruction: either due to a genetic disposition or to acquired factors (remission phase of anemia, polycythemia, psoriasis or leukemia, yet massive cell necrosis accompanies the cytostatic therapy of tumors, hemolysis, polytraumatization, or extensive size of AMI);
- in hypoxia, the xanthine-dehydrogenase turns into xanthine-oxidase, thereby it elevates the urate level (in addition to the acidic pH due to anaerobic glycolysis, and this promotes the precipitation of urate);
- alcohol-consumption (Fig. A5.1.) enhances the function of xanthine-oxidase (acidic metabolites also cause precipitation);
- a disorder of the “salvage” pathway (e.g. defect of the hypoxanthine-guanine phosphoribosyl transferase enzyme = Lesch-Nyhan syndrome*) prevents re-utilization of hypoxanthine during the synthesis of purine bases, therefore, the metabolic processes are shifted to the direction of urate production.

Causes of decreased urate excretion:

Urate levels may increase if its excretion is insufficient. This happens primarily since all hypoxanthine is transformed to xanthine, due to an enzyme disorder. The rise is more often secondary, in states with decreased renal excretion: low GFR, volume depletion, tubular injuries (e.g. lead-intoxication), high level of organic acids (lactate, ketone-bodies, alcohol-metabolites, salicylates, etc.), and some di-

* Lesch-Nyhan syndrome: It occurs among young men with hyperuricemia, mental retardation, muscle spasticity and obligatory self-destroying tendencies (e.g. biting off a finger phalanx). In other cases, the phosphoribosyl pyrophosphate may also accumulate, which blocks the transformation of hypoxanthine to guanine and thereby, increases urate level.

uretics enhance urate reabsorption. The enteral excretion may also be low (e.g. nicotinic acid that is used to decrease lipid levels, may also inhibit urate breakdown), shifting the load onto renal excretion. The renal excretion is also low in stressful situations (perhaps due to low renal perfusion and GFR, or to hypoxia-induced lactate).

Pathomechanism of gout:
The lasting elevation of urate level leads to urate accumulation in the body. The urate excess precipitates at location of relatively low pH and low temperature, particularly in the kidney (acidotic urine) and the peripheral joints (low temperature), causing *urolithiasis* (the risk is 1000-fold higher), tubular/collecting duct obstructions, injury of the renal parenchyma (*urate nephropathy*, chronic sclerotizing interstitial nephritis) or *arthroses*. Urate precipitation is easier if the kidney is already damaged.

The precipitated urate crystals behave as foreign bodies and induce an inflammatory reaction. However, the accumulating neutrophil granulocytes and monocytes cannot destroy the phagocyte crystals, and, inevitably these cells will be destroyed. This will release free radicals and lysosomal enzymes, which will lead to local cellular damage (e.g. synovial cells and chondrocytes), along with starting an immune reaction. The developing acidic milieu prediposes to an increase in urate precipitation. In acute inflammation, the extremities are red and swollen and this is accompanied by fever, anorexia and malaise. Alcohol consumption (acidic metabolites) and bigger feasts (more purine intake) may provoke the development of **acute gouty attacks**. Repeated flare-ups my result in chronic inflammation and foreign-body granuloma = **tophus** development (crystals surrounded by fibrotic tissue and macrophages). A characteristic location are the first metatarso-phalangeal joints, and their gouty inflammation is also referred to as **podagra**. Additionally, joints of the hand, elbow, ankle and the arch of the foot may all be affected, and they are chronically deformed and painful. Chronic

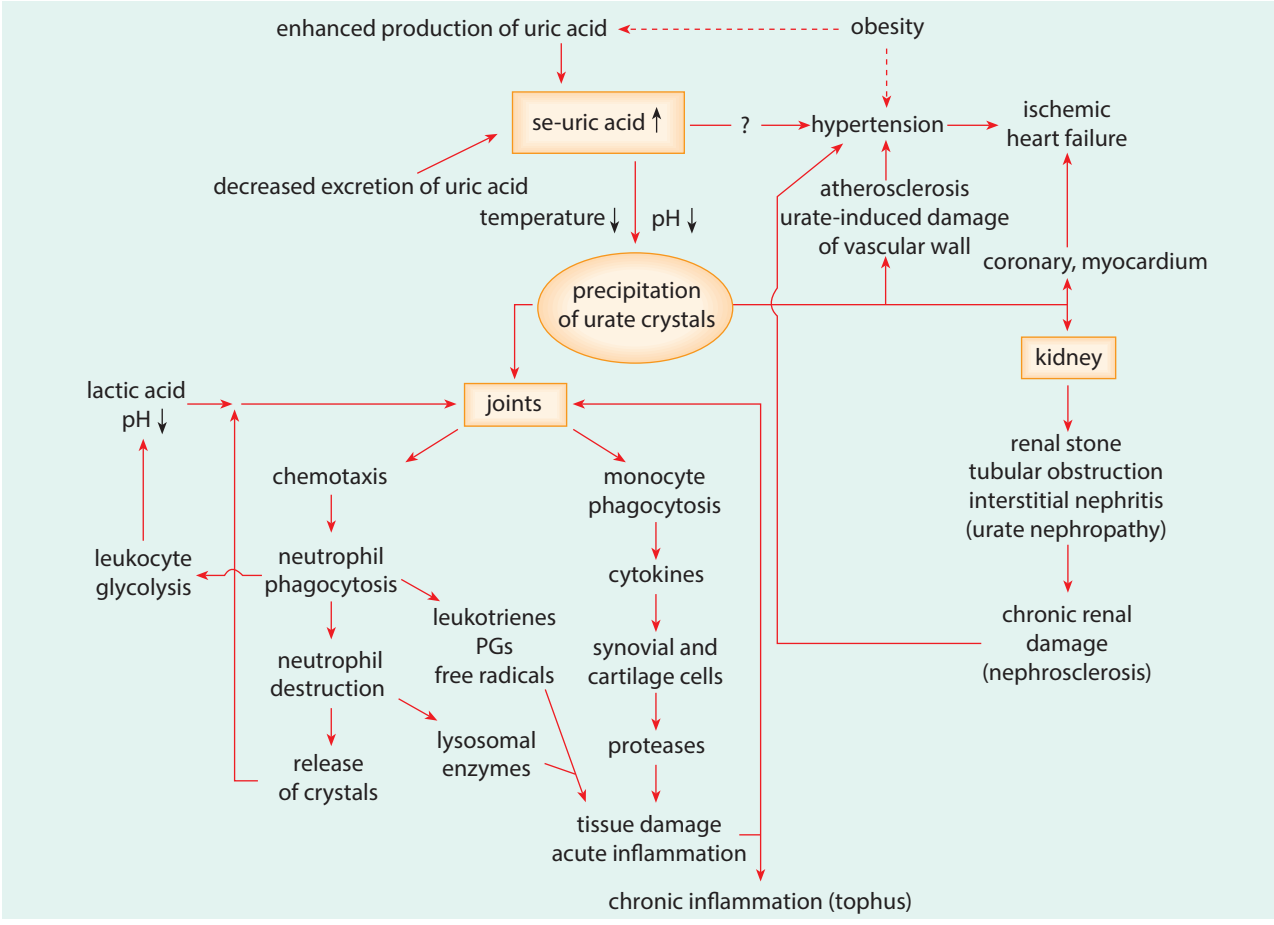

Fig. 9.3.: Ways and most important consequences of urate accumulation.

inflammation from urate precipitation is possible in many other locations (except the brain), and with different consequences:

- arterial wall – *atherosclerosis and hypertension*,
- coronaries – *ischemic heart disease*,
- pyelon – *urate stones*,
- renal parenchyma (the tubular pH is low) – *urate-nephropathy,* which is later followed by renal hypertension and chronic renal failure.

The chronic inflammatory process and the complications of all these are coupled with a high mortality rate. The consequences are shown in Fig. 9.3.

No direct connection was found between the high se-urate level and the development of gout: the normal 1.5 g urate amount of the body may even exceed 20-fold without causing gout. The elevation of urate level is rather frequent, it is present in about 5-20% of the population (more in men), while the incidence of gout is 1-2%.

Urate has an antioxidant property, which, theoretically, may imply an evolutionary benefit in the defense against free radicals (the slowdown regarding aging, prevention of atherosclerosis, cancer, etc.), and in theory, this may explain why it has tubular reabsorption.

Therapy in support of gout:
Blockers of xanthine-oxidase, e.g. the competitive antagonist hypoxanthine-analogue allopurinol (Milurit®), prevents the production of urate, and instead, hypoxanthine is produced, which has a good solubility and can be easily excreted. Colchicin is administered in acute gouty attacks to suppress the activity of leukocytes and inflammation. Colchicin is a strong toxin, it stops cell mitosis, and this is why it is used for studying chromosomes. To relieve extreme pain as seen in acute cases of gout, steroids are used (NSAID cannot be applied, since they inhibit urate excretion).

9.1.3.2. DISORDERS OF PYRIMIDINE METABOLISM

Hereditary orotaciduria:
In the course of the synthesis regarding pyrimidine-nucleotides, during the last step, the uridine production is defective due to enzyme deficiency. Through the biochemical bypaths, orotic acid is produced, which causes macrocytic hyperchromic anemia (resistant to folic acid, vitamin B_{12}, or Fe), and also causes severe mental retardation. The administration of uridine may help.

9.2. CARBOHYDRATE METABOLISM AND ITS DISORDERS

9.2.1. REGULATION OF BLOOD GLUCOSE, GLUCOSE TOLERANCE TESTS

In healthy individuals, during fasting (ca. 12-h after food intake), the plasma glucose level is in the range of 3.9-6.0 mmol/l (70-108 mg/dl). Values in the range of 6.1-6.9 mmol/l are defined as impaired fasting glucose (IFG), while values reaching 7.0 mmol/l or higher, the diagnosis is diabetes mellitus (DM).

Traditionally, glucose level is measured in the plasma, or in the capillary blood taken from finger-tip or earlobe, and frequently, from mixed venous blood. Due to the presence of morphological elements, the glucose content of the full blood is lower than that of the plasma, and the difference is ca. 11%. The fasting glucose level in the capillary and mixed venous blood are similar, however, postprandially (e.g. in oral glucose tolerance test, OGTT) the glucose level in the capillary blood may be significantly higher.

At the lower end of the normal range, signs of activation of the physiological counter-regulation can be observed. Although se-insulin starts decreasing at 4.7-4.4 mmol/l, the compensatory rise of glucagon and adrenaline can be observed only once the glucose level is 3.9-3.6 mmol/l. The upper end of the range is the value which, according to epidemiological data, does not show any pathological alteration of carbohydrate metabolism and does not suggest a high risk for the development of diabetes mellitus. According to the WHO, this "safe" value is 5.6-6.0 mmol/l. Recent investigations suggest somewhat lower glucose levels should be accepted: accordingly, definitely lower (5.6 mmol/l) fasting glucose levels are proposed to be the upper limit of the normal range in the mixed venous blood, however, the American Diabetes Society is even more severe, as the ADS advises this level for the capillary blood as the normal maximum.

Due to the continuous high glucose utilization of the tissues, proportional glucose replacement is necessary. Glycogenolysis from the liver is the immediate source of this, in turn, the liver glycogen is produced partly from the glucose intake by food, and partly by gluconeogenesis. The utilization of glucose in tissues and replacement from the liver are influenced by various factors (Fig. 9.4.), a dynamic balance between the two processes determines the actual value of blood glucose.

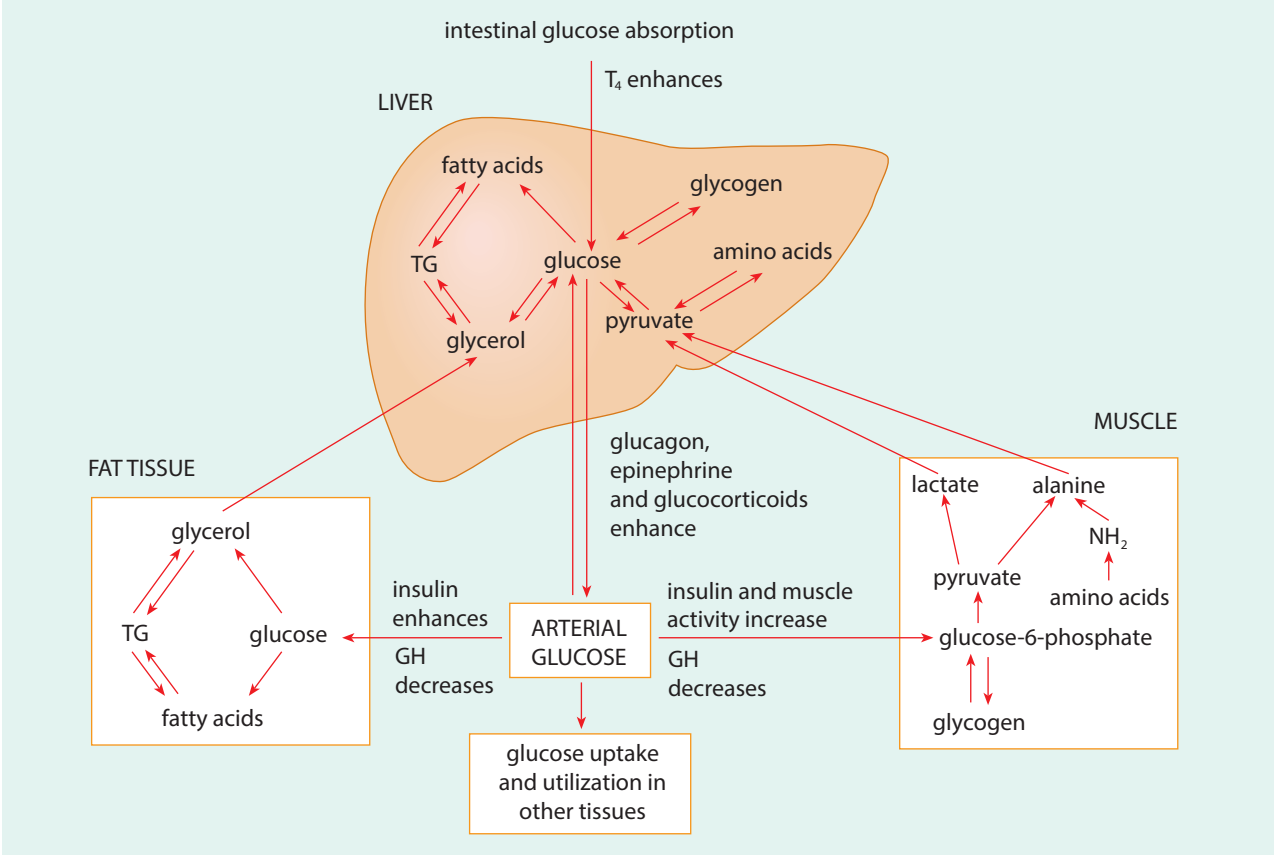

Fig. 9.4.: Glucose-exchange and the effects of hormones influencing blood glucose levels, in the liver, muscle and fat tissues.

Accordingly, the blood glucose level is not strictly standard, but fluctuates between the given limits. Upon food intake, particularly carbohydrate-containing food or refined sugar, the blood glucose rises (alimentary hyperglycemia).

The extent of alimentary hyperglycemia varies with the variety of foodstuff. (Fig. 9.26.). The **glycemic index** (GI) indicates the measure in the rise of blood glucose upon ingestion of the given food. The refined carbohydrates (easily absorbable di- and mono-saccharides) have a high, while the complex, fiber-rich carbohydrates possess a lower glycemic index.

In order to determine the glycemic index of a certain food, a 50 g carbohydrate-containing portion of it is ingested and the area under the 2-h postprandial glucose tolerance curve is compared with the reference area after consuming 50 g glucose (GI = 100). For different substances, the GI value is given in the form of percentage of the reference value. Vegetables and cereals have a low GI (below 55%) and induce a slow lasting, yet smaller rise in the blood glucose than the high extraction rate bread (56-69%), or the white bread and desserts (70-99%). Individuals of any form of diabetes or prediabetes (e.g. obese) are advised to consume food of low glycemic index.

The rise in blood glucose is detected by pancreatic β-cells, that release insulin, which in turn, promotes the entry of glucose to the insulin-dependent muscle, fat, and other cells, (e.g. hepatocytes, some hypothalamic or hippocampal neurons) also enhances the glycogen production in the liver, thereby, the excess glucose disappears from circulation. A moderate decrease in blood glucose initiates processes acting in the other direction: the insulin release decreases, while there is an increase in the release of contrainsular adrenaline and glucagon, which promote the glucose release from the liver (glycogenolysis), glucagon simultaneously stimulates gluconeogenesis in the liver (and somewhat also in the kidney). Through enhancing gluconeogenesis, the carbohydrate metabolism is also influenced by glucocorticoids of the adrenal gland, e.g. cortisol, but apart from this, thyroxine of the thyroid gland enhances the intestinal glucose absorption, as well as the growth hormone and in large amounts (by a spillover mechanism) the prolactin, too.

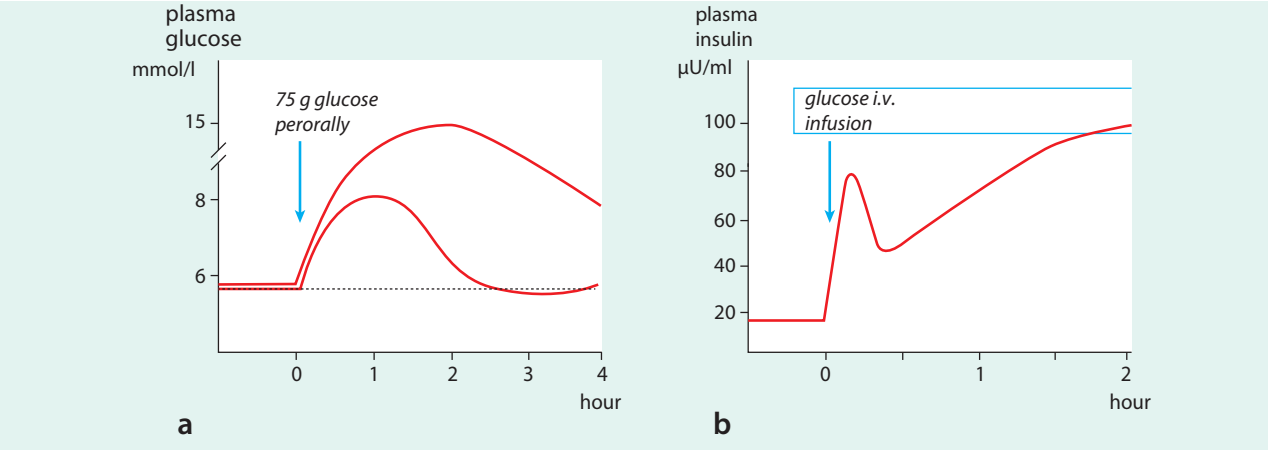

Fig. 9.5.: "A": normal biphasic and diabetoid oral glucose-tolerance tests. **"B":** the glucose load induces an insulin-secretion response – in early phase of type 2DM the resting (and maximal) insulin levels may be higher, but the early phase of the reaction is missing.

Pathological alterations of the regulation of blood glucose can be investigated by oral or intravenous **glucose tolerance tests**.

1. Most often the simple version of oral glucose tolerance test (OGTT) is applied. In fact, this is a specifically standardized form of alimentary changes of blood glucose. In order to get comparable results of measurements performed in different institutions, at different times, it was necessary to standardize the parameters of OGTT. After 3 days of diet containing minimally 150 g/day carbohydrate and normal physical activity, a fasting period of 12-h (10-16-h) follows, then a solution containing 75 g glucose (at some places 1 g/kg, for children 1.75 g/kg, but maximally 75 g) is to be consumed by the resting patient within 5 min (Fig. 9.5.a.). In order to avoid physical activity, the patient remains seated. Prior to ingesting the solution, the fasting se-glucose level is measured, then a blood sample is taken every 30 min for the ensuing 4 hours. The internationally accepted criteria emphasize the importance of the value at 2-h of the test. The maximal value is reached normally by the end of the first hour. The 2-h level is optimally already at the descending part of the OGTT curve, its normal value is below 7.8 mmol/l (140 mg%). The se-glucose level returns to the fasting value within 2-2.5-h. This is followed by a moderate (10-15%) symptomless postalimentary hypoglycemia, and by the 4th hour, the se-glucose level becomes the same as the initial value.

If the extent or dynamics of insulin secretion is insufficient, or insulin-antagonist factors are active, the curve is modified: the peak is higher and develops later, the hyperglycemia lasts longer and the hypoglycemic phase is missing. This is defined as a diabetoid curve (Fig. 9.5.a), since it occurs most frequently among prediabetic-diabetic individuals, e.g. in obese persons. In the event the 2-h se-glucose value is above 7.8 mmol/l (but below 11.1 mmol/l), impaired glucose tolerance (IGT) is diagnosed. Diabetes mellitus is the diagnosis, if the 2-h value reaches or exceeds 11.1 mmol/l. Too flat of an OGTT curve suggests abnormality regarding absorption.

Indications of standard OGTT:

- impaired fasting glucose level (IFG)
- earlier detected abnormal glucose tolerance and the test has to be annually repeated, since this state usually progresses to diabetes.
- in pregnancy*
- mother of neonate if and when the body weight is exceptionally high
- following age 45-y, the test is advised in 3-y intervals, particularly if the BMI >27
- prior to age 45-y, if other components of the multimetabolic X syndrome are present (ch. 9.2.2.3.), e.g. hypertension, hyper-/dyslipidemia (cholesterol >5.0 mmol/l, HDL cholesterol <1.2 mmol/l, LDL cholesterol >3.5 mmol/l, triglyceride >1.7 mmol/l).
- following age 40-45-y, the test is also advised in cases marked with a high risk of developing diabetes, e.g. polycystic ovaries (causing infecundi-

*Screening during pregnancy: In the 24–28th week of pregnancy, there is a compulsory (in Hungary) screening by using 75 g glucose, if the 2-h se-glucose level exceeds 7.8 mmol/l, gestational diabetes is diagnosed, which should be treated immediately. In the USA and Japan, 50 g glucose is used, if it is abnormal (the 1-h value exceeds 7.8 mmol/l), the diagnostic test is repeated by 75 or 100 g glucose (in the event of 100 g, the 2-h value is regarded abnormal if it exceeds 9.1 mmol/l).

ty), or the patient was born with a very large (or very low) body weight, and also if close relatives (brother/sister, parents, child) have 2DM.
- increased tendency for infections, for purulent dermatitis, for sty, etc. suggest immune deficiency typical for DM.
- microalbuminuria, proteinuria and retinopathy with IFG.

In manifest diabetes (e.g. fasting se-glucose above 7.0 mmol/l) there is no value or benefit to administering the OGTT. Similarly, the test is considered meaningless in the event of less than 5.6 mmol/l fasting se-glucose levels.

A **false positive** result of OGTT may be due to longer fasting or low-carbohydrate diet (e.g. slimming diet), since the insulin-producing capacity of the pancreas is transiently lower than normal. Stress, trauma, early postoperative or febrile states may also result in diabetoid curves, due to an enhanced production of insulin antagonist hormones/cytokines (adrenaline, cortisol, IL-1, IL-6, TNF). In contrast to this, physical activity during the test may result in a **false negative** test.

More rarely applied is the **double glucose tolerance test**. In this test, 20 g glucose is administered twice within a 1-h interval. Naturally, the hyperglycemic reaction is smaller when compared with a standard OGTT. The hyperglycemic peak after the second administration is normally smaller than the first peak, since the pancreatic β- cells have already been activated and had an enhanced responsiveness to the repeated glucose administration. If the second peak exceeds the first one, or the hyperglycemia is prolonged, there is a high probability of defective insulin producing capacity.

2. Directly, the responsiveness of β-cells is analyzed by the **venous glucose tolerance test** (Fig. 9.5.b.). In these examinations, the glucose-induced changes of insulin level are recorded. Normally the se-insulin exhibits a sudden increase and return towards the initial level within 30 min, what is followed by a slower, more pronounced rise. The early rapid reaction can be explained by the release of the stored insulin, while the later gradual increase is due to the enhanced production of insulin. In 2DM, the insulin resistance maintains a high resting insulin level, yet the hormone is not stored and the first fast reaction is missing. In the early (but not late) phase of diabetes, the continuously elevated insulin secretion can be further enhanced and the maximal insulin level may be higher than that compared with healthy individuals. The method can be applied in the diagnostics of e.g. the 2DM in young individuals (MODY = maturity onset diabetes in the young).

9.2.1.1. EFFECTS OF INSULIN ON CARBOHYDRATE METABOLISM

Insulin is a peptide hormone including 51 amino acids, and its two chains (21 vs. 30 amino acids) are connected by two disulfide bridges. During its production, a large prohormone features a helical structure, the two end-parts are connected by two disulfide bridges, while the mid-portion is cleaved by endopeptidases as C-peptide (connecting chain), thereby creating the final, effective insulin with its two chains. The C-peptide can be detected separately. The insulin hormone exerts its action by binding to its membrane receptors. The insulin receptor is a glycoprotein, which forms an $\alpha_2\beta_2$ heterotetramere, and its β subunit has a latent receptor protein tyrosine kinase (Tyr-kinase) activity. Several growth factors have a similar structure, e.g. the insulin-like growth factor-1 (IGF-1) which mediates the effects of growth hormone, or the IGF-2 receptor which mediates the effects of human choriogonadotropin (hCG). In the course of hormone binding to the receptor, the Tyr-kinase is first activated, and this phosphorylates and activates the receptor itself, or the Tyr side-chain of some of the several (thus far, 12) known insulin receptor substrates (IRS), thereby the Ser/Tyr kinases, e.g. 3-OH-kinase, protein kinase C (PKC), activate a phosphorilation cascade. [Such substrate may include the followings: IRS-1-6, Gab-1 (growth factor receptor bound 2/Grb2/] associated binder-1), 3 subtypes of Shc (Src – a sarcoma oncogene, with Tyr—kinase product – homology and collagen protein), $p62^{dok}$ (Tyr-kinase substrate adaptor protein family), APS adaptor protein (PH /pleckstrin homology/and SH2 /Src homology 2/ domain containing adaptor molecule)]. The activation of the phosphorylation cascade has three global consequences within the cell:

1. in association with intracellular reorganization, the insulin-dependent glucose transporter GLUT4, formerly closed in the vesicles, now becomes released and transported to the surface of the cell, thereby initiating the insulin-dependent glucose uptake;
2. through posttranslational covalent modification, the cAMP-splitting phosphodiesterase enzyme is activated, thereby inhibiting the effect of glucagon (it acts upon cAMP as a secondary messenger);
3. as the result of a process in which several steps modify gene expression, e.g. the translation of proglucagon gene decreases.

Following insulin binding of the receptor, the receptor-insulin complex enters the cell by endocytosis, then due to the vesicle membrane H-ATP-ase activity, an acidic pH develops, tconsequently the insulin is detached from the membrane and it is disintegrated, while the insulin receptor reaches the cell surface again (Fig. 9.6.).

Regarding the facilitated glucose uptake of the cells, a transmembrane protein family is responsible, the glucose transporter molecules (GLUT). Presently, 14 types of GLUT molecules are known, which possess various functions in various tissues, using dissimilar transport parameters (GLUT 1-4 and GLUT 14: glucose transporters, GLUT 5,7,9,11: fructose transporters, GLUT 6,8,10,12,13: /H^+ myoinositol transporter/: structurally atypical transporters).

The most important transporter proteins:

GLUT 1: RBC, BBB, retina, placenta (its transport maximum is reached at relatively low glucose concentration)

GLUT 2: hepatocytes, pancreas β-cells, kidney (the glucose transport proportionally changes with the glucose concentration)

GLUT 3: brain, nervous tissue (higher affinity transporter than GLUT 1, it ensures the glucose flow from the BBB towards the neural cells)

GLUT 4: muscle, heart, fat tissue, connective tissue, others: insulin dependent transporter (however, e.g. the muscle, during physical activity is able to recruit GLUT 4 on the surface of muscle fibers, without insulin)

GLUT 5: small bowel and kidney (fructose transporter)

GLUT 6: part of the genome, yet not expressed

GLUT 7: liver microsomes

GLUT 4 is the only glucose transporter which is insulin dependent. Originating from the intracellular vesicles, the transporter protein reaches the cell surface with the aid of insulin (recruitment), and, upon the effect of insulin, the cellular glucose uptake exhibits a 10-20-times increase within 5 min. GLUT 4 is present on the cells of striated muscle, fat tissue and cells of the heart, liver, pancreas, placenta, etc. The GLUT 4 dependent glucose uptake is characteristic primarily for the cells of resting striated muscle, myocardium, fat tissue, and these are unable for glucose uptake without insulin, yet many other cells of the body also belong to this group. (In working muscle recruitment of GLUT 4 on cell surface does not need insulin.) The membrane dependent effect of other GLUT types is not determined nor dependent on the presence of insulin. Therefore, these cells are suitable for glucose uptake without insulin, and they can use glucose according to the actual function of the cells, e.g. the brain cells use it for obtaining energy, the liver cells for glycogen synthesis, the pancreatic β-cells for insulin release by glucokinase, etc. The brain is the greatest glucose consumer, and it is responsible for nearly 70% of the resting glucose consumption. It uses primarily GLUT 3 and is independent of GLUT 4 or insulin.

Insulin can influence not only the membrane transport of glucose, but also enhances the cellular uptake of amino acids and potassium.

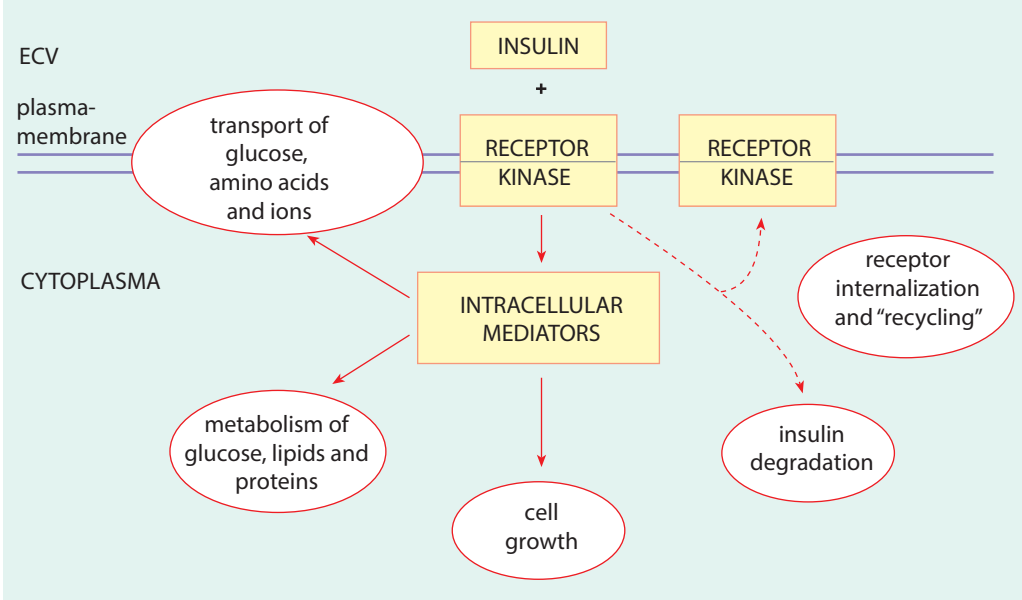

Fig. 9.6.: Effects of insulin on cell-membrane transport processes and various intracellular processes

Table 9.3a.

Interactions of insulin and metabolic-enzymes: *Carbohydrate metabolism*

Name of the enzyme	Function of the enzyme	Insulin effect	Consequence of the insulin effect
Glucokinase	First step of glucose metabolism	activates	Start of metabolism of the gluccose that had entered the cell, this results in ATP production, with insulin secretion in β-cells and enhanced insulin synthesis.
Phosphofructokinase	Glycolysis	activates	Helps ATP production from glucose.
Pyruvate-kinase	Glycolysis	activates	Helps ATP production from glucose.
Citrate-liase	TCA-cycle	activates	Helps ATP production from glucose.
Fructose-2,6-bisphospha-tase*	It breaks up fructose-2,6- bisphos-phate, which would activate glycol-ysis and inhibit gluconeogenesis	inhibits by decreas-ing the level/effect of glucagon	By promoting glycolysis, it helps ATP production from glucose, simultaneously inhibits gluconeogenesis.
Glucose-6-phosphate dehy-drogenase	First step of pentose-phosphate metabolic pathway (direct oxida-tion of glucose)	activates	Helps the NADPH2 production from glucose, what is important in synthetic processes, e.g. in synthesis of fatty acids.
Phosphoenolpyruvate-car-boxykinase	In gluconeogenesis it synthesizes phosphoenolpyruvate from oxa-lo-acetate.	inhibits	By inhibiting gluconeogenesis, it inhibits glucose synthesis from proteins, lactate, glycerol. By elevating the level of the substrate (oxalacetate) of citrate-synthase it helps the function of TCA cycle.
Fructose-1,6-bisphospha-tase	Gluconeogenesis	inhibits	By inhibiting gluconeogenesis, it inhibits glucose synthesis from proteins, lactate, glycerol.
Pyruvate-carboxylase	Gluconeogenesis	inhibits	By inhibiting gluconeogenesis, it inhibits glucose synthesis from proteins, lactate, glycerol.

* The fructose-2,6- bisphosphate regulator simultaneously stimulates the activity of the glycolytic phospho-fructo-kinase enzyme and inhibits the gluconeogenetic fructose-1,6-bisphosphatase. In the glycolysis the phosphofructokinase transforms fructose-6-phos-phate to fructose-1,6-bisphosphate, whilst the other enzyme of gluconeogenesis, fructose-1,6-bisphosphatase catalyzes the opposite reaction, i.e. it transforms fructose 1,6-bisphosphate into fructose-6-phosphate. This shows the coordinated regulation of carbohy-drate metabolism. The amount of the regulator is regulated by glucagon, by enhancing the activity of antagonist fructose-2,6-bis-phosphatase enzyme.

Table 9.3b.

Interactions of insulin and metabolic-enzymes: *Fat metabolism*

Name of the enzyme	Function of the enzyme	Insulin effect	Consequence of the insulin effect
Hormone sensitive-lipase	Breakup of TG-s in fat tissue	inhibits	Defense of fat stores.
Lipoprotein-lipase (LPL)	Th enzyme bound to the vessel wall splits fatty ac-ids from the triglycerides.	activates	Helps decreasing triglyceride content of chylo-micron and VLDL, to turn them to chylomicron remnant and IDL, respectively.
Acetyl-CoA carboxylase	Fatty acid synthesis, malo-nyl-CoA production	activates	Increases FA synthesis. Product of the process, mal-onyl-CoA inhibits the function of carnitine-palmy-toil-acyl-transferase, the entry of FA-s to mitochon-dria for metabolizing fatty acids.

Another group of insulin effects are the activating or inhibiting actions on intracellular enzymes (Fig. 9.6.). It enhances the intracellular glucose utilization (activation of phospho-fructo-kinase, pyruvate-kinase, citrate-synthase enzymes; Fig. 9.6.), and enhances the activity of the TCA cycle. In the liver, the inhibition of the phosphorilase activity decreases the breakdown of glycogen to glucose and through the activation of glycogen-synthase, more glucose is used for glycogen synthesis, while inhibition of fructose-1,6-bisphosphatase decreases gluconeogenesis. Insulin inhibits proteolysis and increases production of proteins (ribosomal effect). In fat cells, insulin inhibits hormone-sensitive lipase and release of triglycerides, yet it enhances the glucose uptake, utilization and direct oxidation (pentose-phosphate shunt, which provides pentose for nucleic acids, NADPH for synthesis of fatty acids), while through increased acetyl-CoA carboxylase activity, it increases the synthesis of fatty acids which, with glycerol, form triglycerides. Another intracellular effect is the influence upon the cell nucleus to increase the growth of the cell.

Glucagon is produced in the α-cells of the pancreas. Its effect on cellular enzymes leads to catabolism and mobilization of glycogen and fats, it enhances the gluconeogenesis and proteolysis and enhances the catabolism of fats in the cells. It inhibits acetyl-CoA-carboxylase and malonyl-CoA synthesis (this would be a basic step for producing fatty acids from acetyl-CoA), thereby more acetyl-CoA is used in support of ketogenesis.

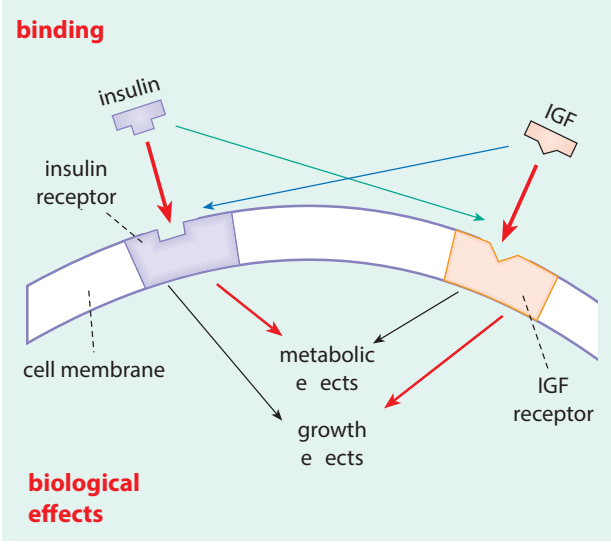

Fig. 9.7.: Binding of insulin and insulin-like growth factor (IGF) to their own receptors is mor pronounced, but they cross-react with the other receptors. Accordingly, besides a weak insulin-like effect of IGF, the insulin has a weak effect to enhance growth.

Adrenaline and glycogen enhances the metabolism and utilization of fats, thereby the glucose level of the blood quickly increases. The similar rapid elevation of se-glucose is seen in cases of increased sympathetic activity.

The effect of cortisol or ACTH markedly increases the protein catabolism, the gluconeogenesis, the utilization of amino acids, altogether leading to elevation of se-glucose. The lipolysis is also significantly enhanced.

Growth hormone inhibits the cellular glucose uptake, and this is how it elevates se-glucose and increases the burden on the β-cells. The IGF-s (insulin-like growth factors also known as somatomedins) bind to insulin receptors (Fig. 9.7.), however, they have a weak effect, therefore, inevitably they act as functional antagonists of insulin.

The somatostatin inhibits insulin secretion, and the adequate insulin level can be seen only at higher se-glucose levels.

In summary: While insulin enhances glucose utilization by tissues, inhibits the glucose replacement from the liver and decreases the blood glucose level, other (contrainsular) hormones act in support of the elevation of blood glucose. Insulin aids the production, other hormones rather promote the catabolism of proteins. Insulin enhances the production of fats and inhibits their mobilization, and other hormones enhance their catabolism and inhibit their synthesis. The production of ketone bodies is inhibited by insulin, while this is promoted by hormones (particularly glucagon) which cause enhanced fat catabolism.

9.2.1.2. SECRETION OF INSULIN AND GLUCAGON

The quantity of insulin secretion is essentially determined by the level of blood glucose. The glucose entering the β-cells with the aid of GLUT 2 (or glucose bound to glucoreceptors) stimulates the release of stored insulin and the synthesis of new insulin. Physiologically, the insulin release exhibits an oscillation of 3-6 min periods. This likely inhibits the development of receptor down-regulation and consequent insulin resistance. In addition to glucose, other substances increase insulin secretion: other carbohydrates (fructose), certain aminoacids (e.g. leucine), gastrointestinal hormones (gastrin, secretin, CCK, VIP, glucagon, glucose-dependent insulinotropic peptide /= GIP, also named gastric inhibitory polypeptide/, glucagon-like peptide-1 /GLP-1/), other hormones (GH, ACTH), additionally, the sulfonylurea (oral antidiabetic) and biguanide derivatives also increase the secretion (the last versions also inhibit gluconeogenesis in order to decrease the blood glucose

level). In contrast, the insulin secretion is decreased by catecholamines, somatostatin and inhibitors of protein synthesis. The secretion of insulin may be insufficient due to the severe damage of β-cells, either since the glucose cannot bind to the surface glucoreceptors of the pancreas or it cannot exert its intracellular effects.

The process of insulin secretion (Fig. 9.8.): With the aid of GLUT2 receptors, glucose enters the pancreatic β-cell, from glucose utilization ATP is produced, and its effect is to close the ATP-dependent K^+ channels. Therefore, the cell is depolarized, and this leads to the opening of the voltage-dependent Ca^{++} channels. The rise in IC Ca^{++} initiates the insulin secretion.

The glucagon production is also regulated specifically by the blood glucose level: upon the decrease in glucose levels, the glucagon secretion is enhanced, and the other way round, following food intake, its secretion decreases. In fasting, the glucagon secretion increases more than when compared with diabetes. The catecholamines, VIP stimulate and somatostatin effectively blocks glucagon secretion.

Following food intake and during stress situations, a rise in blood glucose is physiological, moreover, its eventual absence can be regarded pathological. A disproportionally high elevation of glucose or hyperglycemia at rest, during fasting, is certainly pathological. The most important form of this is diabetes mellitus, however, other endocrine disorders (e.g. Cushing disease/syndrome-cortisol, hyperthyroidism-thyroxin, acromegaly-GH-excess and pheochromocytoma-epinephrine) may also be accompanied by a high blood glucose level. The other basic form of abnormality in regulation of blood glucose is hypoglycemia.

9.2.2. DIABETES MELLITUS

9.2.2.1. DIABETES MELLITUS (DM) SYNDROME

The basic abnormality in the syndrome (Fig. 9.9.) is the absolute or relative insulin deficiency. Decreased cellular glucose uptake and increased glucose output result in *hyperglycemia* and high osmotic pressure. The high glucose content of the glomerular filtrate leads to *glucosuria*, the high osmotic pressure of the filtrate causes osmotic diuresis (ch. 5.1.3.) a combination of *polyuria* and hyposthenuria. The syndrome was named after these symptoms (diabetes mellitus: the classic expression = "flood of sweet urine" – the "mellitus" /sweet taste/ was added by the physician of Charles II of England, though the phenomenon had first beendescribed by Avicenna). The polyuria-induced hypovolemia and the hyperglycemia-induced hyperosmolarity cause thirst, and that causes secondary *polydipsia*. Gluconeogenesis leads to protein loss, while to counterbalance the decreased glucose utilization, the cells metabolize more fat (or fat-related ketone bodies), therefore, the *body weight* decreases, according to the calorie content of glucose and ketone bodies lost by the urine. In young, lean, type-1 DM patients, polyuria, polydipsia and an enhanced appetite are adjoined by severe physical weakness, loss of body weight and ketoacidosis. In obese type-2 DM patients, the weight loss is transient and moderate (they lose more muscle and water, less fat), characteristically, without ketoacidosis. Apart from glucose metabolism, the protein and fat metabolisms are also affected in diabetes, as well as the salt- and water, and the pH balance.

Osmotic diuresis in diabetes (cf. ch. 5.3.2.):
An increasing number of nephrons become unable to completely reabsorb the glucose in the proximal tu-

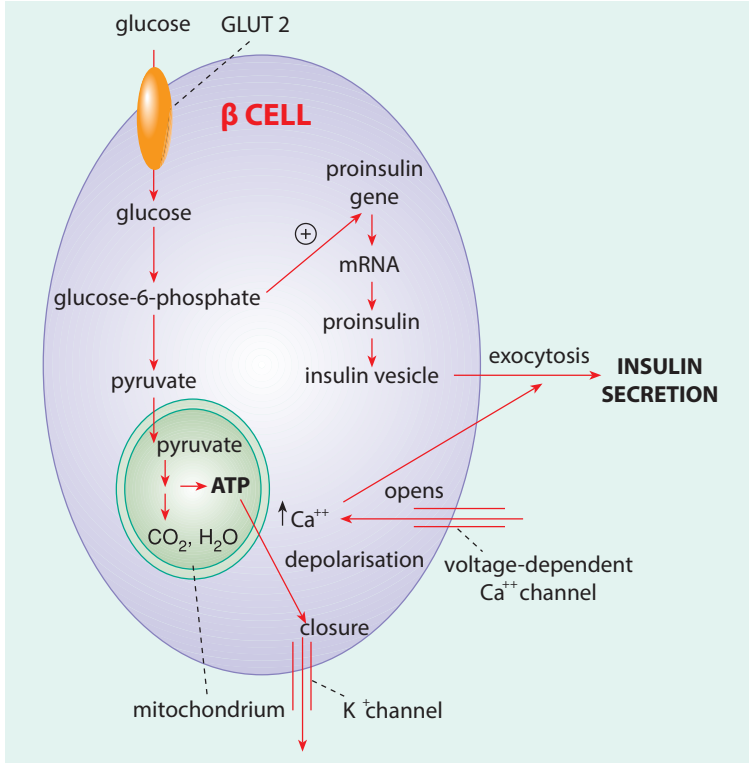

Fig. 9.8.: Mechanism of insulin secretion in pancreatic β-cells.

bules (from ca. 11 mmol/l se-glucose). At the end of the proximal tubule, a large quantity of glucose remains, together with water (i.e. in larger volume), and, this glucose cannot be reabsorbed later. In consideration of the enhanced proximal glucose (and symport Na) reabsorption, this explains a definite decrease in the NaCl-concentration (although the osmotic pressure is still high, as in the filtrate, however, glucose, rather than NaCl, is responsible for the high pressure). An increase in the quantity and diminished NaCl-concentration fluid reaches the loop of Henle. The low NaCl concentration and rapid flow rate make it difficult for the salt reabsorption in the ascending part of the loop, therefore, in the ascending part of the dilution, in the descending one, the concentration cannot be performed normally, thereby the cortico-medullary osmotic gradient of the entire kidney decreases. An increased larger volume reaches the end of the loop of Henle and the distal tubule, including an increase in salt amount (though smaller salt concentration). This, by way of the tubuloglomerular feedback, leads to the enhancement of SNGFR, and in the beginning, the total GFR. In the distal tubule, there is scarcely any possibility for compensation, yet even a moderately increased salt-reabsorption (by secondary hyperaldosteronism) results in potassium loss. A pronounced polyuria ensues, despite high ADH-levels, and the urine is less concentrated (as compared with the filtrate) than normally, although according to the renal concentration gradient, it is maximally concentrated. However, the concentration of the filtrate is much higher than normal, and the final urinary concentration may be extremely high. In summary: Due to hyposthenuria, the range of concentration-dilution is narrower and, due to the high osmotic pressure of the filtrate (e.g. 1.030), it is shifted to higher values (e.g. 1.025–1.040). Not only water and glucose are lost, but an immense quantity of NaCl (and some potassium) too, explaining the development of real exsiccosis and hypovolemia. Although potassium is lost and the potassium content of the whole body is low, the se-K level is rather high, due to ketoacidosis.

In the diagnostics regarding diabetes, the most important elements are the fasting blood glucose level (hyperglycemia), the glucosuria and the OGTT (IGT).

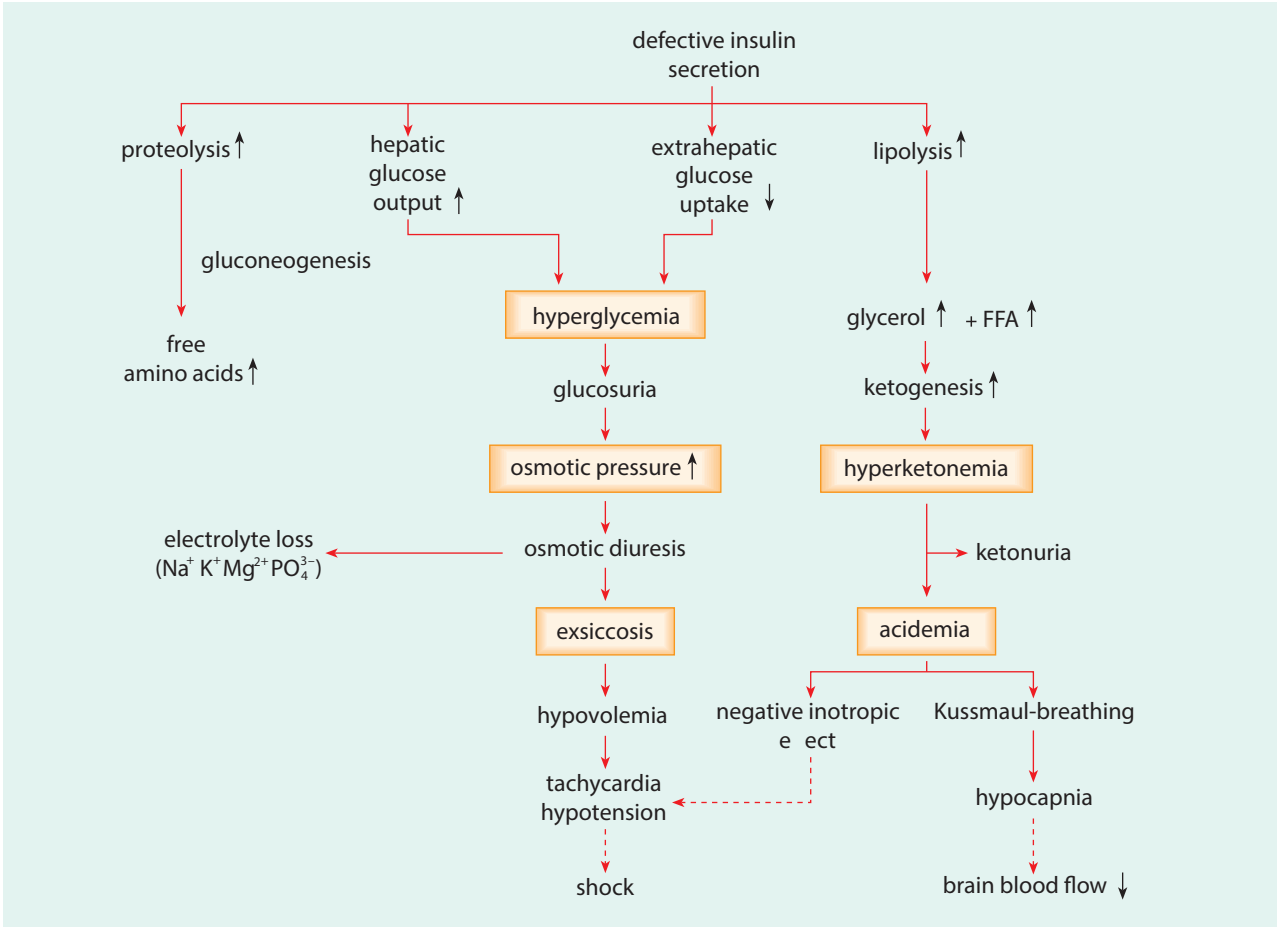

Fig. 9.9.: Development of basic symptoms of diabetes mellitus syndrome.

9.4. table

Comparison of characteristics of 1DM and 2DM

	1 DM	2 DM
% of all DM cases	10%	90%
Inheritance	HLA-associated	independent of HLA
Gene localization	chromodome 6	chromosome 11 (?)
Concordance	ca. 40%	ca. 80-90%
Evoking mechanism	frequently infection, toxin	infection is not characteristic
Time of appearance	before 25-y of age	after 30-y of age
Seasonality	peaks at autumn/winter	no seasonality
Geographical distribution	N>S	N<S
Beginning	fast	gradual
Body weight (initial)	low-normal	generally high
(later)	decreases	slight decrease, then it may increase
Symptoms	polyuria, polydypsia, polyphagia, weight loss, weakness	polyuria, polydypsia
Blood glucose level	very labile (depends on diet, growth)	stabile, controllable
Insulin need	always	rarely, but in this case a lot
Oral antidiabetic drugs	cannot be used	in most cases effective
Beta-cells	generally completely destroyed, se-insulin is missing or little	produce variable quantity/quality insulin, finally destroyed
Ketoacidosis	frequent	hardly any occasion (lactate!)
Hypoglycemia tendency	high	little
Insulin resistance	none or little	usually pronounced
Diet	plenty of energy, lot of complex carbohydrate, little fat	less energy, lot of complex CH, little fat (diet alone may be enough)

However, the severity of glucosuria is not a reliable index of the severity of the disease.

General classification of diabetes mellitus syndrome:

I. Type-1 primary DM (1DM, according to classification prior to 1997: I. type, insulin-dependent DM / IDDM/, juvenile DM)

A. Immune-mediated (5-10% of all DM)
(Its subtype in adults: LADA – latent autoimmune diabetes in adults)

B. Idiopathic (a minority of DM patients, mainly in patients of African or Asian origin)

II. Type-2 primary DM (2DM, 90–95% of all DM, according to classification prior to 1997: II. type, non-insulin-dependent DM /NIDDM/, maturity onset DM)

III. Other specific DM (*earlier "secondary" DM*):

- genetic (monogenic) β-cell defects (e.g. neonatal DM, mitochondrial DNS disorder, or MODY – maturity onset diabetes in young: e.g. the defect of chromosome 12, 7, 20, 13, 17, 2, with various subtypes)
- pancreatogenic DM (e.g. chronic pancreatitis and cystic fibrosis)
- endocrinopathies (Cushing's disease/syndrome, prolactinoma, hyperthyroidism, acromegaly and pheochromocytoma)
- medications, drugs (cortisol, β-adrenergic agonists, in the course of HIV/AIDS treatment, or following organ transplantation)
- some infections (e.g. cytomegalovirus)
- certain genetic syndromes (Down's, Turner, Prader-Willi, Laurence-Moon-Biedl, etc.)

IV. Gestational DM

Diabetes or pathological OGTT, which manifests itself among healthy women during early phases of pregnancy. It is supposedly related to the endocrine changes occur-

ring in pregnancy: it is thought to develop in women in whom the risk of 2DM is high. During pregnancy, an increased production of insulin antagonist hormones (e.g. progesterone, human placental lactogen, and prolactin) necessitates a very pronounced increase (up to 3-times the normal amount) of insulin secretion. In nearly 5% (3–14%, depending on the investigated population) of healthy pregnant women, at or about the 2nd-3rd trimester, gestational diabetes develops, which usually spontaneously disappears following delivery (ch. A13.1.3.). In additional pregnancies, it may again transiently re-appear in nearly 30-80% of the cases, and in 40% of cases, 2DM develops 10-15 years later. It is assumed that the temporarily significantly elevated insulin need "unveils" the hidden tendency for 2DM and causes symptoms. The GFR is normally elevated during pregnancy, and this increases the probability of glucosuria.

During pregnancy, the DM (irrespective whether it is gestational DM or it is a known primary or secondary diabetes pre-existing prior to the pregnancy) must always be treated by insulin in order to gain better glycemic control.

Primarily, two basically distinct subtypes of diabetes mellitus are typically distinguished. One of these begins characteristically at a young age, causes cachexia (despite polyphagia), and there is total/absolute insulin deficiency and sensitive to exogenous insulin. This form is described as type-1 DM (1DM). Its *main subtype (1A)* develops due to the fact in which the pancreatic β-cells are destroyed by T-cell mediated autoimmune mechanisms. In 85-90% of the patients, autoantibodies can be detected, and the disease exhibits a strong HLA-association. The speed of β-cell damage may be rather variable, in children and teenagers it is rapid, and among adults, it is slower. At the beginning of the disease, patients are characteristically lean or cachectic, although slight overweight does not exclude the possibility of this diagnosis. The disease may adjoin other autoimmune diseases, such as autoimmune thyroiditis, Addison disease, myasthenia gravis and/or pernicious anemia. In the *idiopathic subtype (1B),* there is also an absolute insulin deficiency, yet without any sign of autoimmune processes or HLA-association. The disease exhibits a strong hereditary character (although the precise mechanism, or the responsible genes are not known) in patients among African or Asian origin.

The other form begins later in life, primarily following 40-y of age, often in obese patients. The insulin production does not stop for a long time, it may even be elevated, yet it cannot exert its effects normally (insulin resistance). Understandably, the patient can scarcely react to the exogenous insulin. This form is defined as type-2 DM (2DM).

The differences of the two types are summarized in Table 9.4.

More recently, these forms are regarded as two endpoints of the spectrum of development of DM. In this sense, the often mentioned "double diabetes", which starts typically with insulin deficiency, however, in an obesogenic environment and in the presence of the appropriate genes, insulin resistance develops. In contrast, over time, the β-cells may be destroyed in 2DM, and then, and in addition to the insulin resistance, total/absolute insulin deficiency may develop.

9.2.2.2. BIOCHEMICAL CHANGES IN DIABETES

Due to the similarity associated with the biochemistry regarding starvation, the diabetes is often characterized as a form of intracellular starvation. Most changes can be explained by insulin deficiency, and several others due to an excess in glucagon (and insulin antagonist substances).

In complete insulin deficiency (1DM), the insulin-dependent (facultative glucose-consuming) cells cannot take up glucose, since the GLUT4 activity is missing. In the event in which glucose still enters somehow a given cell, the glucose metabolism is also suppressed, since the insulin dependent enzymes of the glycolysis and the TCA cycle are also inactive. The decreased glucose utilization is only one cause in the rise of blood glucose. The other is the inhibition of glycogenolysis by insulin, what is also defective, the cessation of inhibition by fructose-2,6-bisphosphate promotes the glucagon-stimulated gluconeogenesis (mainly from muscle protein origin), thus, the liver forwards more glucose into the circulation.

The insulin-dependent cells are "forced" to metabolize something else instead of glucose (cf. ch. 8.4.2.1.). This need of some other source of energy is supported by the fact that the intracellular action of insulin is also absent in fat cells: therefore the lipolysis increases, and so does the free fatty acid (FFA) level and, in fact, more albumin-bound non-esterified fatty acid (NEFA) is forwarded to the circulation. These can be metabolized by β-oxidation in the peripheral cells, and as a result, increased amounts of acetyl-CoA are produced, however, this cannot be utilized normally within the TCA cycle, due to the defective function of the insulin-dependent citrate-synthase enzyme. This need of some

other source of energy is supported by the fact that the intracellular action of insulin is also absent in fat cells: since the glycolysis is disordered due to insulin deficiency. The synthesis of fats similarly decreases, thus, from the excessive amount of acetyl-CoA, ketone bodies (aceto-acetic acid, beta-hydroxy-butyrate, acetone) are produced in the liver. In contrast to starvation, now the brain (or other tissues) does not utilize the ketone bodies, since the brain cells are able to take up glucose with the aid of GLUT3 and they do not need anything else. Consequently, the ketone bodies accumulate, they induce ketoacidosis, and are partly excreted through the urine.

The cellular uptake of amino acids and the protein synthesis decreases, while an increased amount of proteins are decomposed. The amino acids (particularly the glucoplastic Ala) are used for gluconeogenesis or they are directly metabolized.

The obligatorily glucose-utilizing tissues (e.g. brain, retina, kidney and red blood cells) are able to take up glucose without insulin and GLUT4 (in fact, due to hyperglycemia, they take up too much), however, the intracellular metabolism of the immense quantity of glucose is often pathologically modified (e.g. polyol pathway and sorbitol production).

Somewhat different changes are seen if insulin is available, but it cannot exert all of its possible effects (2DM). Various insulin effects (Fig. 9.6., Table 9.2.) will be altered unevenly, with dissimilar severity: the lack of membrane transport is pronounced, while the intracellular insulin-effects are maintained, at least partially. It is nearly impossible for glucose to enter the insulin-dependent cells by specific (GLUT4 mediated) way, yet in consideration of the extreme hyperglycemia, some may enter by other means, and this can be used in the cell. There is no need for the enhanced utilization of fats, and the possibility in support of this is also considerably limited, since the inhibition of hormone-sensitive lipase is nearly maintained. In fat cells, the fat synthesis may be significantly increased (the insulin can exert this effect), and the body weight typically increases. No ketoacidosis is expected. The protein catabolism is strongly increased, further enhancing the gluconeogenesis and the hyperglycemia, all of which are very pronounced. The non-insulin-dependent cells (that utilize glucose in an obligatory manner) take up so much glucose that they are no longer able to normally metabolize it, thus, sorbitol is produced intracellularly.

9.2.2.3. PATHOMECHANISM OF THE DEVELOPMENT OF DIABETES

The etiology and pathomechanism are dissimilar in the two types of diabetes. Hereditary and acquired/environmental factors play various roles in both types, however, these roles differ from one another.

Etiology and pathomechanism of 1DM

The destruction of pancreatic β-cells and the consequential complete lack of insulin presents the fundamental problem, irrespective of the cause of the destruction. Other endocrine functions of the pancreas (glucagon and somatostatin) are usually maintained. The hereditary factors are not as important as when compared with 2DM: the concordance between brothers/sisters is 5%, and, even among homozygotic twins, it is only 40-50%. This suggests that the environmental/acquired factors have greater importance. In special cases, such factors alone /e.g. total pancreatectomy due to tumor, pancreatitis/ may be sufficient to explain the complete lack of pancreatic β-cells, and the situation corresponding to 1DM. The genetic propensity for autoimmunity appears to be still fundamental.

Steps of pathogenesis of 1DM:

- genetic susceptibility
- exogenous effect (e.g. infection, toxic effect)
- insulitis, "self" antigens turning to "non-self" and development of autoimmunity
- autoimmune damage of pancreatic β-cells
- manifestation of the disease

In the presence of certain genetically determined HLA-antigens (DR3, DR4, DQw8), 1DM occurs more frequently, in other cases (DR2, DQw7) rather rarely. These HLA antigens are responsible for the formation and surface expression of MHC II type antigen structures. Certain MHC II structures can easily be modified by external damaging environmental factors, and following modification the body reacts to the neoantigens (converted from "self" antigens by the damaging external factors) as if it was "foreign", i.e. autoimmunity develops. With regard to the autoimmune reaction, both the cellular immune processes (cytotoxic lymphocytes, cytokines of macrophage origin: e.g. IL-1, IL-2, TNF), as well as the humoral immune response (upon the effect of β-cell-bound antibodies, the complement system is activated and the consequent reaction causes inflammatory tissue damage of immune origin) participate.

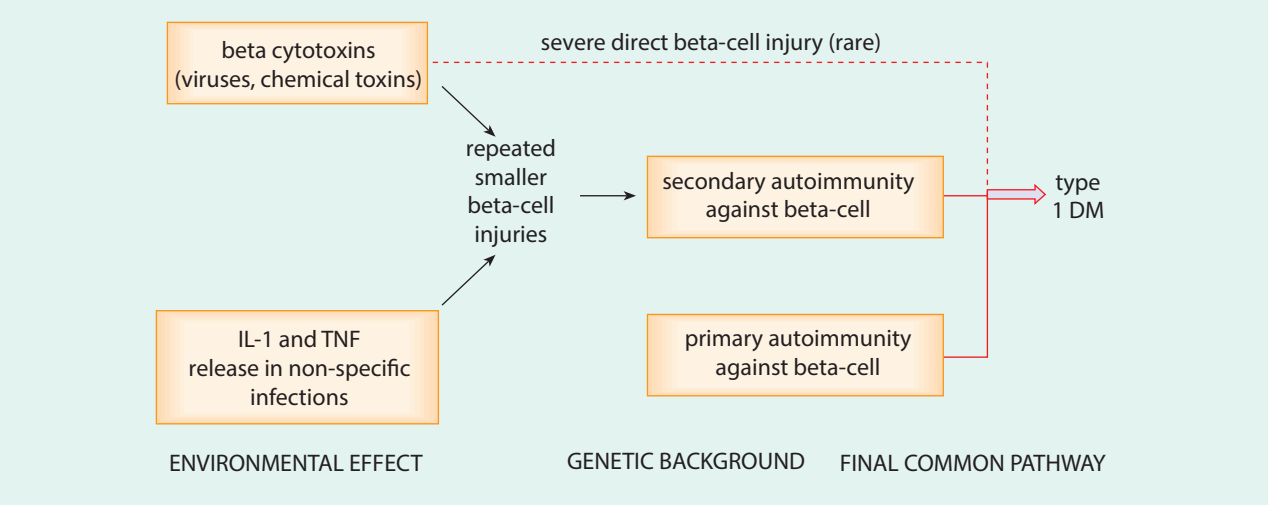

Fig. 9.10.: Mechanism of development of 1DM.

The external damaging factors injure or destroy the β-cells, either by a *direct* action or by the effect of a not necessarily specific *insulitis* (Fig. 9.10.). Becoming a neoantigen is characteristic for these cells, and the autoimmunity develops against them. As an autoimmune disease, 1DM exhibits familiar concordance with other autoimmune diseases, e.g. Addison's disease, Graves' disease and pernicious anemia, and not necessarily with diabetes itself (this explains that "only" 40% is the concordance of 1DM among homozygotic twins).

In consideration of the environmental factors, the geographical distribution of the disease (more frequent among the population of the Northern countries) and its seasonal character (peaks during autumn and winter) suggest a possible role for viral infections in the development/appearance of the disease. Parotitis, Coxsackie, rubella and cytomegalo-viruses appear to be the most important among the possible infections. With regard to viral infections, on the surface of β-cells viral antigens are expressed, and this results in an inflammatory process and lymphocytic infiltration. If the MHC structure is suitable, the distortion of self-antigens initiate an autoimmune process. Indeed, after many of such viral infections, various antibodies may be demonstrated in the circulation (well before the manifestation of the disease; Fig. 9.11.), like ICA (= islet cell cytoplasmic antibody), ICSA (= islet cell surface antibody), GADA (= glutamic acid decarboxylase autoantibody – a homologous protein of this enzyme is expressed upon the surface of β-cells, and likely, its similarity to Coxsackie viral protein evokes the antibody production). The enzyme is also present in neurons, where it is responsible for GABA production, and this is why it is assumed to have a role in the development of diabetic neuropathy, Sometimes, IAA (insulin autoantibody) may also be detected. In the course of further progression, the amount of antibodies decreases or they completely disappear (the cells serving as antigens also disappear). At the beginning of the disease, cellular immunity is also activated, however, this also disappears with the progression of the disease.

The (cellular and humoral) autoimmune inflammatory damage of β-cells induces manifest clinical symptoms (or prior to this, the abnormality of OGTT), when nearly 90% of β-cells are destroyed (the specific antibodies may be demonstrated earlier). After this, however, the clinical symptoms of 1DM rapidly develop, particularly if the insulin production suddenly decreases (e.g. due to an acute infection) and the contrainsular factors are activated. Such acute manifestations of diabetes (following the acute infection) are often followed by a transient improvement ("honeymoon period") due to normalization of contrainsular factors, however, at the end of the process, the β-cells completely disappear, soon even C-peptide cannot be demonstrated (= no endogenous insulin is present). The full process (including the latent period) may take months or years (Fig. 9.11.).

Similar damaging and/or an autoimmunity-inducing role may be ascribed to toxins which injure mainly the pancreas (alloxan, streptozotocin, nitrosamines, cyanides /e.g. consumption of tapioca, horse-bean/), particularly in protein deficient states, when the detoxication is defective.

Damage of β-cells may develop in other ways, too. For example, in Coxsackie infection, a role for *molecular mimicri* is assumed: the protein of the pathogen

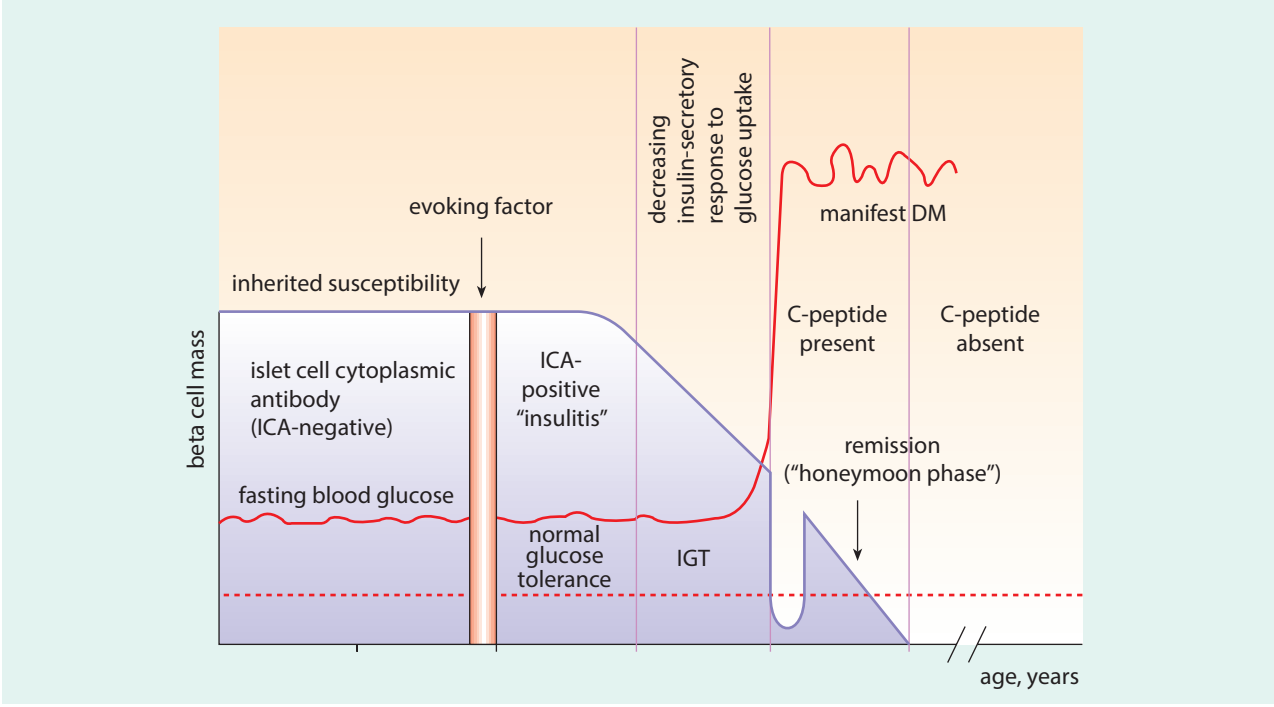

Fig. 9.11.: Changes of β-cell mass and fasting blood glucose in the process of 1DM development. The genetic susceptibility causes no changes as yet. After presentation of an evoking factor various antibodies appear against the pancreatic cells, the β-cell mass starts decreasing, but no disorders of glucose metabolism can be demonstrated (even OGTT is normal). Later the glucose-induced insulin secretion decreases and the OGTT is abnormal (IGT), but the fasting glucose level is still normal. At manifestation of the disease the insulin secretion suddenly decreases below the minimally needed amount (ca. 10% of normal, designated by an interrupted line in the Fig), by now the fasting glucose level is also high, although some insulin production can still be detected (there is C-peptide). In this case sometimes transient improvement can be observed ("honeymoon"), but finally the insulin production is completely finished (there is no C-peptide) and the fasting glucose is very high.

(against which antibodies are produced) resembles the surface ("self") antigen of Langerhans islet cells, therefore, the antibody binds also to these cells, and these "signalized" cells will be destroyed by killer T-lymphocytes. A similar role has been described in association with the proteins of cow-milk: if, at an early age (at the age when not only amino acids, but also proteins can be absorbed) infants are fed with such milk, antibodies may be produced against the milk-proteins, which later may give a cross-reaction with the islet cells.

Only the β-cells are injured, not the glucagon-producing α-cells, the somatostatin producing δ-cells or the pancreatic polypeptide-producing PP-cells. It is characteristic for early diabetes that the glucagon level does not decrease proportionally in response to the elevation of blood glucose and does not increase in hypoglycemia, this phenomenon apparently contradicts the original idea and suggests that, in this phase, the α-cells are also affected. However, this does not last long, and inevitably, only the β-cells are injured.

Etiology and Pathogenesis of 2DM

The fundamental problem characteristic of 2DM is not the lack of insulin (the insulin level is usually higher than normal), it is the fact according to which the insulin cannot exert its effects, at least not all of them, i.e. insulin resistance develops. In other cases, sufficient amount of insulin to block a further rise in blood glucose and establish a new balance can be observed only at actual blood glucose levels much exceeding the normal.

In the etiology of 2DM, the role of **hereditary factors** has been long well-known, although its complex background has yet not been fully clarified. It is highly likely that several hereditary mechanisms may participate. Frequently, the multigenerational accumulation points to hereditary factors, as seen in homozygotic twins, in whom the concordance is 90%, or in brothers/sisters in whom it is 20-40% (much higher than in 1DM). In certain populations (mainly in those near the Equator), the occurrence is higher, and in obese Pima Indians, as much as one third of the population beyond age 5-y was found to have 2DM. In Hungary 8-10% of the total population has diabetes (mainly 2DM), however, the ratio is increasing.

Proposed gene defects and mutations:

- insulin gene (chromosome 11 and abnormal insulin-forms)

- glucokinase gene (chromosome 7, too little glucokinase enzyme in the pancreas, the glucose can increase insulin secretion only at high blood glucose levels. It is likely to be responsible for some forms of diabetes, designated as MODY; see later)
- amylin gene (chromosome 12, islet amyloid polypeptide = IAPP, the peptide is secreted together with insulin, and slows gastric emptying, glucose absorption, enhances the effects of insulin and the leptin effects to enhance insulin sensitivity, yet the deposited amylin damages the pancreas and promotes the β-cell apoptosis).
- GLUT genes (chromosome 17, due to decreased GLUT 2 the pancreas is less able for glucose uptake and produces disproportionately less insulin as compared with the blood glucose level. Due to more GLUT 4 among the fat cells, the glucose uptake increases, and, in contrast, there is less GLUT 4 among the muscle cells).
- insulin-receptor gene (chromosome 19, diminished receptor synthesis, decreased transport/binding to the insulin receptors, decreased internalization and recirculation and disordered signaling within the cell)
- hepatocyte nuclear factor (HNF) gene (chromosome 20, factor of gene-expression regulation, its genetic disorder stands e.g. in the background of MODY 1).
- calpain 10 gene (the cysteine-protease contributes to the arrangement of cytoskeleton, it may play a role in the translocation of GLUT4, and also in the insulin secretion of β-cells /exocytosis/).
- the genetic background of the multimetabolic X-syndrome is not clarified, however, its fundamental feature is insulin resistance.

Out of the **environmental/acquired** factors, *obesity* exhibits primary importance (Fig. 9.12.), since it causes insulin resistance (high level of several contrainsular hormones and factors, while the number and density of insulin-receptors decrease). Equally relevant is the role regarding *feeding habits* (increased levels of carbohydrates and frequent hyperglycemia: the "glucotoxicity" leads to the rapid exhaustion of β-cells). Similar is the effect regarding *physical inactivity*, the advanced *age*, the contrainsular *endocrine effects* (e.g. stress-hormones, growth hormone and thyroxine), and disorders associated with *fetal development,* in which the 2DM is more frequent among individuals, who were stunted as neonates – likely due that fetal starvation decreases the total number of β-cells. On the other hand, many of the overweight neonates of diabetic mothers become later diabetic.

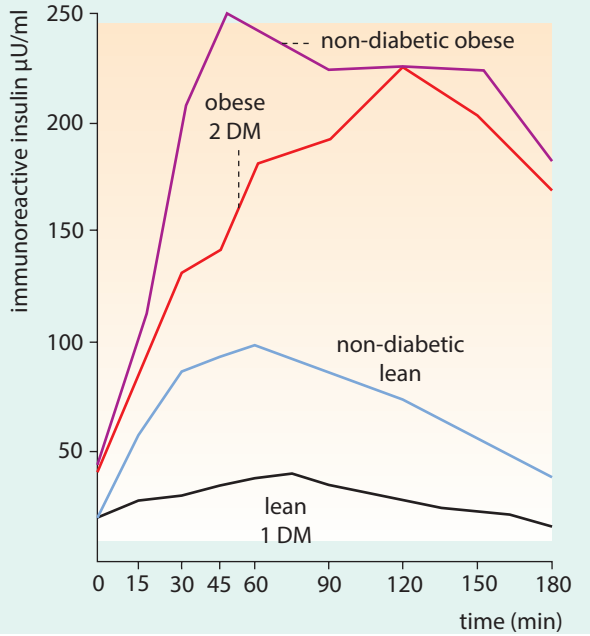

Fig. 9.12.: The insulin-secretion response to i.v. glucose load in lean 1DM patient is minimal, while in obese non-diabetic patients several times more than normal, in obese 2DM patients it is still higher than the normal value, although smaller than in the non-diabetic obese. (A first phase of a normal response would take place in the first 15 min, which cannot be shown here). Later on, in 2DM patients the insulin secretion may decrease, even below the normal levels, but the peripheral insulin resistance persists.

Insulin resistance caused by disorder of the target cells: Genetic origin: genetic modifications of the peroxisome proliferator-activated receptor γ* (PPAR-γ), which have a fundamental influence upon insulin sensitivity, alterations of insulin receptor substrates (IRS), which may modify the intracellular signal transduction of insulin (Fig. 9.13.).

*The PPAR is a member of a nuclear receptor family and is similar to thyroid and steroid hormone receptors. It forms a heterodimer with the RXRa receptor of retinoic acid (cf. mechanism of A-vitamin action, ch. A6.2.3.1.), and in this form it is connected to the peroxisome proliferator response element of special genes, and modifies the gene expression. Some subtypes (alpha, beta, gamma and delta) of these receptors are present in all tissues. PPARγ receptors can be found in fat tissue (they aid in the differentiation of fat cells, but decrease the TNFα production of the adipocytes), and they are present in the muscle tissue, the liver, and also in epithelial cells. As endogenous ligands of the receptors of thus far identified fatty acids, flavonoids from food, and certain prostaglandins (e.g. PGJ_2), and some pharmacological substances increased their activity, e.g. substances which enhance the insulin sensitivity, compounds of the thiazolidinedione (TZD) group (troglitazone, rosiglitazone), which are used in the therapy of 2DM. Inhibition of the angiotensin system (ACE inhibitors, angiotensin receptor blocking drugs) also enhance the PPARγ receptor activity.

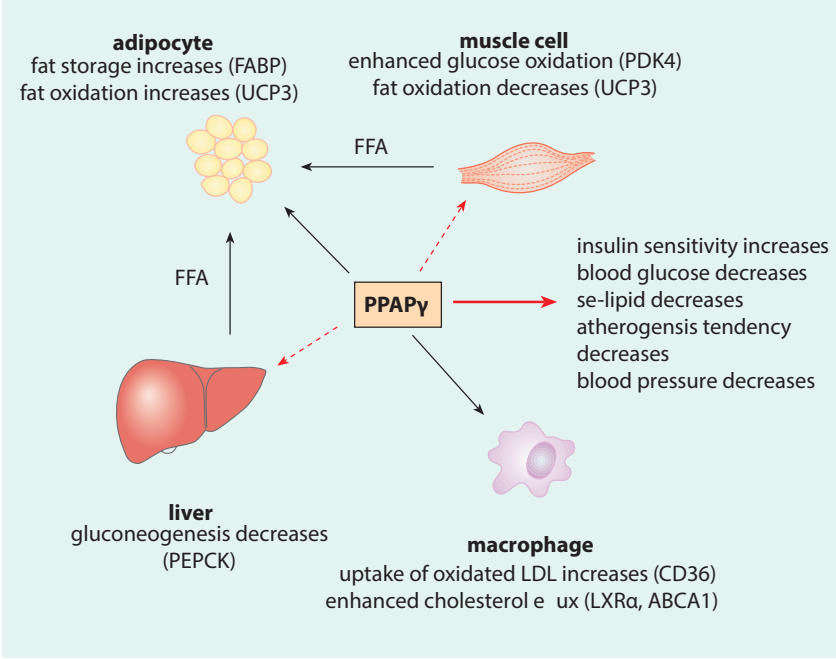

Fig. 9.13.: Effects of peroxisome proliferator activated receptor γ (PPARγ). FABP: fatty acid binding protein, UCP3: uncoupling protein 3, PDK4: pyruvate-dehydrogenase-kinase 4, PEPCK: phosphoenolpyruvate-carboxy-kinase, LXRα: liver X receptor, CD36: transmembrane glycoprotein (this belongs to B type of scavenger receptors), ABCA1: ATP-binding cassette transporter A1 protein, or gene.

Pathological: stress, sepsis, fever, trauma (alterations of cell metabolism, effect of stress-hormones and inflammatory mediators), malnutrition, starvation, uremia, cirrhosis, ketoacidosis (histotoxic hypoxia and metabolic disorder of the tissues), obesity. The adipocytes produce resistin (it decreases the insulin effect), and other proinflammatory cytokines (TNF, IL-1, 6) of similar effect, the number and density of insulin receptors decrease on the surface of the cells, the fat tissue also activates contrainsular hormones (e.g. from weak androgens of the adrenal cortex more estrogen is produced, and from cortisone cortisol is activated that also, elevates blood glucose). Although adiponectin enhances the insulin action, it cannot counterbalance the previous effects.

Physiological: puberty, pregnancy (endocrine changes), old age (the responsiveness of the pancreas decreases, together with the peripheral insulin-sensitivity).

Hormonal/humoral factors: excess of glucocorticoid or growth hormone, overproduction of glucagon, hyperthyroidism, catecholamine overproduction, amylin, hyperinsulinemia (due e.g. to insulinoma, or to frequent consumption of sweets and sweetened soft-drinks, the number of insulin receptors decreases = "down-regulation"), hyperglycemia and hyperlipemia.

Insulin autoantibodies (IAA) may bind the insulin, thus, they may also inhibit the binding of insulin to its receptor.

Upon the basis of environmental/acquired factors of 2DM, insulin resistance first develops with hyperinsulinism. The high insulin level at the beginning is able to secure the normal fasting blood glucose, yet the OGTT is already abnormal: although the insulin increases to a higher level than normally, however, the extent and speed of the increase are not sufficient to maintain a normal OGTT. With special analyses, the decreased glucose utilization of peripheral cells can already be detected at this stage. Later on, the fasting glucose level also rises, yet the sustained higher level of insulin is not sufficient for maintaining even the normal level of blood glucose. In the next stage, the β-cells become exhausted, and the so far high insulin secretion starts declining (Fig. 9.15.). The peripheral insulin resistance persists without change – extremely high insulin dose is

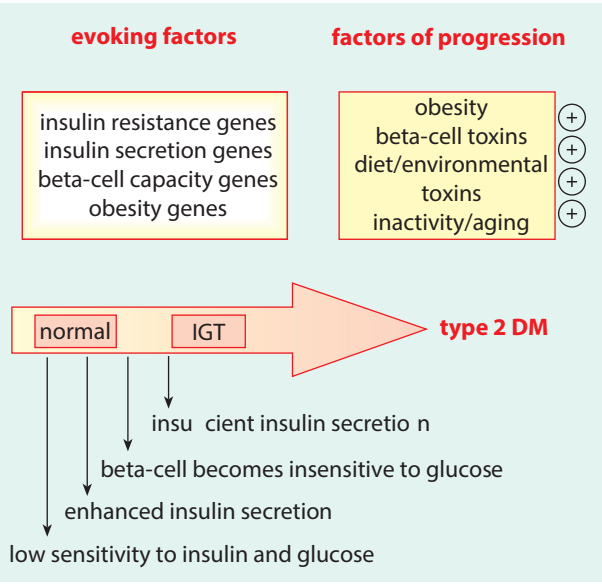

Fig. 9.14.: Factors playing a role in the initiation and progression of 2DM.

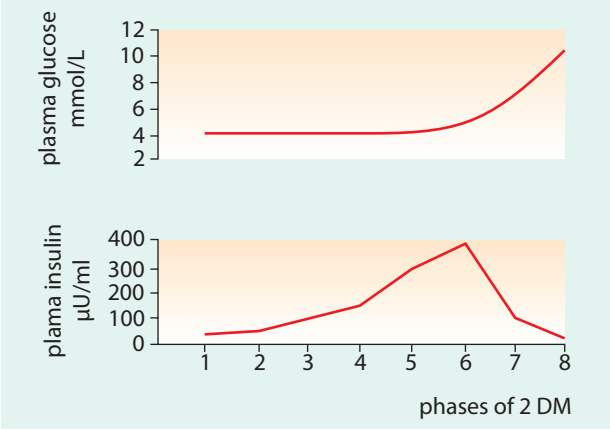

Fig. 9.15.: Values of se-insulin and fasting glucose in the course of development of 2DM. Blood glucose remains normal with ever-increasing insulin levels, when it cannot increase any more, glucose levels start increasing. IGT develops earlier. The rise of glucose level speeds up at the exhaustion of β-cells and gradual fall of insulin secretion.

needed for the therapy. PPARγ agonists increase the insulin sensitivity, their preventive administration during IGT may delay the manifestation of 2DM, yet due to the enhanced insulin action, it may progressively increase obesity.

SPECIAL FORMS OF DIABETES

MODY (maturity onset diabetes of the young, in which these rare genetic forms are responsible for 1-2% of all DM).

MODY starts at a young age and corresponds to 2DM: despite the presence of insulin, hyperglycemia develops. It is accompanied by obesity, severe hyperglycemia (without ketoacidosis), and their early/late consequences. There is no serious hyperinsulinism, nor insulin resistance. In some genetic disorders, various subtypes of MODY were observed.

MODY 1 – HNF-4α defect (the protein known as hepatocyte nuclear factor-4α, or transcription factor 14, participates in the early development of pancreas and, among others, in the expression of insulin or GLUT2 gene).

MODY 2 – glucokinase defect (this enzyme plays a role in the glucose utilization of β-cells, thus, it is needed for the initiation of insulin secretion. Although these patients can produce insulin, they begin insulin secretion only at very high levels /7-8 mmol/l/ of blood glucose. The fasting glucose level is high, however, the OGTT is less pathological than expected).

MODY 3 – HNF-1α defect (this factor is a part of the system mentioned at MODY 1).

MODY 4 – insulin promoter factor defect (this transcription factor is necessary for adequate gene expression of insulin, GLUT2, glucokinase).

MODY 5 – HNF-1β defect (this factor is a part of the system mentioned at MODY 1 and 3).

MODY 6 – neuro-D1 defect (appropriate function of the neurogenic differentiation 1 is necessary for the transcription of insulin gene, and for the development of β-cells, and certain neural cells).

MODY 7 – mitochondrial DNS damage (a very rare form, and it may appear as Wolfram syndrome /diabetes insipidus, diabetes mellitus, optic nerve atrophy, deafness/, and as thiamine-responsive megaloblastic anemia syndrome, rarely as maternally inherited combination of deafness and diabetes).

MODY 8 – proinsulin defect.

MODY 9 – PAX4 defect (this transcription factor damage affects the development of pancreas islet cells, and differentiation of β-cells. Less than 5 families are affected worldwide.

MODY 10 – CEL defect (defective expression of carboxyl ester lipase) leads to the lack of glycoprotein, which normally is secreted into the gastrointestinal system. It may participate in the hydrolysis of cholesterol and fat-soluble vitamin-esters. Less than 5 families are affected worldwide regarding this aspect of DM in adults. In addition to the obvious exocrine insufficiency, the mechanism of damage of endocrine function is unknown.

GESTATIONAL DIABETES (please see classification of DM, ch. 9.2.2.1.)

MALNUTRITIONAL DIABETES

Long-lasting, severe protein-malnutrition and consumption of toxic substances (e.g. cyanate-containing tapioca, horse-bean) leads to fibrotic or calcification disorder of the pancreas and insufficiency regarding the endocrine/exocrine pancreas. Although its characteristics correspond rather to 1DM, there is no complete insulin deficiency, nor a tendency for ketoacidosis.

STEROID DIABETES

In the event of corticosteroid overproduction, or more frequently during steroid treatment, the hormones increase gluconeogenesis, and through its permissive effect upon other hormones, also the glycogenolysis, thus, the blood glucose level increases. Enhanced lipolysis, ketosis may develop, although otherwise the clinical picture corresponds rather to 2DM. The diabetes remains after cessation of the corticosteroid excess.

HYPOPHYSEAL DIABETES

Hypophyseal diabetes is based on the insulin-antagonist activity of GH, in which the β-cells become exhausted due to the lasting overload. The relatively high incidence of 1DM in adolescence may be explained by the high GH level (the precedent

latent diabetes is manifested upon the acute effect of GH rise/resembling to acute infection). In acromegaly, the diabetes is quite common, and the clinical picture corresponds rather to 2DM. The overproduction of ACTH in the pituitary gland may also contribute to the development of diabetes. The diabetes is sustained after cessation of the hormonal excess (metahypophyseal diabetes).

MULTIMETABOLIC X-SYNDROME (Reaven-Modan syndrome)

The metabolic X syndrome (multimetabolic syndrome) was described in 1988, its basic characteristics are the insulin resistance and defective glucose-tolerance, and in severe forms it may lead to 2DM. Other features include the following: 1. Visceral obesity (BMI >30, the waist/hip ratio in men > 0,90, in women > 0,85); 2. Hypertension (> 140/90 mmHg, or more recently >130/85 mmHg); 3. Dyslipidemia (se-triglyceride > 1,7 mmol/l, and/or low HDL-cholesterol < 1,1 mmol/l in men, < 1,2 mmol/l in women (according to WHO criteria), and other parameters may also be abnormal, e.g. total cholesterol > 5,0 mmol/l, LDL-cholesterol > 3,5 mmol/l); 4. Microalbuminuria (>20 μg/min or the albumin-creatinine ratio is 30 mg/g). Other abnormalities may also be present in metabolic X syndrome, else (e.g. hyperuricemia/gout, cholelithiasis, coagulation abnormalities, elevated PAI-1-level). In some developed countries, ca. 25% of the population suffers from this syndrome. In the USA, among individuals above 50-y of age, the prevalence is 40%, in Hungary the prevalence is nearly the same.

DIABETES ADJOINING PANCREAS INJURY

Trauma of the pancreas, its surgical removal, tumor, inflammation (chronic pancreatitis, cystic fibrosis), or hemochromatosis ("bronze diabetes") naturally also results in diabetes, mainly similar to 1DM.

9.2.2.4. CONSEQUENCES OF DIABETES

Due to its consequences, diabetes inevitably shortens the expected lifespan. Prior to the availability of effective insulin treatment, the expected survival rate among young diabetic patients was 1.5-2.5 years, and it was only slightly better when diabetes was diagnosed among older individuals. Most patients, particularly the young ones, succumbed due to diabetic coma. Although the diabetic coma is still an acutely life-threatening state, life is more frequently endangered due to the functional disorders induced by chronic consequences. Today, with an early diagnosis, the appropriate treatment and strict glycemic control, the expected lifespan is incomparably longer, yet still shorter than when compared with non-diabetic individuals.

9.2.2.4.1. ACUTE DIABETIC COMPLICATIONS

Diabetic ketoacidosis - DKA (ketoacidotic coma)

It is a characteristic acute consequence of the metabolic disorder in 1DM (Fig. 9.10.). It is most common among young, insulin-treated patients during their inflammatory diseases, following a surgical or accidental trauma. In the event of an enhanced secretion of contrainsular hormones (adrenaline, cortisol), or inflammatory cytokines (TNF-α, IL-1, 6) the usual insulin dose is insufficient, therefore, this must be considered prior to elective operations! The gradually worsening disorder of consciousness turns into a coma within a few days, in which the patient does not react even to painful stimuli; ch. A.11.). During the development of the coma, the patient experiences a strangeness regarding the respiration, referred to as Kussmaul breathing, and its unusual "aromatic" odor is reminiscent of acetone, while the mental disorder and aggressive behavior prior to the coma resembles the symptoms of acute alcohol intoxication.

Explanation of the disorders of the nervous system:

The **osmotic diuresis** of hyperglycemic origin leads to exsiccosis, hypovolemia and to circulatory disorders (with hypotension) characteristic regarding circulatory shock. The brain circulation primarily is not affected (vasodilation due to autoregulation), however, there is also ketoacidosis, which is compensated by Kussmaul breathing, which leads to hypocapnia and causes vasoconstriction in the brain. Thus, the cerebral circulation may certainly decrease, and the oxygen supply of the brain may be insufficient.

The **cerebral ischemia** causes enhanced release of the excitatory neurotransmitter glutamate (ch. 2.5.2.2.). The oxygen need of the brain increases, due to the consequent hypoxia, the intracellular Na and Ca levels increase, however, the myoinositol uptake and the Na^+/K^+ ATP-ase activity decreases.

The neuronal **intracellular hyperosmolarity** induced by hyperglycemia (28-30 mmol/l) also suppresses brain metabolism, since it causes non-specific enzyme inhibition. In the background of a high osmotic gap (ch. 4.1.1.) the accumulation of ketone bodies can be demonstrated. In a hyperosmolar envi-

ronment, the tertiary structure of proteins is altered, causing functional damage (non-specific enzyme inhibition). In severe cases, the proteins are precipitated. Since glucose enters the brain cells (even in excess, since the GLUT3 is a non-insulin-dependent transporter), the cell volume does not decrease, but the IC osmotic pressure is high. The neural cells are able to produce idiogenic osmoles (of unknown/variable origin) to counteract hypertonicity (e.g. taurine, glycine, inositol, glutamine, however, the sorbitol also belongs here). The cell volume may still increase, due to the high intracellular Na^+ and water, and as a result of abnormal cellular metabolism (and it certainly increases in the event of too fast rehydration therapy, which causes brain edema).

The utilization in excessive amounts of IC glucose cannot be carried out by the normal way, a part of it is transformed to sorbitol and fructose with the aid of aldose-reductase (**polyol pathway**). In the course of this, NADH is produced in excess, while the NAD level decreases, as in hypoxia. This is the **hyperglycemic pseudohypoxia**, which inhibits the cellular metabolism and ATP formation, similarly as seen in hypoxia.

Due to the disordered cellular metabolism, K^+ is lost from cells, replacing the EC K^+ loss by polyuria and secondary hyperaldosteronism, and the se-K^+ may be elevated, even above the normal level (due to the renal K^+-loss the total potassium amount of the body may be low, and following the initiation of insulin treatment /and cellular re-uptake of K/, may result in **hypokalemia**). This hypokalemia is acutely life-threatening due to its cardiac consequences.

The **diabetic ketoacidosis (DKA)**, through inhibiting the IC metabolic enzymes (the IC buffering causes non-specific inhibition), also causes a disorder of brain cell function, similarly to the rise in IC osmotic pressure. The severe metabolic acidosis (pH may decrease to 6.9), and results in decreased contractility of the myocardium, are further worsening the brain circulation/oxygenation. The mortality rate of DKA is at or about 5%.

PATHOPHYSIOLOGY OF THE THERAPY

- Infusion of insulin, due to the rapid decrease in blood glucose may induce cerebral edema with a high mortality rate (up to 20%). Its clinical sign (Cushing reflex) is the disproportionate rise in blood pressure and bradycardia ($\leq$ 50/min). Additional clinical symptoms of brain edema include irregular respiratory patterns, incontinence, eventually abnormal body positions (e.g. decerebrate rigidity: head rigidity, shoulders adducted, arms pronated in extension, plantar flexion and hands flexed, or the decorticate rigidity: shoulders adducted, arms in flexion, legs in pronated extension, plantar flexion and hands flexed). In the event of brain edema mannitol therapy or, if not successful, an additional slow hypertonic (3%, 5-10 mg/kg) NaCl-solution may be necessary.
- In addition to electrolyte replacement (saline), the continuous monitoring of se-K^+ is necessary. In this coma, the K^+ content of the body is low. The chronic osmotic diuresis and adjoining secondary hyperaldosteronism leads to pronounced K^+ loss, and eventual vomiting or diarrhea makes it more severe. In contrast, the se-K^+ may be high partly due to the lack of insulin, partly to the concurrent acidosis (both cause K^+-outflow from cells and elevation of EC K^+ level).
- Treating the acidosis and administration of insulin itself lead to K^+ movement into the cells, causing a potentially lethal EC hypokalemia.
- Bicarbonate administration is advised only in the case of a very low pH (below 7.0) (risk of alkalosis, and this shifts the Hb oxygen saturation curve to the left, hypoxia develops, and due to alkalosis the brain perfusion may decrease).

Ketone-body analysis (*acetone plus acetoacetic acid-AcAc*) may overestimate the real ketoacidosis during treatment; since, prior to treatment, the AcAc/Hb (β-hydroxy-butyrate = Hb) redox system is shifted to the latter one, while once the pH improves, it is shifted toward AcAc; indicating a mistakenly high ketosis in the course of treatment.

Hyperglycemic hyperosmolar syndrome – HHS (non-ketoacidotic) coma

This is a complication characteristic regarding 2DM, occurring mainly among the elderly, often decrepit and infirm patients. Typically, the patient vomits and/or suffers from diarrhea, sweating, unable to effectively replace the loss in salt and water, and this ignites the HHS development. This coma develops more slowly than DKA, it may take several days or up to a week. The development of ketoacidosis is not expected, and the mechanism of coma partly differs from the coma seen in 1DM.

The development is so slow that relatives only note the deterioration of consciousness and believe it as a sign of senility, thereby delaying the appropriate medical intervention. The dryness of the skin and mucosa (tongue!) may be noticed, and then hyperosmolarity and pronounced exsiccosis may be suspected.

The **osmotic diuresis** is more pronounced than when compared to that in DKA, and the exsiccosis is far more severe (up to 10 liters of loss from the EC+IC volumes). Due to the severity of the exsiccosis, the disorder of the circulation is also more pronounced; this may affect also the brain perfusion, particularly since the entire process is often seen in elderly, atherosclerotic patients with a limited capacity of the brain vessels to compensate hypovolemia and hypotension with vasodilation.

Cerebral ischemia (and enhanced Glu activity) similarly to DKA.

There is no ketoacidosis, yet the disorder of tissue metabolism (hypovolemic tissue ischemia, hyperglycemic pseudohypoxia, etc.) may result in **lactic acidosis.** Although this is less severe than ketoacidosis, it may strongly influence the *intra*cellular pH. Lactic acidosis typically predicts a very poor prognosis, and the mortality rate may reach 50%.

The hyperglycemia is more pronounced (55-60 mmol/l, i.e. 10-15-times higher than the normal fasting glucose level). Very pronounced **hyperosmolarity** may develop, the enzyme-inhibiting effect of hyperosmolarity is far more severe, when compared to that seen in the DKA type coma. The pseudohypoxia and the inhibition of Na^+/K^+-ATP-ase is also increasingly, far more severe.

The **K^+-metabolism** is disordered much like that as seen in a DKA coma: high se-K at the beginning, turning into low se-K during treatment.

The polydipsia is not sufficient regarding the compensation of polyuria.

Seizures accompany the gradually deepening coma, which is characteristic, and without hyperventilation.

High overall mortality rate (>15 %).

PATHOPHYSIOLOGY OF THE TREATMENT

Careful insulin infusion (due to insulin resistance, a very high dose of insulin is required), and very slow fluid replacement (risk of brain edema, in its presence the mortality rate may reach 50%). The K-administration is very important, however, it should be achieved with continuous monitoring of its level. Bicarbonate administration is usually not needed.

Hypoglycemia

It is a frequent complication of not so much the diabetes itself, but its treatment (it more often develops in 1DM, but may also occur in 2DM). It can be regarded as a diabetic complication, which occurs more often in diabetic patients. Its characteristics and forms will be discussed in the following chapters.

9.2.2.4.2. LATE DIABETIC COMPLICATIONS

Non-specific changes

Essential hypertension is frequent, particularly in 2DM. Factors of pathogenesis include the following:

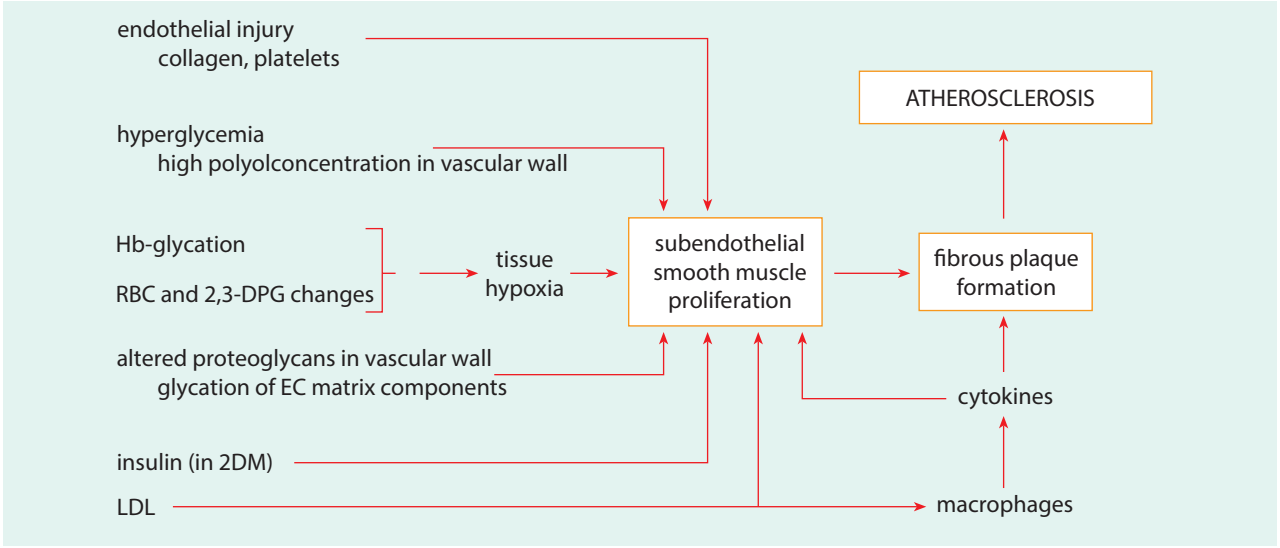

Fig. 9.16.: Factors of atherosclerosis development in diabetes.

1. Hyperinsulinism leads to enhanced renal salt- and water-reabsorption and to increased levels of ECV. It also elevates the Ca-content of vascular smooth muscle cells, thereby they become sensitized to sympathetic influences. Insulin, as a growth factor, elicits the hypertrophy of vascular smooth muscle. These mechanisms are particularly important regarding the hypertension accompanying 2DM. (In obese leptin-resistant 2DM patients, the high leptin level contributes to the increase in blood pressure through enhancing the sympathetic tone, and by pathological microvascular changes. The cortisol activated by the abnormal adipocytes, and the angiotensinogen produced by such adipocytes leads to an additional rise in blood pressure).
2. Atherosclerotic plaques develop (Fig. 9.16.) in a generalized way (this, in itself, may cause hypertension) and in the renal vessels (narrowing of the renal artery leads first to the activation of the RAAS, and the diabetic nephropathy causes depressor deficiency). This may be an important mechanism regarding hypertension of both 1DM and 2DM patients.

Macroangiopathies (progressive atherosclerosis, vascular stenosis, acute myocardial infarction, stroke, ischemic gangrene of the lower extremities). Factors of pathogenesis include the following:

1. Hyperlipoproteinemia: Due to the increased activity of hormone sensitive lipase, the free fatty acid supply (NEFA) increases, thus the VLDL synthesis increases in the liver (type IV hyperlipoproteinemia; ch. 9.3.2.), while the VLDL catabolism decreases, since the lipoprotein lipase enzyme is insulin-dependent. The LDL level also rises. From the excess LDL, more abnormal variants are formed in the circulation. The oxidated and glycated forms of LDL are not recognized by the physiological LDL receptors. Abnormal LDL receptors may also occur. The excess VLDL and abnormal LDL forms will be taken up by the scavenger cells: foam-cells are produced, which mean a basis for atherogenesis.
2. Non-enzymatic glycosylation (NEG = glycation): the glucose normally, reversibly binds to the amino groups of proteins, in lasting hyperglycemia, the binding is irreversible and protein cross bindings are formed (advanced glycation end-product = AGE) (Fig. 9.20.). Both the LDL receptors and the LDL apoprotein are affected, of which, damage to the vascular wall provides the basis of atherosclerosis. The AGE-activated receptor-mediated mechanisms lead to ROS production in macrophages and elicit activation of nuclear factor κB (NFκB) transcription factor, and evoke the enhanced production of inflammatory mediators. Both the free radicals, and the inflammation speed up the progression of atherosclerosis.
3. Hypertension is associated among the major risk factors of atherosclerosis.
4. The pseudohypoxic damage of the endothelial cells (polyol pathway), and the enhanced production of free radicals may also contribute to the development of atherosclerosis.

Lipotoxicity: In DM, the excessively released NEFA from fat tissue accumulates in ectopic cell types, independent of fat tissue, such as that of the myocardial and skeletal muscles, liver cells, or pancreas cells, and inflict important functional and morphological damage. Among the elderly, non-diabetic individuals, ectopic fat deposition can also be observed.

The fatty acids accumulating in the muscle and liver cells negatively affect the insulin responsiveness of these cells, thereby promoting the development and progression of insulin resistance. In hepatic steatosis, the activation of diacyl-glycerol (DAG)-protein kinase C (PKC) pathway (see later) can be detected, and due to this activation, the PKC binds to the insulin-receptor-kinase, inhibits its function and contributes to the decrease of insulin action. Steatosis of the liver, in addition to enhanced insulin resistance, may also lead to non-alcoholic steatohepatitis, even to cirrhosis. In the background, the activation of a special cytochrome enzyme (P4502E1) may be observed (fatty acids and ketone bodies are also substrates of this enzyme), together with consequently increased production of free radicals, TNF-α secretion, inflammation and liver cell injury.

The fatty acids deposited in the myocardial fibers lead to secondary dilative cardiomyopathy. Apart from the apoptosis-inducing effect of the lipid metabolites, the disorder of intracellular carbohydrate metabolism of insulin resistance of myocardial fibers (what results in substrate deficiency and tissue damage) is thought to be in the background. The greater the injury to the heart, the more it utilizes carbohydrates for energy production, as a more effective substrate instead of fats.

The accumulation of fatty acids is also observed in the pancreatic cells, and in the long run, may also damage the insulin-producing ability of β-cells, thereby inhibiting – as seen in 2DM – the development of the appropriate degree of compensatory hyperinsulinemia.

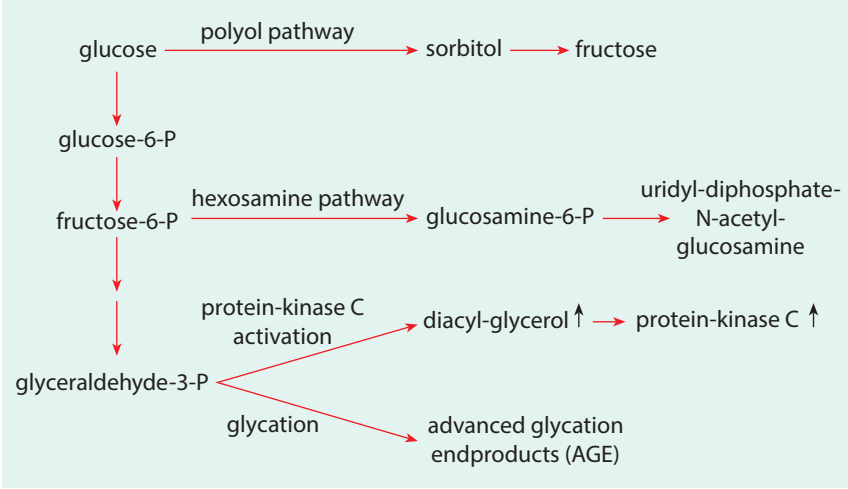

Fig. 9.17.: Pathobiochemistry of chronic complications in diabetes mellitus.

Specific changes:

Microangiopathies *(affecting various organs):*
Primarily, the vessels of the retina, the kidney and the brain are damaged, since their glucose uptake is not insulin-dependent. In the event of poorly controlled (long-lasting or repeated hyperglycemia) diabetes, after 5-20 years of presence, such microangiopathies occur with a rate of 40%. First, the dilation and enhanced permeability of the vessels cause disorders (the PKC and DAG activities are still low; see later), then the constrictor mechanisms take over, and inevitably, progressive vascular obstructions and widespread tissue injuries develop. In addition to the microvessels, the function of neural elements may be damaged directly (neuropathy).

The biochemical processes in the background of the damages are detailed below (= the pathobiochemistry of the chronic complications of diabetes; Fig. 9.17.):

1. The pseudohypoxia induced by the *polyol pathway (poly-ol = containing multiple alcoholic OH-groups)* among these organs damages the capillary endothelial cells, or the neural cells. The normal utilization of pathologically large amounts of glucose uptake is impossible. Through the activation of an otherwise insignificant metabolic pathway, sorbitol is produced (this process is catalyzed by aldose-reductase that utilizes NADPH, therefore there is not enough NADPH for producing vasodilator NO and for regeneration of the antioxidant glutathione – the activity of glutathione dehydrogenase is not adequate, either). Sorbitol is transformed to fructose with the aid of polyol-dehydrogenase, in parallel with this, NADH is formed from NAD. A decrease in the NAD/NADH ratio creates a redox state in the cells resembling to hypoxia (= hyperglycemic pseudohypoxia, Fig. 9.18.). Among the affected cells, which have the enzymes of the polyol pathway, the ATP production decreases. The aldose-reductase pathway may be experimentally inhibited by aldose reductase inhibitors (e.g. amino-guanidine, vitamin C, sorbinil).

   In the long run, the accumulation of NADH inhibits the isocitrate-dehydrogenase enzyme of the TCA cycle, thereby further decreasing the defective ATP synthesis. The glycolysis is blocked at the level of glyceraldehyde-3-P, which is ideally, an appropriate substrate in support of glycation (it is glycated with 200-times greater probability, when compared to that of glucose).

   As a result of enhancement of the polyol pathway, the decrease of the function of the PKC system has also been described, what may partly explain the vasodilation and enhanced vascular permeability observed in the early phase of diabetic microangiopathy, and what is followed later by increased activity of the PKC system with consequent vasoconstriction and further damage of the function of the Na^+/K^+ ATP-ase.
2. The situation is further worsened, since in certain tissues which would need the uptake of some myoinositol from exogenous sources for the operation of Na^+/K^+-ATP-ase, due to the disorder of *myoinositol* uptake the Na^+/K^+-ATP-ase activity further decreases. As a result, the IC Na^+-content increases, the cellular water uptake increases, cellular swelling can be observed, including functional and morphological damage. This may cause particularly severe disorders in the neural cells.
3. As a result of other disorders of inositol-metabolism, the diacyl-glycerol (DAG) and the subsequently evoked PKC activity increases, which decreases the activity of nitrogen-oxide synthase

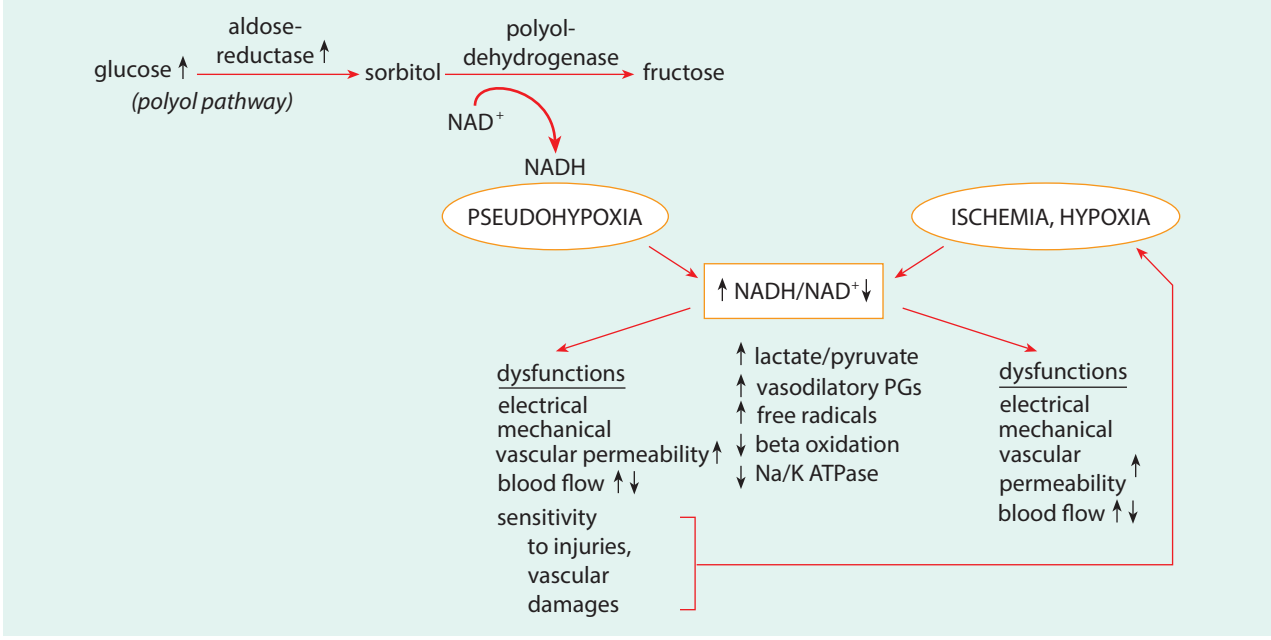

Fig. 9.18.: The polyol pathway leads to production of sorbitol and fructose, plus development of pseudohypoxia. The features and consequences of the redox state correspond to those seen in real hypoxia. The developing vascular damage promotes the manifestation of real hypoxia

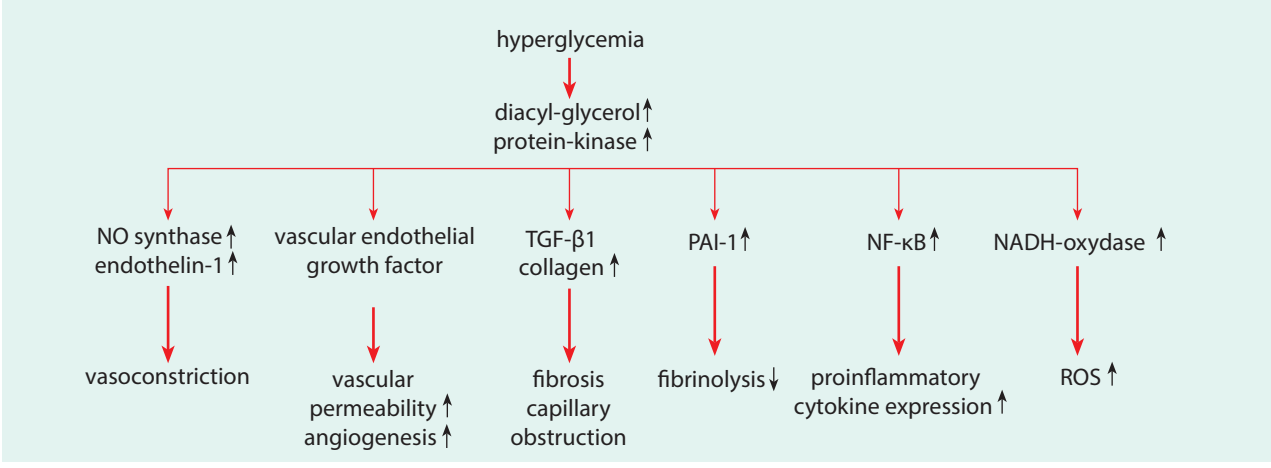

Fig. 9.19.: Consequences of activation of the protein-kináz C system.

(NOS), evokes the production of vasoconstrictor prostanoids, endothelin-1 (ET-1), the higher appearance of free radicals, enhances the permeability and the production of angiogenesis-inducing vascular endothelial growth factor (VEGF), the production and activity of transforming growth factor-β-1 (TGF- β-1) which is responsible for the fibrotic processes, the NFκB which enhances the expression of inflammatory cytokines, and the production of plasminogen activator inhibitor (PAI-1) (Fig. 9.19.). At the same time, DAG also activates the IP_3 signal-transduction system, thereby, within the cells, exerts the growth effect without the presence of growth factors.

The phosphatidylinositol-bisphosphate (PIP_2) of the cell membrane during coupling of some growth factors to their own receptors and activating phospholipase C (PLC) splits to water-soluble, mobile inositol-triphosphate (IP_3) and DAG. The IP_3 causes Ca^{++} release from special intracellular stores, what evokes the IC effects regarding the growth factor. Normally, the Ca-dependent PKC activation also follows, and moves to the membrane, where it replaces the meantime depleted PIP_2 upon the further activating effect of DAG.

4. The rise in, PKC and the decreased Na^+/K^+-ATP-ase equally inhibit locally, the vasodilator *NO-production* and the relaxation of the vessels.

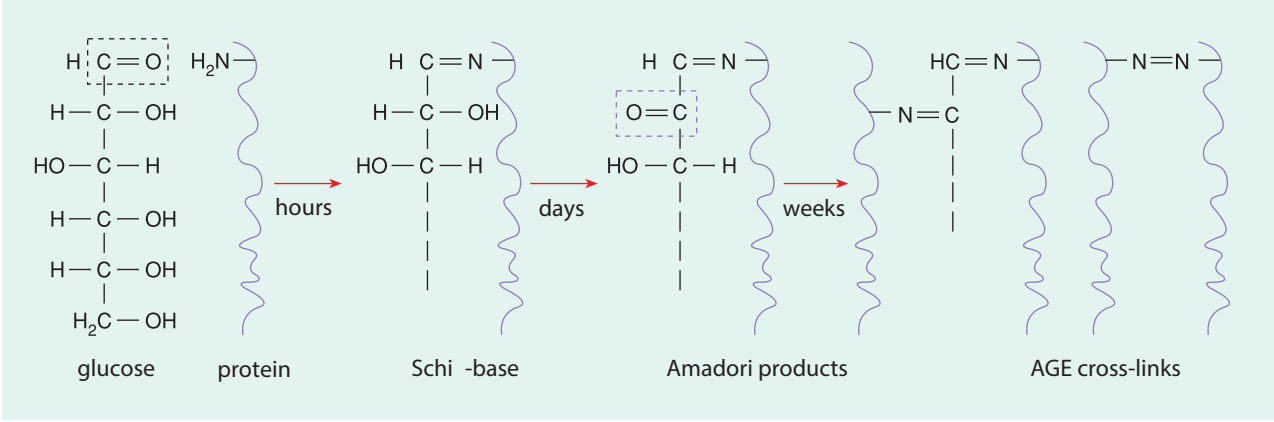

Fig. 9.20.: Formation of protein cross-bridges in the course of glycation.

5. *Non-enzymatic glycosylation* (i.e. glycation), AGE products are released (Fig. 9.20.). The HC=O (aldehyde) group of glucose normally forms a loose, reversible (non-enzymatic) connection (Schiff reaction). In the event of prolonged high glucose concentration, the bonds are more stable (Amadori product), followed by the development of covalent bonds, including new aldehyde groups upon the glucose structure. Therefore, there is a possibility for creating cross-bonds (= advanced glycation end-product = AGE) – the characteristics of the so produced protein-complex differ from the original proteins. The LDL-apoprotein, the collagen (in general, the proteins of long life-span), and the glycation of lens-crystalline may be particularly important. Glycation of Hb is important, from the perspective regarding diagnosis: a rise in its level (normally less than 6.5%) is proportional with the hyperglycemic time-interval during the preceding period (2-3 months), even if the blood glucose is actually normal. At the same time, the oxygen-carrying capacity of the glycated Hb (HbA_{1c}) is not sufficient. Glycation of the collagen of the vascular wall decrease the stability of the basement membrane, now thicker, yet abnormally permeable structures are formed, however, the contractile function of the arterioles also decreases. In the course of phagocytosis regarding the AGE molecules, the activation of macrophages induces inflammatory processes, the consequence may result in an endothelial injury. Contraction of the damaged endothelium increases the permeability of the vascular wall, at the same time, pathological mediators (e.g. VEGF) result in the expression of adhesion molecules, and further endothelial cytokine production. The binding of glycated molecules to AGE-receptor (RAGE) among most cell-types, activates certain signal-molecules (e.g. PKC, microtubule associated protein kinases – MAPk), and nuclear factors of gene expression (e.g. a factor responsible for enhanced production of cytokines: nuclear factor κB – NFkB), which inevitably lead to cellular damage.

   Through a receptor mechanism (RAGE), the AGE molecules significantly increase the mitochondrial production of free radicals, contributing to tissue damage.

   Generally speaking, the AGE substances are present usually in greater amounts with advancing age, among the elderly, and in diseases with higher AGE levels (such as DM) in which the aging process is accelerated.
6. The *hexosamine pathway* is a side-way of glycolysis, which is negligible (3%) under physiological conditions. In this pathway glucosamine, or uridyl-diphosphate-N-acetyl-glucosamine, is produced from fructose-6-phosphate (Fig. 9.17.) with the aid of Gln (NH_2 donor). The activation of this process decreases the insulin secretion upon glucose administration, and increases the formation of glucoproteins, proteoglycans and glucolipids, while due to the simultaneous activation of the PKC system, to the expression of TGF-β1 what leads to mesangial and peritubular fibrosis in the kidney (Fig. 9.23.). Enzymes of the process can also be demonstrated in adipocytes and vascular smooth muscle cells.

   Cells of the endothelium, smooth muscle and the mesangium all can be involved in the processes of microangiopathy. The basement membrane of the capillaries thickens, particularly the type IV collagen accumulates, however, the collagen-structure is considerably abnormal (due to AGE) and the thick basement membrane is more permeable. Due to the high permeability, fluid exits through the capillaries. Processes resembling local inflammatory processes (free

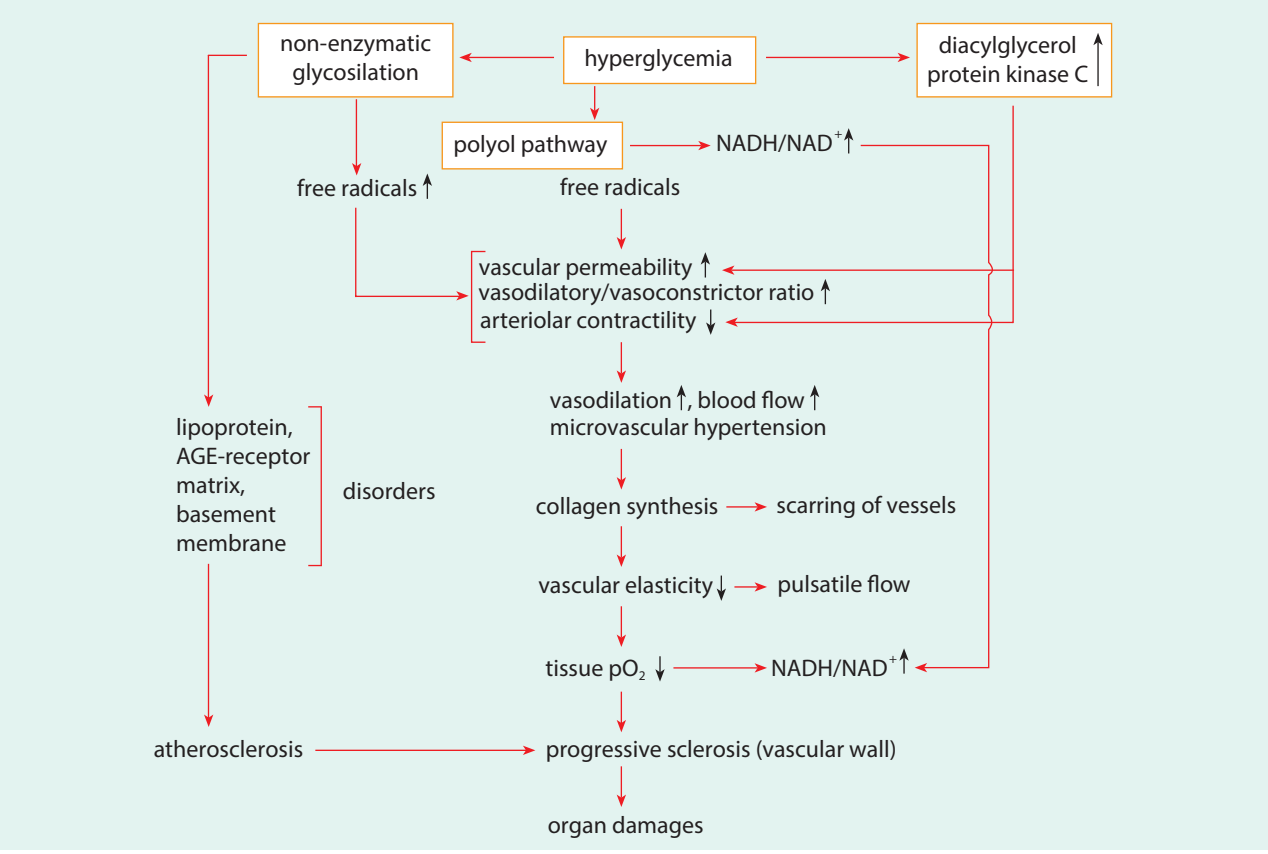

Fig. 9.21.: Reviewing the microvascular effects of hyperglycemia.

radicals, platelet adhesion, etc.) are initiated. As witnessed in inflammation, vasodilation is pronounced, allowing for enhanced perfusion and microvascular hypertension. The endothelial damage and alterations of the basement membrane bear slightly different consequences throughout various organs. The general microvascular consequences of hyperglycemia are demonstrated in Fig. 9.21., and similar disorders participate in the development of neural consequences.

Diabetic retinopathy (slow and a life-long process)_ Earlier, exclusively genetic factors were assumed to be responsible in the development of retinopathy, and it was hypothesized that the glycemic control does not influence the process. Today, regarding 1DM, it can be diagnosed easier and the strict and well-performed blood glucose control initiated at the onset can delay the retinopathy, however, in a long-hidden 2DM, it is still not a resolved problem. According to our present observations, after nearly 15-years, non-proliferative retinopathy can be detected in most (80%) diabetic patients.

In the early stage (retinopathy simplex, non-proliferative retinopathy), changes of the perfusion (obliterated and over-perfused areas), exudation, arteriovenous shunt formation, microaneurysm formation, the disappearance of pericytes of mesangial origin that should support the small vessels of the fundus of the eye from the outside, and the thickening of basement membrane characterize the picture. Meandering small vessels, string-of-beans aneurysms and small bleedings can be seen on the native picture of the fundus or by fluorescent vessel-tracing. Few microthrombi and small, retinal bleedings are possible, already at this stage. A serious decrease in vision is not yet characteristic at this stage, unless the bleeding affects the region of the macula.

Later on, in the vasoproliferative period (proliferative retinopathy), due to the progressive hypoxia, abnormal coils of capillaries develop in the fundus of the eye. These vessels may originate from the vitreous body of connective tissue origin or from the vascular layer of the eye (choroid). The newly formed capillary coils may cause bleedings into the vitreous body or, upon retraction, they cause retinal detachment. The detachment, vitreous body bleeding, macula-edema results in blindness. Today, in the developed countries the DM is most fre-

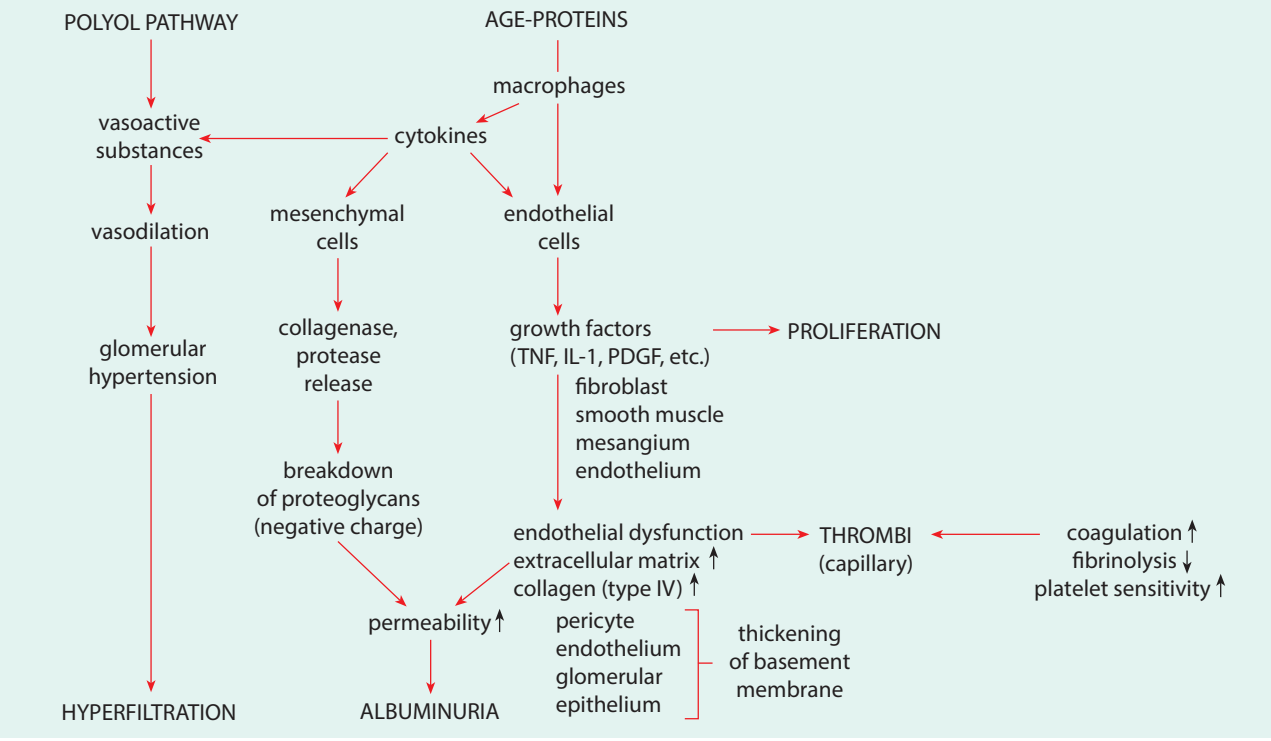

Fig. 9.22.: Pathomechanism of the development of diabetic nephropathy.

quently lurking in the background in causes of blindness developing in adulthood.

Prior to this stage, there may be smaller vision problems due to bleeding and patchy cicatrization/scar-formation in the retina, early crystalline glycation of the lens and development of cataract.

Abnormalities of the retina have great diagnostic importance, since they supply non-invasive, indirect information regarding the state of the concordantly damaged vessels of the kidney, and perhaps the brain. **Diabetic nephropathy** (earlier: Kimmelstiel-Wilson syndrome) (Figs. 9.22-9.23.)

At the beginning of the process, it is characteristic that the kidney is enlarged, its perfusion enhanced, and the filtration pressure and GFR are enhanced. This is first followed by microalbuminuria (200-500 mg/day), then

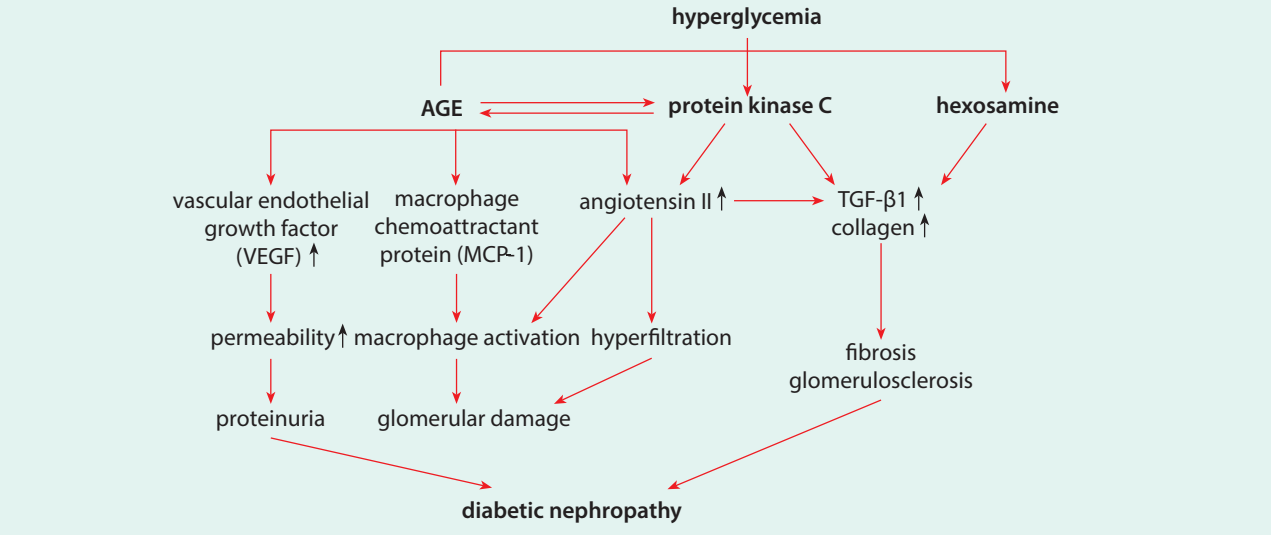

Fig. 9.23.: The role of biochemical processes in the development of diabetic nephropathy.

by an increase in proteinuria. Later, due to permanent hyperfiltration (ch. 5.1.1.; Fig. 5.16.), the kidney is damaged, and gradually, the picture of chronic renal failure develops. In the USA and Europe, 40% of end-stage renal failures can be of diabetic nephropathy origin.

In the background of early morphological disorders, mainly the enhanced TGF-β1 activity, or other renally accumulating growth factors (CTGF = connective tissue growth factor – other growth factors /particularly TGF/ are activated by IGF = insulin-like growth factor – glomerular and tubular cells, PDGF = platelet derived GF – mesangial cells, VEGF – podocytes, EGF = epidermal GF – produced in tubules, their role has been proven). In the activation of TGF-β1, the high blood glucose level, the evoked intrarenal RAAS, and the elevated PKC activity all play a critical role. In the background of originally high renal perfusion, various pathogenic factors may remain: glucosuria may increase via tubulo-glomerular feedback filtration, the hyperglycemia enhances the intrarenal RAAS activity, and blocks the voltage-dependent Ca^{++} channels in smooth muscles of vas afferents, therefore, it induces vas afferent vasodilation.

At the same time in the kidney, the polyol pathway can be present, together with the RAAS, the DAG, the consequent PKC activation, and chronic activation of the hexosamine pathway. With regard to the development of lesions, the AGE receptor mechanism (RAGE) induced increase in NADPH-dependent free radical production also has a contributory role.

The morphological picture of diabetic nephropathy is characteristic. The glomeruli are hypertrophic at the beginning, the capillary membrane is thick, the number and density of the podocytes decrease, thus the selectivity of the permeability decreases, mesangial changes develop, the glucosaminoglycanes of negative charge are glycated and pathologically bind to the basement membrane, what allows enhanced filtration of proteins. The tubular basement membrane also thickens, and the epithelium is hypertrophic. In the interstitium, lymphocytic infiltration is often seen (the AGE activates the macrophage-chemoattractant protein-1, MCP-1). The damaged endothelium slowly regenerates, however, in the meantime, the capillary becomes obstructed. Due to the enhanced protein filtration and deposition, mesangial inflammation and consequent mesangial proliferation develops (cicatrization-glomerulosclerosis), and this also worsens the glomerular function. Due to the presence of glycation, and the activity regarding the hexosamine pathway epithelial injury, inflammation and scarring (tubuloepithelial-mesenchymal transdifferentation) join the glomerular injury. Thus, the filtering

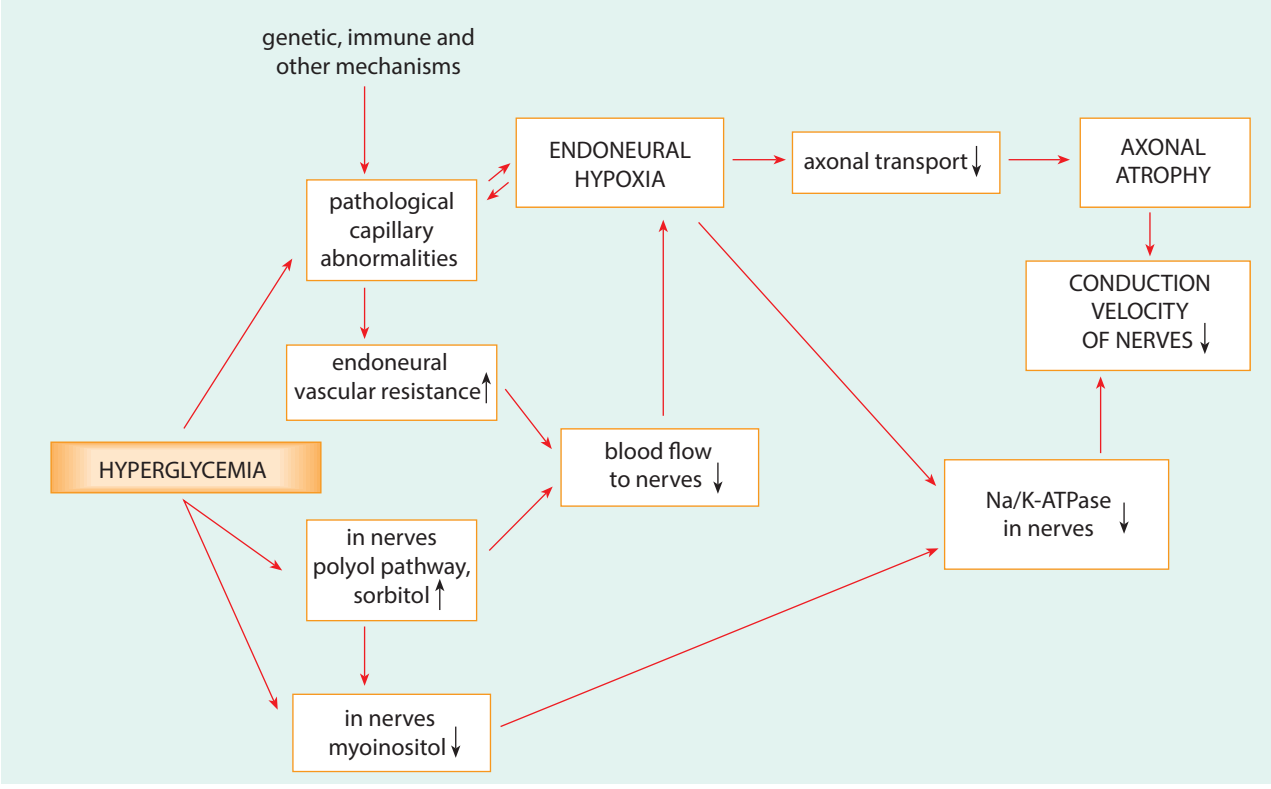

Fig. 9.24.: Pathomechanism of the development of diabetic neuropathy.

surface decreases, the progressive glomerular and tubular damage leads to chronic renal damage. The early selective microproteinuria is replaced by non-selective proteinuria (containing also >65 kDalton proteins). Additionally, the gradually worsening macroproteinuria (>2g/day) is accompanied by similarly worsening hypertension. In late stage, papilla necrosis may occur.

Diabetic neuropathy (Fig. 9.24.)
In the background stands the glycation of neural elements (e.g. myelin-proteins), tubulin glycation (damaging the axon-transport) and axon-degeneration, the last one primarily due to pseudohypoxia and real hypoxia, and the lack of myoinositol. The endothelium of local capillaries is injured, the circulation decreases and transudate production is characteristic. The acceleration regarding neural conduction significantly decreases.

First of all, sensory and autonomic, least of all the motoric functions are damaged. Symmetrical polyneuropathies, paresthesias and disorders of pain-sensation (decreased pain-sensation in the case of normoglycemia, and, increased pain-sensation in hyperglycemia) are characteristic. In addition to these various aspects, the damage of the autonomic nervous system (DAN – Diabetic Autonomic Neuropathy) is shown by the tendency for orthostatic hypotension (disorder of vasomotor reflex), for fainting, the disorder of sweating, disorders of gastrointestinal motility (slowdown and obstipation tendency), paralytic ileus-tendency (e.g. gastroparesis diabeticorum), and urinary or fecal incontinence severely diminishes the quality of life. According to recent surveys, 46% of men suffering from 2DM diagnosed at least 5-y earlier, admitted erectile dysfunction, definitely due to neuropathy.

In diabetic neuropathy, the hypoglycemia unawareness (= lack of prodromal vegetative symptoms), unresponsiveness, or silent myocardial ischemia occur more frequently.

Unilateral damage is also possible, including severe sensory/motor disorders, e.g. foot injury, ulcer, infections, in which neuropathic gangrene may develop, often followed by amputation (Fig. 9.25.).

Diabetic cardiomyopathy
Diabetic cardiomyopathy can be attributed to the degenerative damage of the myocardium. Chronic acidosis, pathologic microvascular changes, hypertension and coronary-sclerosis causing chronic ischemia (the mac-

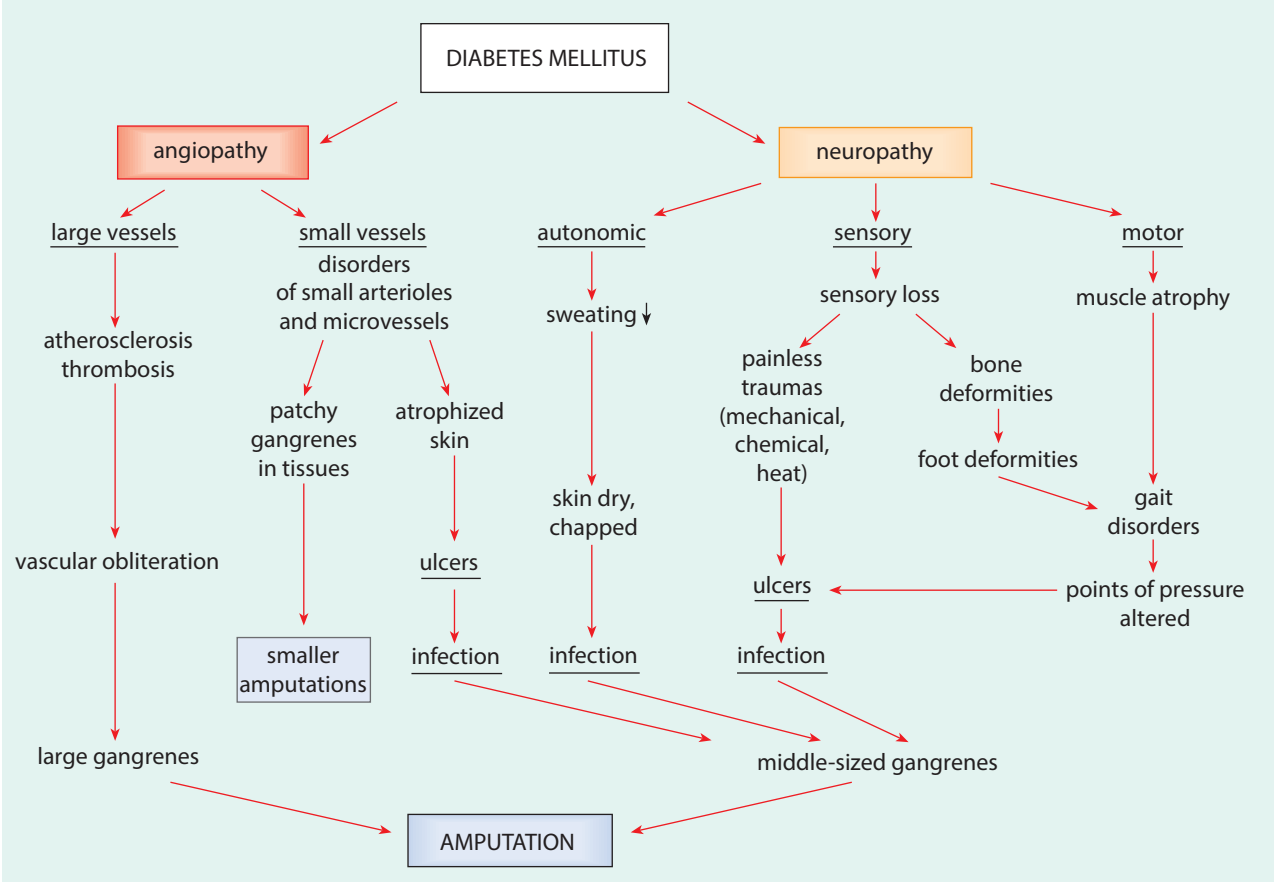

Fig. 9.25.: Pathological processes in the development of diabetic foot.

roangiopathic consequences) contribute to the complex pathogenesis of diabetic dilated cardiomyopathy.

Increased tendency for infection
In diabetic patients, an enhanced infection tendency demonstrates the weakness of the immune system, often appearing in a prolonged sty-formation, protracted therapy-resistant purulent or fungal skin infections.

Diabetic foot
This is explained partly by the often occurring peripheral angiostenosis (macroangiopathy), or other disorders of tissue perfusion (microangiopathy), partly by phagocyte dysfunctions, frequent infections and neuropathic abnormalities (thin, dry skin, trophic disorders) (Fig. 9.25.) – these very often make amputation necessary.

Due to the asymmetric motor disorder, the statics of the foot becomes altered (pathologic pressure points and ulcer formation), therefore the patient now has a tendency for falls and stumblings. Healing regarding open wounds is slow, the infection tendency is increased, therefore non-healing wounds, deep ulcers and/or gangrene may develop. Due to the sensory neuropathy, the injury may be painless, thereby the diagnosis may be delayed. Prevention must be emphasized, since the cure is prolonged, expensive and, unfortunately, the prognosis is poor. Among diabetic patients, prolonged, recurring and incurable osteomyelitis may explain the necessity of amputation.

9.2.2.5. PRINCIPLES OF THERAPY IN DIABETES

General principle of therapy is securing adequate glycemic control. On the basis of HbA_{1C} level the aim of the therapy is the 7% value (in case of patients with low hypoglycemia risk and good compliance the 6.5% is more optimal, causes less chronic complications, while patients with higher risk of hypoglycemia or in those with difficult cooperation the 7.5% value is acceptable.

Type 1 DM:
Exogenous insulin is always needed. Oral antidiabetic drugs that stimulate insulin secretion are of no help. (Application of biguanides inhibit intestinal glucose absorption and gluconeogenesis may decrease the insulin need, their administration may be imagined, but it is not used in practice.)

Most patients are treated by several daily injections of basal (medium- or long-acting) insulin, or at food intake (considering the carbohydrate content of the food) fast or ultrafast acting insulin injection, eventually with continuous subcutaneous insulin infusion (insulin-dosing pump, IP). Such therapy secures low incidence of hypoglycemia and improved metabolic control. The recent international trend is that the correctly informed and motivated patients are taught to correct their fast-acting (not basis) insulin dose, considering their blood glucose before food intake, the carbohydrate content of the food, and the planned physical activity. Also new trend is that those aging patients who had already successfully used the IP, should continue using it even after age 65-y.

In extremely severe, therapy-resistant cases, or in those awaiting renal transplantation (invariably needing immune-suppression) among 1DM patients arises the possibility of pancreas/islet cell transplantation. Although this usually normalizes the blood glucose, but continuous immunosuppression is needed and due to the probability of repeated autoimmune islet cell damage this is used only in exceptional cases.

Regarding the diet, it is important to synchronize the food intake and insulin administration together with providing sufficient energy intake to maintain the body weight or in children to provide enough energy for growth and development. Of course the easily absorbed carbohydrates should be avoided, but the complex carbohydrates should cover half of the energy need. Important is the ample protein intake (but mainly from vegetables), and sufficiently large intake of polyunsaturated fatty acids. Proteins and fats of animal origin should be limited.

Type 2 DM:
In early phase of 2DM it is of primary importance to decrease the calorie intake and body weight preferably to the normal level (at least 7% lasting decrease is advised). Also advised is the increase of physical activity, at least 150 min/week medium intensive activity (e.g. walk with slightly accelerated pace). Successful treatment of obesity and the physical activity have a positive influence on insulin resistance and decreases the requested medication. (Due to the late complications, foods rich in fat or containing lots of protein are not an optimal calorie source, and the sugar intake must be strongly reduced. Accordingly, the optimal source of calorie is the complex carbohydrate, what is not followed by as high elevation of blood glucose level as after glucose intake (Fig. 9.26.).

In the pharmacological treatment of 2DM (oral antidiabetic drugs) various agents are used.

In the group which **slows down or blocks the glucose absorption** the **amylin analogues** (e.g. Pramlintide,

which slows the gastric emptying, enhances satiety, and decreases the glucagon secretion of the pancreas) and the α-**glucosidase inhibitors** (e.g. Acarbose, Miglitol, Voglibose also increase the GLP-1 level) are important.

The **biguanide** substances (e.g. metformin) decrease the blood glucose level by inhibiting the intestinal glucose-absorption and the gluconeogenesis. Metformin is used with good results in the polycystic ovarium syndrome (PCOS) which is accompanied by insulin resistance and infertility. Metformin decreases the cardiovascular risk and it is suitable for the prevention of 2DM. It is assumed that prolonged metformin treatment slows down the aging process.

In the group of **insulin secretion enhancing** substances the **sulphanyl-urea** derivatives (e.g. Gliklazid, Glibenklamid, and **meglitinides** e.g. Repaglinide, Nateglinide), which increase the insulin secretion by closing the ATP-dependent K^+-channels on the β-cells are well-known. These also enhance the insulin secretion and production (but at the same time they enhance the β-cell differentiation and inhibit the β-cell apoptosis). Substances of the incretine system **GIP/GLP-1 analogues** (e.g. Liraglutid, Exenatid), or substances which extend the action of endogenous GLP-1 **dipeptidyl peptidase-4 (DPP4) inhibitor** gliptins (e.g. Sitagliptin, Vildagliptin, Saxagliptin) are peptides that can be applied in the form of subcutaneous injection.

Improving insulin efficacy - PPARγ agonists (thiazolidinedione-TZD, e.g. earlier Troglitazone /now withdrawn due to its toxic side-effects/, Rosiglitazone, Pioglitazone) may contribute to the treatment of 2DM or to the prevention in some high-risk group by improving insulin sensitivity. Further positive effects are the decrease of lipid- and cytokine- (TNFα, IL-6) levels, enhancement of adiponectin secretion, decrease of hypertension. Their side-effects of obesity or liver failure may limit their use. Inhibition of the angiotensin system (ACE inhibitors, angiotensin receptor blockers) also enhances the activity of PPARγ system, without the toxic side-effects of glitazones.

New substances applied in the treatment of 2DM are the **Na^+-glucose cotransport (SGLT2) inhibitors** (e.g. Dapagliflozin, Empagliflozin, Canagliflozin), which decrease the glucose reabsorption in the kidney, thus much more glucose may leave the body by the urine.

Recently **GLP-1 agonist** substances are investigated in clinical studies: they may be useful partly by decreasing obesity, partly by enhancing insulin-production and -sensitivity.

At the beginning of the disease, beside the lifestyle changes, Metformin monotherapy is advised, if it is not sufficient for the glycemic control, additional substances (e.g. sulfanylurea, or PPARγ agonists) should be given. If the 2-component treatment is still not sufficient, then a 3rd substance (e.g. DDPP4 inhibitor) administration is advised.

In severe cases, insulin treatment has to be advised even in 2DM, but due to the insulin resistance in significantly larger doses than in 1DM.

9.2.2.6. SUBSTANCES APPLIED FOR PREVENTION OF LATE DIABETIC COMPLICATIONS

The late complications of DM have extraordinary clinical importance, accordingly very intensive studies are going on for the prevention of these complications. Hereby the most promising possibilities are shown, without giving a full picture of the studies.

Thiamine (B_1 vitamin) is a coenzyme for many enzymes (e.g. transketolase, pyruvate dehydrogenase and α-ketoglutarate dehydrogenase) participating in the carbohydrate metabolism, and it is necessary for appropriate insulin production. The B_1 vitamin related substances e.g. **benfotiamine** (Milgamma, Benfogamma) enhance glycolysis, the activity of the TCA cycle, the direct oxidation of glucose, and inhibit the aldose-reductase and the hyperglycemia-activated other pathological metabolic pathways (polyol pathway, glycation, DAG-PKC, hexosamine pathway), thereby have a protective effect on the endothelial-, the micro- and macroangiopathies, the retina, the kidney, the peripheral nervous system (both in diabetic and in other neuropathies), the heart, and have a positive influence on the lipid profile. Other **aldose-reductase inhibitors** (Sorbinil, Zopolrestat) can be applied in the prevention of neuropathy and microvascular damages. The B_6 vitamin (pyridoxamine) **inhibits the production of AGE substances**, by this way it contributes to the prevention of late complications. Other AGE production inhibiting substances are also used for delaying the development of late complications, e.g. aminoguanidine, metformin (in vitro decreased the AGE receptor expression and inhibited the signal transduction).

Inhibitors of the angiotensin system (**ACE inhibitors**, angiotensin receptor blockers /**ARB**/) positively influence the blood pressure, the proliferative retinopathy and the nephropathy.

Since diabetic patients have an extremely high cardiovascular risk, for them the suppression of se-cholesterol, particularly that of LDL-cholesterol (<2,5 mmol/l) is very important. This is why the **statin treat-**

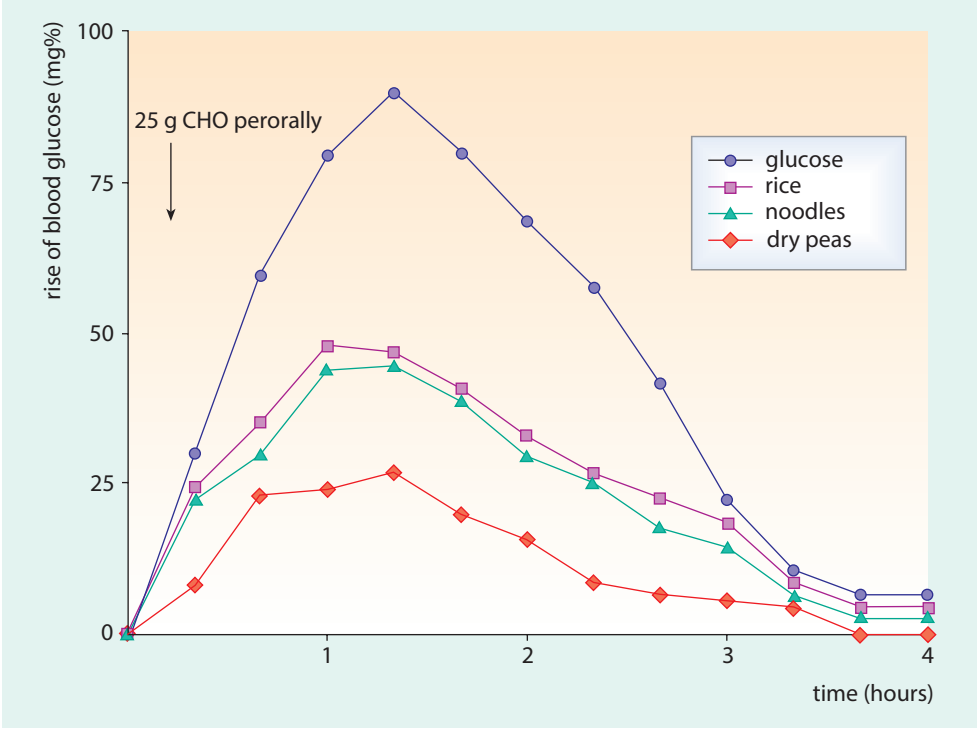

Fig. 9.26.: Various carbohydrates of the same calorie content result in different rises of blood glucose levels (different glycemic indices): the rise is greatest when refined sugar is consumed.

ment is important specially for diabetic people (e.g. Atorvastatin, Simvastatin), although a recent study raised the possibility that in elderly women statin treatment enhanced the risk of diabetes. To prevent the cardiovascular complications, the **aspirin treatment** (inhibition of platelet aggregation) is advised also for diabetic patients.

Antioxidants (e.g. alpha-lipoic-acid) can be used in the treatment of diabetic neural damage-induced pain and sensory disorders.

9.2.3. HYPOGLYCEMIAS

In practice, hypoglycemia means such low blood glucose level, which gives clinical symptoms (i.e. <2.5-3.0 mmol/l). Most often it joins insulin excess, but lack of the contrainsular factors (e.g. pituitary deficiency, disorder of sympathetic functions) may also be a causative factor, as well as the defective replacement from the liver. Low blood glucose, particularly acutely developing hypoglycemia is more dangerous than a rise of se-glucose.

It follows from the mode of blood glucose regulation that to prevent a decrease the first step of counter-regulation is a fast glucose mobilization from the liver (decrease of insulin and activation of glycogenolysis via active adrenergic mechanisms), what is coupled with enhanced gluconeogenesis (this needs glucagon). The counter-regulation needs intact liver and sufficient amount of substrates for gluconeogenesis. It is possible, however, that all these are not enough to normalize the se-glucose level, therefore the lack of glucose for the brain leads to clinical symptoms (Fig. 9.27.). Therefore, the consequences of hypoglycemia may be classified as 1) symptoms of adrenergic activity, 2) neuroglycopenia (glucose deficiency of the nervous system).

ad 1) As a sign of **adrenergic hyperactivity**, in addition to the signs of "panic-reaction" (tachycardia, sweating, weakness, tremor) the feeling of **hunger is very characteristic**. With the development of diabetic autonomic neuropathy, these signs are lessened, the patient does not notice the signs of developing hypoglycemia ("unawareness") – without the prodromal symptoms hypoglycemia and the neuroglycopenic disorder of consciousness develops suddenly.

ad 2) On the basis of neuroglycopenia, headache, dizziness, disorders of speech, affective disorders, eventually aggressiveness, ataxia, worsening confusion, seizures, finally coma may develop, and the patient may die. The **hypoglycemic coma** may develop

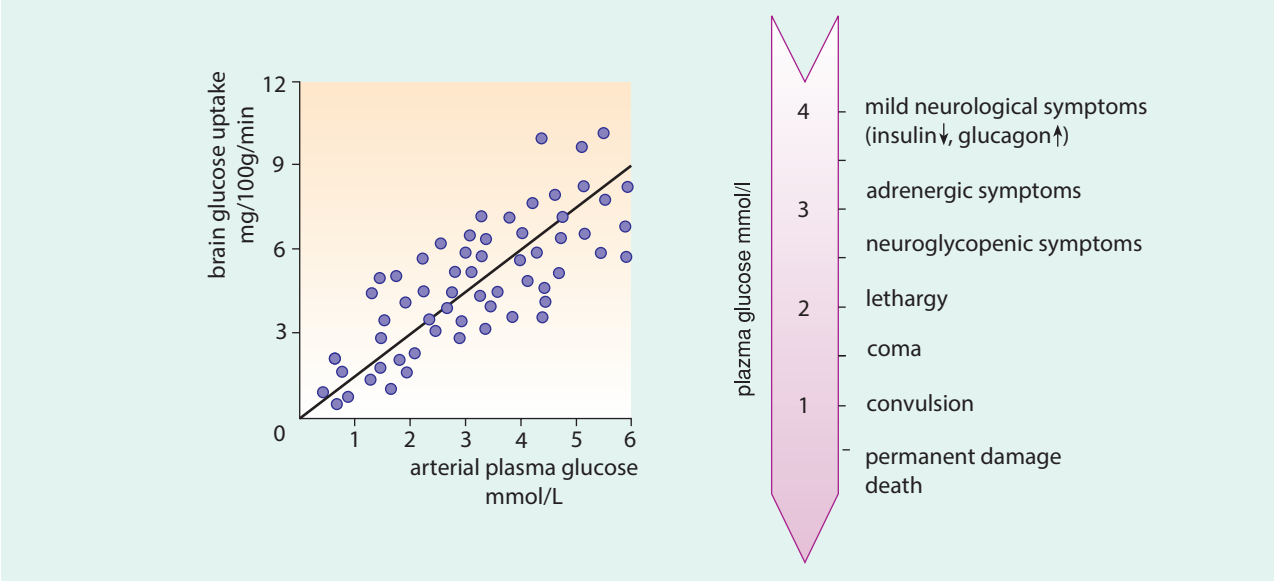

Fig. 9.27.: Glucose uptake by the brain decreases parallel with the fall in blood glucose. The other part of the Fig demonstrates the main neurological symptoms observed with falling blood glucose. In the Fig "counter-regulation" refers to decrease of se-insulin and rise of glucagon, what alone cause no symptoms.

very quickly (1-2 min), particularly in 1DM, although development of coma is not unavoidable even at extremely low blood glucose (particularly not if the decrease of blood glucose is relatively slow, like in 2DM). In the explanation of coma, one, but not the only factor is the glucose deficiency of the brain (the local ATP level is relatively maintained: it may be 25-30%, while in ischemia it is below 5%), local (cerebral) circulatory changes and transmitter disorders also play a role. Eventually, a swelling of brain cells develop due to membrane transport disorders induced by energy deficit. It is possible that the reticular formation of the brain stem, which is responsible for vigilance and is very sensitive to hypoglycemia, cannot ensure the activity of the cortex (vigilance).

FORMS OF HYPOGLYCEMIA

Postprandial hypoglycemia

- exaggerated postalimentary hypoglycemia and late dumping syndrome
- hereditary disorders, e.g. galactose, or fructose intolerance (1:20 000 birth). Due to the lack of fructose-1-phosphate aldolase, the fructose-1-phosphate accumulates, it inhibits the fructose-1,6,-bisphospha-

Table 9.5.

Characteristics of lipoprotein fractions

	CHY	VLDL	IDL	LDL	HDL
Protein (%)	2	10	19	25	55
TG (%)	86	52	23	10	4
Cholesterol (%)	2	7	9	8	1
CE (%)	3	13	29	37	16
Phospholipid (%)	7	18	19	20	24
Density (g/ml)	0,95	0.96–1.006	1.006–1.019	1.019–1.063	1.063–1.21
Place of production	intestine	liver	in circulation from CHY+VLDL	in circulation from VLDL	gut, liver in circulation from CHY+VLDL
Size of particle (nm)	500	43	27	22	8
Apoprotein	B-48 E, A, CII, CI	(B100) E, A, CII, CI	(B-100) E	B-100	E, A, CI

tase enzyme which is necessary for gluconeogenesis, and also the liver phosphorylase, which is indispensable for splitting glycogen to glucose. The autosomal recessive galactose-1-phosphate-uridyl-transferase deficiency leads to the accumulation of galactose-1-phosphate (hereditary galactosemia), which causes mental retardation, cataract, liver steatosis, in addition to hypoglycemia. These disorders develop only after consuming fructose or galactose, originating at or shortly following birth, and may cause severe developmental disorder of the child. (Due to the severe consequences and relatively frequent occurrence, 1:35 000 birth, a postnatal screening regarding the hereditary galactosemia is compulsory. The affected neonate must not be nursed, as maternal milk, through breastfeeding, may injure the neonatal brain resulting in severe hypoglycemia).

Fasting type hypoglycemia (there is no food intake, however, starvation is not necessary):

- decreased glucose production (end-stage of starvation and accelerated starvation)
- premature, atrophic (stunted) neonate
- acute liver failure
- adrenal gland, pituitary, glucagon deficiency (lack of counter-regulation)
- alcohol intoxication (accelerated starvation and defective gluconeogenesis)
- increased glucose utilization (insulinoma, production of too much insulin, due to leucine
- insulin overdose (this is the most frequent), particularly if combined with physical activity, since the active muscle takes up glucose without insulin
- oral antidiabetic treatment (e.g. lasting overdose, without food intake)
- neonate of diabetic mother
- maple syrup disease: the accumulating leucine (in addition to the specific consequences) causes hypoglycemia repeatedly due to enhancement of insulin secretion. In addition, there is a lack of glucoplastic amino acids in this disorder.
- pentamidine treatment: from damaged β-cells the stored insulin is released (leaking)
- without insulin excess, it is possible, e.g. in malignant tumors, in sepsis

Hypoglycemia adjoining insulin-treatment in 1DM:
Patients of 1DM do not produce insulin, yet they are sensitive to insulin. In theory, the glucose balance can be maintained by replacing the daily insulin production. However, in practice, the insulin need is not continuously standard, e.g. following food intake, it is greater, and during physical activity, it is lower (the active muscle takes up glucose without insulin). From an exogenous source, a relatively stable insulin level can be secured, yet in cases of inadequate adjustment in the level to the actual need both hyperglycemic (e.g. following food intake) and hypoglycemic periods may occur. Hypoglycemia typically develops if the insulin injection is not followed by food intake in time, or if the well balanced insulin-treated patient performs an unusually robust form of exertion or exercise. This form of hypoglycemia characteristically develops suddenly and often leads to a coma. The rapid development of a coma is not due exclusively to neuroglycopenia (the blood glucose does not drop to a critical value within 1-2 min), however, changes in the regional distribution of brain blood flow is assumed, including changes in the transmitter functions and in the function regarding reticular formation. The coma state is quickly normalized upon an i.v. injection of glucose (K-supplementation may also be necessary).

Hypoglycemia evoked by oral antidiabetic drugs in 2DM:
There is insulin resistance among 2DM patients and, despite hyperinsulinism, the insulin effect is insufficient. Since the insulin production is maintained and it is even somewhat adjusted to the actual needs, and since the insulin action is limited, hypoglycemia develops less easily. However, it may develop in the case of a lasting overdose of oral antidiabetic drugs (particularly sulfanylurea), or if such drugs are continuously administered, despite a lasting disorder of food intake (anorexia, vomiting, etc.). This occurs particularly often among the infirm, elderly yet well-cared for patients. The pharmaceutical drug in the circulation lastingly stimulates the β-cells, evoking lasting insulin secretion, far exceeding the need, the blood glucose slowly decreases and may reach extremely low (e.g. about 1 mmol/l) level, causing very severe confusion, yet neither unconsciousness, nor coma. With regard to treatment, one glucose injection is not enough (the drug cannot be quickly excreted from the body and stimulates the β-cells for a longer period), therefore, glucose infusion and K-replacement are necessary over 1-2 days. However, this requires immobilization, and causes severe volume-load, and its consequences (hypertension, heart failure and pulmonary edema). The mortality rate is high.

Neonatal hypoglycemia:
Following the cessation of glucose supply via the placenta, the neonate must rely on glucose released from the glycogen stores. Physiologically, the blood glucose declines transiently to 2.2-2.8 mmol/l, with-

out causing symptoms (the hypoglycemia tolerance is better among neonates when compared to that of adults), and normalizes by the end of the first day. Lasting or severe hypoglycemia develops if the amount of liver glycogen is not sufficient. Provided that the placental circulation is good, glycogen is produced in the fetal liver during the last month of pregnancy. In premature and atrophic neonates, the amount of liver glycogen is insufficient. The cellular glucose utilization may be too high in heavier neonates of diabetic mothers, since the fetus produces more insulin (in response to the maternal hyperglycemia), and following delivery, the maternal glucose supply is stopped and the high insulin level leads to life-threatening hypoglycemia.

Hypoglycemia in alcoholics, or in alcohol intoxication: Patients in the state of accelerated starvation (after at least 24-h fasting) can secure glucose supply exclusively by gluconeogenesis, however, in acute alcohol intoxication this is not possible, since the alcohol blocks glugoneogenesis (the alcohol-dehydrogenase results in a redox-state /NAD decreases, NADH increases/, in which the pyruvic acid is turned to lactic acid and cannot be used for gluconeogenesis). In severe alcohol intoxication, this may occur without previous fasting, particularly among children.

PRINCIPLES OF THERAPY IN HYPOGLYCEMIA

As long as the patient is conscious, oral glucose intake (tea with sugar, sugar-cube, etc.) aids in preventing the development of a coma. In a coma state, i.v. glucose therapy immediately normalizes the blood glucose. An exception is the hypoglycemia evoked by oral antidiabetic substances in 2DM, when long-lasting glucose infusion may be necessary, and the peril is hypervolemia.

”Dawn phenomenon”: at dawn, the elevation of GH- and cortisol-level leads to hyperglycemia.

”Somogyi phenomenon”: after several hours following increased levels regarding the dosage of insulin (evening), reactive hyperglycemia develops due to the over-enhanced effect of counter-regulatory hormones (by morning). The mechanism: too large an insulin dose evokes a hypoglycemic episode, of which, is not noticed during nocturnal sleep, and this initiates the counter-regulation. The additional increase in the evening insulin dose does not help, but worsens the blood glucose in the morning.

9.3. PATHOPHYSIOLOGY OF LIPOPROTEIN METABOLISM

9.3.1. LIPOPROTEIN CLASSES AND OUTLINE OF THEIR METABOLISM

Lipoproteins (LP) are complex particles built from lipids and the commonly referred to *apo*proteins. Their main role is to transport (carry) the bulk of hydrophobic lipids in the aqueous phase of plasma. They contain all the fat molecules transported in the plasma except those bound to albumin. The latter ones include non-esterified fatty acids (NEFA), being quantitatively the most relevant. (These are often erroneously referred to as “free fatty acids” /FFA/ though they are not circulating *free* in the plasma, yet are indeed albumin-bound).

THE COMPONENTS OF LIPOPROTEINS

Triglycerides (TG): Are defined as 3 fatty acid (FA) molecules, each forming an ester bond with one of the 3 OH groups of a glycerol molecule. Characteristics of FAs include the following:

- saturated FA: **No** double bond in the aliphatic side-chain (e.g.: palmitic and stearic acids)
- monounsaturated FA: **One** double bond in the aliphatic side-chain (e.g.: oleic acid)
- polyunsaturated FA (PUFA): Such as linolic (n-6/ω-6)* and eicosapentaenic (n-3/ω-3) acids

Phospholipids (PL): Are defined when 2 FAs each form an ester bond with 2 vicinal OH groups of a glycerol molecule (*apolar tail*), the third OH being esterified by a polar phosphor containing acid residue (*polar head group*). For example, phosphoryl choline is in the best known phospholipid, which is lecithin. Hence, PLs are partially of hydrophilic (head) and of lipophilic (tail) nature, thus, they form micelles in an aqueous phase.

In the lipid bilayer of the cell membrane, the apolar PL tails face each other, while the polar head groups on both surfaces of the membrane “*bathe*” in the aqueous phase of the extra- and intracellular space.

* The n/ω number represents the position of the first double bond from the last carbon atom of the chain. The ω-6 PUFAs are mostly plant-derived, while most ω-3 PUFAs originate from fish. TG provides the majority of fat in our menu (bacon, lard, butter, vegetable oil, margarine, etc.) TGs are apolar lipids, i.e. they have no charge, thus, they have a hydrophobic/lipophilic nature.

A monolayer *shell* of PLs comprises the surface of the LP particles in which head groups face outward in contact with the aqueous phase of plasma, while the tails turn inward, in contact with the hydrophobic lipids (e.g. TGs) which give the bulky *core* of the LP.

Cholesterol (Chol): It is found exclusively in menu items of animal origin, yet all cells of the human body are able to synthesize it, hence, we are not dependent on alimentary cholesterol. Its non-esterified form (polar *free cholesterol:* **FC**) is a very important component of the cell membrane, in which its free OH group is aligned with the head groups of the PLs, while its polar sterane skeleton is aligned with the apolar PL tails.

The often referred to "*lipid rafts*", float like vessels in the "sea of the lipid membrane" and contain rich quantities of cholesterol, since its presence decreases membrane fluidity, i.e. makes these rafts rigid. Several membrane proteins are anchored to these rafts, e.g. receptors for hormones/adhesion molecules, etc.

If the OH group of FC is esterified by a FA, the polarity is lost in the product, and this is cholesterol ester (**CE)**. In the LP particle, FC resides in the shell, while CE is found in the core.

Apoproteins: These special proteins are surface components of LPs. Some are restricted to only one particular type (class) of LPs, while others are building blocks among several different ones, yet often in a variable proportion.

TYPES OF LIPOPROTEINS, THEIR ROLE AND DISTRIBUTION

The various types of LP particles are formed and built at different sites throughout the human body, and these also differ regarding their structure and functionality. Chylomicrons (Chy) and very low density lipoproteins (VLDL) are rich in TG, while low density lipoproteins (LDL) and high density lipoproteins (HDL) are more cholesterol rich LPs.

LPs are metabolized on **3 main pathways** (routes): the **exogenous** and the **endogenous pathways,** plus the **reverse cholesterol transport**, which are, however, not operating fully isolated from one another.

1. ***Exogenous*** lipids absorbed from food in the intestine are transported in chylomicrons via lymphatic vessels and eventually enter into the blood circulation through the lymphatic duct. Vascular endothelium-bound lipoprotein lipase (LPL) cleaves FAs off from TGs of Chy to serve the needs of tissues whether it be either oxidation (muscle cells) or storage (adipocytes). LPL gets activated by the C-II apoprotein of Chy. As lesser levels of TG can reach the Chy particle, its core becomes increasingly smaller, yet parallel to it are several surface components which are also lost through the process of *shedding*: e.g. PL, apo-AI and other apoproteins. These will be built into the newly formed HDL (nascent HDL). A lipid exchange also takes place in the circulation: TG is transported from Chy to HDL and Chy receives CE in exchange from HDL. This is mediated by the cholesterol-ester transport protein (CETP), or in other words: the lipid transport protein (LTP). As a result of the constant combined action of LPL and of CETP, the lipid composition of Chy shifts away from the original: it will contain much more CE and much less TG. This lipoprotein particle having also lost some surface components, now qualifies as Chy-remnant. It still has, however, the proteins apo-B48 and most importantly, Apo-E which is recognized on the hepatocyte membrane by the well- known ***remnant receptor,*** which mediates the uptake of remnant LPs into the hepatocytes for final disassembly.

2. ***Endogenous*** lipids synthesized in the liver are released into the circulation by the hepatocytes packed into the VLDL particle, to serve the needs of peripheral tissues. The fate of VLDL is basically identical to that of Chy: its surface apo-CII activates LPL, therefore, its TG content decreases, takes part in the lipid exchange and loses surface components through *shedding*. The particle, as a result, becomes richer in CE and poorer in TG, yet still has apo-E on its surface, hence, it can be considered a remnant LP. Actually, a portion of it is eliminated from the circulation by the liver remnant receptor. However, it is defined as an intermediate density lipoprotein (IDL), and most of it is further metabolized into LDL, of which, does not have any apoproteins but only apo-B100. Having no apo-E, the remnant receptor cannot eliminate LDL from circulation. A special domain on apo-B100 is recognized by the LDL receptor (LDL-r) which is found on most peripheral tissues and the liver and mediates LDL uptake to the cells.

   When LDL is bound to its receptor, the LDL-receptor complex enters the cell via endocytosis, the LDL becomes disassembled (digested) in the endosome (lysosome) and the LDL receptor enters back to the membrane (recycling). The CE of LDL is hy-

drolyzed and cellular FC levels rise. This is the often referred to, ***specific route of LDL uptake*** to cells. Once cholesterol is taken up by the specific route, it has three main consequences concerning cellular cholesterol homeostasis:

1. FC is esterified by the acyl-CoA cholesterol acyl transferase (ACAT) enzyme, since cholesterol's intracellular storage form is CE.
2. LDL receptors on the cell surface are down-regulated, whereas is the transcription of the LDL-r gene and the translation of its mRNA diminishes, since ample cholesterol is already available for the cell.
3. It halts the intracellular cholesterol synthesis by down-regulating one of the first key enzymatic step, the HMG-CoA reductase.

3. **The reverse cholesterol transport** takes FC from the peripheral cells, converts it into CE and transports it towards the liver. Its key actor is the HDL particle, formed from apo-AI synthesized by the liver and the intestine and from surface components (PL, apoproteins, FC) shed from VLDL and Chy. Therefore, the newly formed (*nascent*) HDL takes the shape of a disc, since apolar lipid, which forms the bulky core, is not yet packed into it. While circulating, CE is formed from FC by the HDL-bound lecithin cholesterol acyl transferase (LCAT). Its substrates are FC and lecithin and the products are lysolecithin and CE. The other apolar lipid, TG, enters into HDL via the CETP mediated lipid exchange, during which CE gets from HDL- to VLDL/IDL and Chy. Eventually both, CE and TG enters into the hepatocytes via the remnant receptor, while hepatic uptake of HDL is mediated by a specific *scavenger receptor* (SR-BI) and lastly, LDL is taken up by the hepatocytes via their LDL-r.

The liver is the only organ which can rid the body of the superfluous cholesterol by secreting it into the bile in the form of cholesterol proper or in the form of bile acids. Both undergo enterohepatic recirculation, and the recycled amount bears a regulatory feed-back effect on the excretion.

9.3.2. HYPERLIPIDEMIAS

Elevated plasma levels of LPs, i.e. hyperlipidemias have been classified by D.S. ***Fredericksen*** into five major types, according to the particular LP fraction affected and denoted with ascending roman numbers from I to V. This respected phenotypic classification of Freder-

9.6. table

Types of hyperlipoproteinemias (Frederickson)

Phenotype	Elevated lipoprotein	Strongly elevated lipoprotein	Mildly elevated lipoprotein	According to genotype	
				primary disorders	secondary disorders
I	chylomicron	triglyceride		familial lack of lipoprotein-lipase or apo-C-II	SLE
IIa	LDL (β)	cholesterol		familial hyper cholesterolemia or familial combined hypelipidemia or polygenic hyper cholesterolemia	nephrosis hypothyroidism dysglobulinemia hepatoma Cushing's syndrome
IIb	LDL (β) and VLDL (pre-β)	cholesterol and triglyceride		familial HC, familiary combined hyperlipidemia	same as hyper cholestrolemia
III	LDL(β)	cholesterol and triglyceride		familial dys-beta-lipoproteinemia („remnant" hyper lipidemia)	hypothyroidism SLE
IV	VLDL(pre-β)	triglyceride	cholestrol	familial hypertriglyceridemia „sporadic" hyper triglyceridemia	diabetes uremia nephrosis lipodystrophy
V	VLDL(pre-β) chylomicron	triglyceride	cholestrol	familial hyper-TG familial combined hyperlipidemia	estrogen-effect dysglobulinemia

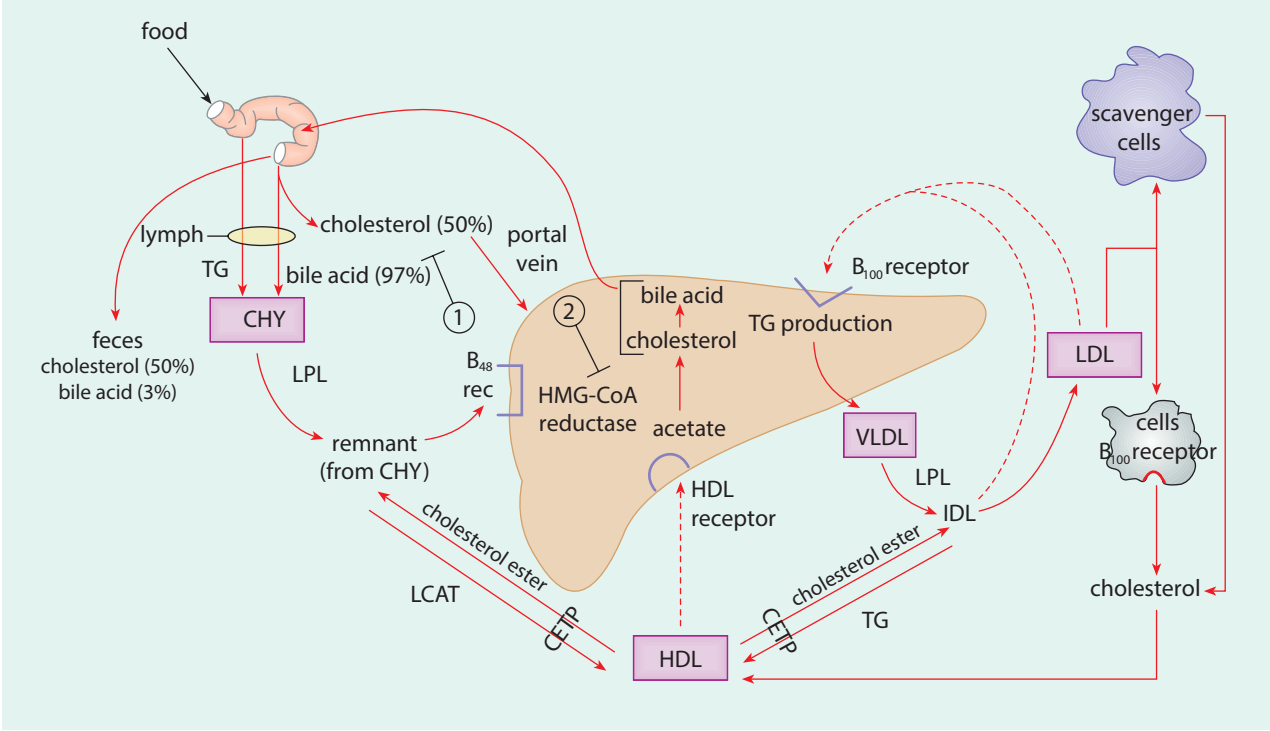

Fig. 9.28.: Lipoprotein metabolism: exogenous and endogenous pathways (centripetal and centrifugal routes).

ickson (Table 9.6.) has been adopted by the WHO and is today, still in use, regardless whether the hyperlipidemia is primary (genetic) or secondary.

Primary hyperlipidemias: These are defects, which directly affect particular steps of the lipoprotein metabolism. Various forms accumulate in certain families (inherited predisposition) which are either monogenic or polygenic genetic determination. The most prevalent forms include the following:

a) *Familial hypercholesterolemia* (FH): In most cases, it causes IIa type hyperlipidemia, due to a defect in the LDL receptor gene (LDLR: 60-80%), smaller parts, due to a defect of the apo B100 gene (APOB: 1-5%), or paraprotein convertase subtilisin/kexin type 9[4] (PCSK9 0-3%) or other, is still not identified as a gene. Monogenic, its inheritance is *co-dominant*, i.e. heterozygotes are also affected, however, their plasma Chol-levels do not reach the extreme high values found in homozygotes. Homozygotes are rare, yet heterozygotes are represented at or about 1:500 in the general population. Homozygotes develop ischemic heart disease due to coronary atherosclerosis during their teenage years or in their early twenties. Heterozygotes usually show these signs later, from 30-35 years of age. Cholesterol depositions in the form of xanthomas surrounding the tendons or on the palms or sole, and also in form of corneal arcus in the eye are discovered early among homozygotes. These may also develop in heterozygotes, yet later, and more often, are missing. In these patients, no functional LDL receptor is available for the LDL particles to be eliminated from the circulation, hence, these accumulate in the blood and enter in a nonspecific manner into the interstitium. There, the LDL becomes modified (oxidized) and invading monocytes/macrophages take it up via their scavenger receptors, since oxLDL constitutes a chemotactic stimulus regarding these cells. OxLDL laden macrophages transform into foam cells which disintegrate and release Chol to deposit in the extracellular matrix (ECM). This event, though not detectable by the unaided eye, is the first step towards the atherosclerotic plaque formation in the subendothelial space of the vessel wall.

b) *Familial combined hyperlipidemia*: Leads to IIb or V type hyperlipidemia. Its inheritance is polygenic. Its consequences rank among the FH heterozygote cases. Its frequency in the European population is at or about 1:300.

Although it is not clinically considered hyper-, a very rare, genetically determined form of **hypo**lipoproteinemia is known as, **Tangier disease**, the importance of

which, is in revealing its molecular mechanism aids in better understanding reverse cholesterol transport. In the vessels of the affected homozygotes, similarly to FH, although in a less pronounced degree, the atherosclerosis is more pronounced, and in some of their tissues (e.g. the tonsils), lipid deposits are formed, while the HDL is nearly completely missing from their blood. The genetic defect affects the ABCA-1 gene, the product of which is referred to as "*cholesterol efflux protein*" (CEP), which functions in the cell membrane. The nascent (discoid) HDL particle can be developed with the aid of CEP, taking up FC (and some PL or other lipids) from cells. Among patients, a complete dysfunction of CEP causes the lack of HDL (and reverse cholesterol transport). The transcription of the ABCA1 gene is regulated, among others, by the LXR (liver-X-receptor) ligand-induced transcription factor (member of the steroid receptor-family). The LXR binds oxysterols, which arise by oxidation of cholesterol. This system, at appropriate HDL levels, is able to remove cholesterol even from cholesterol-containing macrophages of the vascular wall, therefore, determining its potential pharmacological benefits opens new perspectives regarding the treatment of hyperlipemia.)

Secondary hyperlipidemias are accomplices to diseases in which the prime pathomechanism affects lipoprotein metabolism as a side effect. In these cases, inheritance/genetic determination does not play a role. Referred to as, '*secondary*', yet these hyperlipidemias, are of ***prime importance***, since these often associate with diseases which affect a mass population. In these cases, proper and successful treatment of the underlying pathology corrects the lipoprotein anomaly.

Obesity and associated *metabolic syndrome* (and/or type 2DM) causes concomitant Chol and TG elevation (IV, V or I type hyperlipidemia). The decrease of LPL activity due to the insulin resistance, including the activation of hormone-sensitive lipase are key players, since ample FA supply from the periphery is, in part, stored in the liver (cause of *hepatic steatosis*), yet most of it is released and packed into the TG of VLDL. Peripheral "lipid-*clearance*" being suppressed, of which, the result is elevated lipid levels.

Hyperlipidemia caused by *alcohol-abuse* primarily affects TG-levels (IV, V type). Ethanol oxidation in the liver partly runs on the same pathways as FA oxidation (microsomal enzyme system), hence, FA oxidation is suppressed with consequential fat accumulation in hepatocytes (steatosis) and increased TG/VLDL formation.

Liver damage typically causes type III hyperlipidemia due to the remnant lipoprotein-eliminating disability of the liver. In *cholestasis,* the accumulation of an unusual LP fraction of lamellar structure (LP-X) has been described, in which albumin, and increased quantities of phospholipid and non-esterified Chol are found in a covalent bond. The mechanism of its production has not yet been clarified, however, the regurgitation of bile salts or decreased activity of LCAT enzyme may have a role.

Nephrotic syndrome leads to substantial albumin and AI apoprotein loss due to massive proteinuria. The liver responds with increased protein synthesis to replenish losses, yet eventually disordered protein oversupply affects not only the apo-AI, but also the apo-B100 protein, hence the VLDL output is increasing. Apo-AI is continuously lost through the kidney, causing a low HDL level, which is a very atherogenic constellation, even with only moderately elevated LDL. In *chronic renal failure,* apart from the proteinuria, certain uremic toxins contribute to the hyperlipidemia by inhibiting LPL.

Chronic inflammatory conditions (rheumatoid arthritis, SLE, etc.) elevate cytokine levels (IL-1β, TNF-α, IL-6 etc.) which activate peripheral lipolysis, cause insulin resistance of the skeletal muscle and hasten acute-phase protein (CRP) production of the liver. The latter ones and the cytokines, together, cause vascular endothelial dysfunction and inhibit the first step of the reverse cholesterol transport, the entry of FC from the cell into the HDL particle. The result is hyperlipidemia in a particularly atherogenic constellation.

Hypothyroidism elevates Chol level due to the decrease of LDL receptors (type II, III, sometimes IV hyperlipidemia). Proper thyroid hormone substitution rapidly corrects it.

Lipid levels rise statistically in the population among the aged. Women are at lower risk, due to the estrogen's protective effect, and, when compared with the same age in men, their HDL level is higher, and the total Chol level is lower, usually until menopause. The other ovarian steroid, progesterone, however, is not protective, which may explain the common hyperlipemia in *pregnancy*. Corticosteroids and anabolic steroids elevate TG and Chol levels as well as the diuretic thiazides used to be preferred more frequently decades ago. The so called beta-blocker antihypertensive agents may elevate TG levels.

Lipoprotein-a (LP-a) contains apoprotein-a, which is a modified apoB-100, and to which an accessory pro-

tein is linked via an S-S bridge. This is able to interfere with the enzymatic activity of the plasminogen activator, hence, its presence is considered particularly antifibrinolytic and prothrombotic, hence, pro-atherogenic.

9.3.3. THE ROLE OF LIPIDS IN THE ATHEROSCLEROTIC PROCESS

1. Endothelial damage

Damaging agents:

- high blood pressure – causes increased shear forces, especially at bifurcations of arteries or on walls of twisting arteries
- high se-LP levels (especially high LDL, VLDL + low HDL = dyslipidemia)
- smoking – reactive oxygen species (ROS/free radicals) accumulate
- insulin resistance – high glucose level (protein/LDL glycation)

Consequences of the endothelial damage:

- *insufficient vasodilator mechanisms:* **NO** production ↓, **PGI_2** ↓, **endothelin-1** (Et-1) production ↑. Endothelin's effect is also altered: while intact endothelium (due to different Et-receptors available) responds with increased NO production, and with damaged endothelium direct vasoconstrictor effect of Et-1 dominates and stimulates vascular wall smooth muscle cell proliferation (**TXA_2** production ↑).
- *adhesion molecules* appear on the surface of endothelium: P-selectin, E-selectin (leukocyte rolling) **VCAM-1**, ICAM-1 (adhesion and inflammatory cell emigration).
- *leukocyte chemoattractants* are produced in the damaged endothelium: e.g. macrophage chemoattractant protein-1 (**MCP-1**), macrophage colony stimulating factor (M-CSF). These stimulate the emigration of endothelium adherent monocytes into the subendothelial/intimal space.
- *cytokines* are released: **TNF-α, IL-1β, IL-6** which initiate inflammatory events.

Development of fatty streak

Increased levels of LDL enters the vessel wall through the (damaged) endothelium. It binds to ECM fibers and becomes further modified (oxidized, acetylated, glycated etc.). Modified LDL (mo-LDL) constitutes a damaging agent to the endothelium and serves as a chemoattractant and activator for invading monocytes to transform into macrophages, which express copious scavenger receptors (SR). *Modified LDLs do not bind to 'normal' LDL receptors (LDL-r), and these can be taken up exclusively via the SRs into the macrophages.* Today, several classes of SRs are known, such as SRA, SRB1 and CD68 residing mostly on activated macrophages. SRs flood macrophages with cholesterol esters, since the

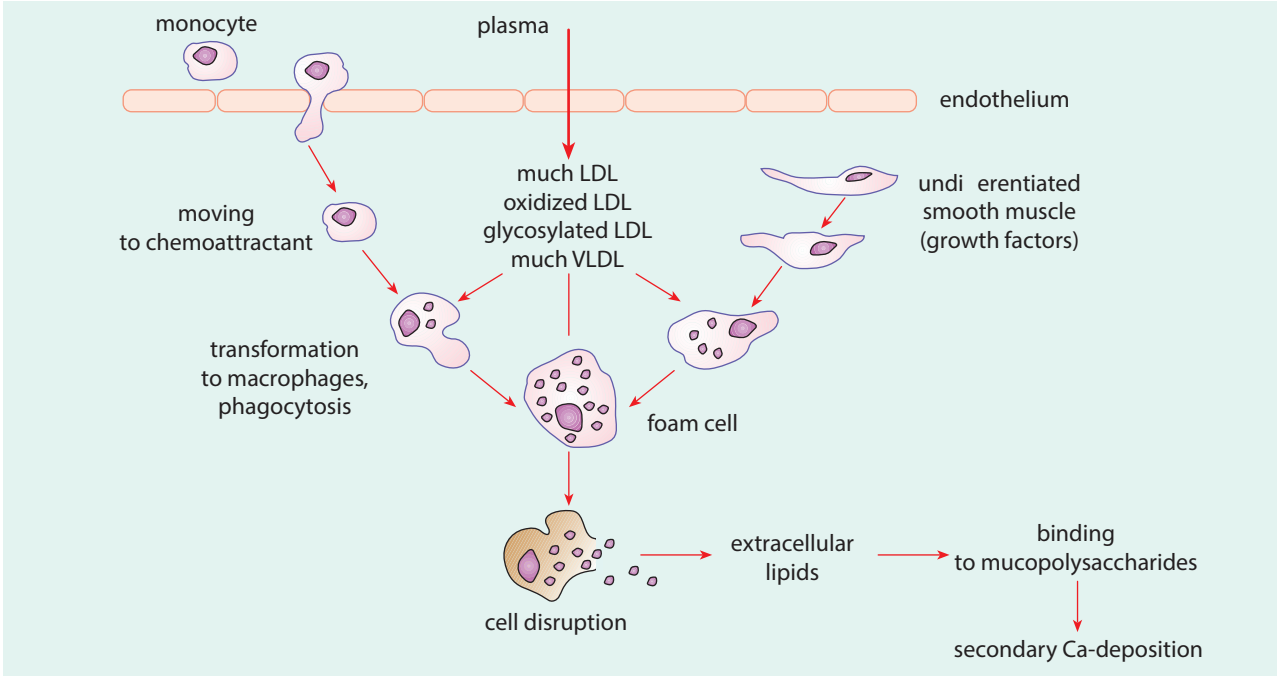

Fig. 9.29.: Role of lipids in atherogenesis.

mo-LDL uptake is concentration-gradient-driven and no negative feedback operates to halt the process. The cholesterol-laden macrophage is referred to as a ***foam cell***. For years, foam cells had been considered irreversibly damaged cells destined to die, due to the enormous LP uptake simply releasing their lipid content into the subendothelial ECM, establishing the lesion, defined as fatty streak. Today, however, research has shown the process is far from being totally irreversible.

Factors retarding the atherogenic process.

- **paraoxonase enzyme** (HDL-attached) is able to regenerate the minimally oxidized LDL.
- **ABCA1 pump** or **Cholesterol efflux protein** (CEP: the protein coded by the **A**TP-**B**inding **C**assette **A1** gene): It mediates the efflux of FC from foam cells – this Chol is taken up by HDL and gets esterified by LCAT en route towards the liver.
- foam cells produce ApoE protein which may aid in the elimination of LPs from circulation.

2. Inflammation – development of the instable plaque

Inflammatory process in the vessel wall is initiated and maintained by the *damaged endothelium*, the *modified LDL* and the *activated macrophages* which recruit other inflammatory cells, most notably, *lymphocytes*.

The endothelium and activated macrophages produce inflammatory cytokines: TNF-α, IL-1β, IL-6, establishing a self-sustaining cascade: SR expression on macrophages is further stimulated, novel monocytes are recruited to the site and are then activated to become cytokine and SR expressing macrophages, etc.

Consequences of inflammation:

Apart from cytokines, *macrophages* produce **ROS** which accelerate endothelial and tissue damage including the modification (oxidation) of the LDL particles. Lysosomal enzymes released from macrophages also contribute to tissue damage, likewise, **the myeloperoxidase (MPO) of macrophages** which may also hasten LDL oxidation.

3. Fibrosis – development of the instable plaque

The inflammatory signals, including growth factors, such as platelet derived growth factor (**PDGF**) derived not only from platelets but also from damaged endothelium and activated macrophages, plus the cytokine interferon-γ (**IFN-γ**), attract and **activate smooth muscle cells of the vessel wall** from the media layer. These transform into **myofibroblasts** which are able to proliferate rapidly and to produce extracellular matrix proteins including ECM degrading enzymes (collagenase, elastase, stromolysine and matrix metalloproteinases / MMP/).

Proliferating myofibroblast and the matrix deposited around them results in the fibrotic transformation of the growing plaque's cap, while a soft fatty core is formed beneath from foam cells and the cholesterol content released from dying foam cells. The cap is exposed to the sheer stress of blood flow, hence, it is prone to rupture in the 'shoulder' (edge) region. Activated macrophages which produce matrix degrading enzymes, promote the probability of rupture, by weakening the edge of the cap or, instable plaque. Once it ruptures, the released plaque content and the denuded vessel wall collagen exposed to the blood induce thrombosis which may result in total and permanent (as in AMI), or partial/fluctuating (as in instable angina) occlusion of the vessel lumen.

4. The complex plaque

At an advanced stage of fibrosis, Ca^{++} is deposited into the plaque making it firm and decreases the risk of rupture, detailed above, however, on the surface, erosion (usuration) of the endothelium may cause repeated platelet aggregation and partial or total thrombotic occlusion of the lumen. Repeated occlusion and recanalization is often evident, during a postmortem examination, in which it can be seen, various layers deposited on top of one another in a longstanding plaque. Chronically repeated, diffuse and multiple, yet transient or partial coronary occlusions often lead to ischemic heart disease (IHD), and referred to, more appropriately, **ischemic cardiomyopathy**.

9.3.4. PRINCIPLES IN THE TREATMENT FOR HYPERLIPIDEMIAS

1. Diet

- optimize caloric intake and attain and maintain the ideal body mass (BMI 20-25)
- calories from fat intake must be kept below 30% of the total caloric intake
- increase the ratio of PUFA containing fats (fish and plant-derived) within the fat intake
- maintain daily cholesterol intake below 300 mg (meat, fat, bacon, eggs etc.)
  (N.B.: 1 egg (hen) contains ca. 300 mg cholesterol)

- prefer menu items with complex carbohydrates and fiber, such as, whole grain cereals and vegetables

2. **Physical exercise** is highly recommended, however, not just regarding weight loss, but it also bears merit on itself (e.g. antiinflammatory).
3. **Medications** need to be introduced, if proper changes in lifestyle fail to decrease the lipid levels to the optimal target values. If no other risk factors for atherosclerosis are present, the following lipid levels are to be attained: ***total Chol <5.0 mM, LDL-Chol <3.0 mM, HDL >1.2 mM.*** The presence of one or more risk factors, other than hyperlipidemia, necessitates more stringent target values.
   - *Statins:* They inhibit the HMGCoA-reductase enzyme, and are, to date, known as one of the most effective strategies in decreasing plasma Chol levels, and increasing the LDL uptake to the liver. In the last 7-9 years, new data show how their anti-atherogenic effect exceeds what might have been expected solely from the reduction of Chol levels. Their anti-inflammatory effect may be in the background. They may cause altered hepatic function (liver enzymes elevated in the blood) and rhabdomyolysis, particularly when joined together with fibrates.
   - *Fibrates:* These act on PPARα receptors, and primarily decrease TG levels. They promote FA oxidation in the liver and the clearance of VLDL particles.
   - *Chol binding resins:* They interfere with the enterohepatic recirculation of Chol and bile acids, hence, promote LDL uptake to the liver and Chol/bile acid excretion to the gut. As a side effect, these bind not only Chol, but also fat soluble vitamins/medicine and cause diarrhea. They are no longer used.
   - *Nicotinic acid derivatives:* These inhibit peripheral lipolysis, decrease the FA supply to the liver, hence, hepatic TG synthesis and VLDL excretion are lowered. In addition, HDL production is increased.
   - *The n-3 fatty acids* (fish): They inhibit VLDL synthesis, hence, decrease TG (and to some extent, Chol) levels.
4. **Future considerations (under development):** Support of HDL's antioxidant capability (paraoxonase). Another means is through the pharmacological manipulation of liver-X receptor (LXR) and farnesoid-X receptor (FXR) of the hepatocytes. The former one binds oxidized Chol derivatives, the latter one recognizes bile acid derivatives: and both are part of the feed-back system which regulates biliary Chol excretion. The PCSK9 proprotein convertase enzyme may yield promising pharmacological benefits in the near future.

9.4. DISORDERS OF INTERMEDIARY METABOLISM IN THE ELDERLY

Among the elderly, various disorders of intermediary metabolism are particularly common. These may affect the protein/nucleic acid-metabolism including the carbohydrate- and lipid-metabolism.

In protein metabolism, the most frequent abnormality is the partial starvation associated with protein loss (ch. 8.4.2.2.), leading to sarcopenia. Moderately elevated levels of protein intake may compensate the poor absorption, yet the incorporation of amino acids is possible, only in the event of appropriate physical activity.

In seniors, hyperuricemia is a far more frequent finding, exhibiting the disorder of purine metabolism. In its mechanism, the lasting (although moderate) hypoxia of several tissues and the consequent activity of xanthine oxidase and increased urate production together, with the poorer renal urate excretion due to the elevated lactate level, are of primary importance (the excretion can be inhibited by some pharmacons, such as salicylates). Among its consequences, arthropathy, with a tendency for atherosclerosis and hypertension, and also the development of urate stones and occasionally urate nephropathy should be regarded.

In the past, 2DM was thought to be a disease exclusively associated with the older generation. Today, it is clear in which 2DM may be diagnosed among younger individuals, yet it is unquestionably far more frequent in older individuals. It may be disputed in which, to varying extent, the physiological, age-related insulin resistance, specifically with regards to the level, when older patients must be treated in the form of diabetic patients. Obviously, it is of considerable benefit to the patient, whether the therapy to be introduced, or its side effects does not cause more harm than a moderately elevated blood glucose level or a shift of OGTT. An obvious characteristic regarding diabetes and its late complications is, naturally, the need to delicately administer effective forms of therapy to senior patients. The well-known aspect of forgetfulness among senior patients necessitates strict control in respect to the treatment, in addition to maintaining the appropriate levels of both food and water. The risk and peril of hypoglycaemia must also be considered,

and, although it is less frequent than in the young, it is far more severe and has a much higher mortality rate among the elderly. Care should be taken regarding the possible drug-interactions and the various, accompanying disorders regarding the salt/water balance (vomiting, diarrhea, hot environment, etc.).

Primary disorders generally associated with lipid metabolism affect not so much the old, but rather the younger generation. In contrast, the secondary disorders of lipid metabolism are more frequent among elderly patients, and the se-lipid levels increase with age. In females, the estrogen-level features a defense action for the procreative era, but not after the arrival of menopause. Several pharmacons often applied in treating elderly patients include diuretics and beta-blockers, meant to increase the se-TG and -Chol levels. Additionally, high lipid levels are often accompanied by cardiovascular complications also seen among the elderly. Unfortunately, certain drugs which decrease the lipid levels in some individuals have very pronounced and severe side-effects (rhabdomyolysis and ATN), and distinctly limits their therapeutic usage. Instead of these, the dietary decrease in lipid levels can be applied, or curing the primary disease infers yet another solution in caring for elderly patients.

Further readings:

Classification and Diagnosis of Diabetes. Diabetes Care 40 (Suppl. 1): S11–S24, 2017. https://doi.org/10.2337/dc17-S005

Feingold K and Wilson DP (Eds.): Diagnosis and Treatment of Diseases of Lipid and Lipoprotein Metabolism in Adults and Children. ENDOTEXT Comprehensive free online endocrinology book, http://www.endotext.org/section/lipids/ or: www.ncbi.nlm.nih.gov/books/NBK305898/

Harrison's Principles of Internal Medicine. I., II., 19th Edition. McGraw-Hill, 2015.

Lipid Disorders. The Merck Manual / Professional Version, http://www.merckmanuals.com/professional/endocrine-and-metabolic-disorders/lipid-disorders/

Lipid Metabolism, UNIT III: pp. 173–244. In Lippincott's Illustrated Reviews: Biochemistry. 5th Ed., (Eds.: Harvey RA and Ferrier DE). Wolters Kluwer / Lippincott Williams & Wilkins, 2011.

Nirosha K, Divya M, Vamsi S, Sadiq M: A review on J Nov Trends Pharm Sci 4: 81–92, 2014.

Parhofer KG: The treatment of disorders of lipid metabolism. Dtsch Arztebl Int, 113: 261–268, 2016.