

Biochemical examinations of the pancreas

In terms of laboratory diagnostics, the examination of the pancreas can be divided into:

- examination of **endocrine functions of the pancreas**;
- examination of the **exocrine part of the pancreas**:
 - evidence of **acinar cell damage**;
 - examination of **pancreatic juice secretion**.

The endocrine function of the pancreas

The intrinsic secretory part of the pancreas mainly produces insulin, glucagon and somatostatin. Disorders of the production of these hormones are discussed in the relevant chapters, especially in connection with diabetes mellitus.

The exocrine function of the pancreas

Examination of pancreatic exocrine function is not widespread. It is used mainly in the diagnosis of chronic pancreatitis. It is based on **direct tests**, which measure the concentration or activity of pancreatic enzymes (chymotrypsin, elastase) in stool, and **indirect tests**, based on the administration of suitable substrates of pancreatic enzymes and the detection of fission products caused by their digestion.

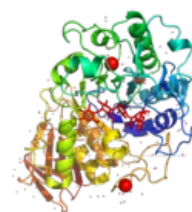
Biochemical examination of the pancreas

Extensive damage to pancreatic tissue occurs mainly in acute pancreatitis. It is a life-threatening sudden abdominal event in which the digestive enzymes of the pancreatic juice are activated, leading to the digestion of the pancreatic tissue. Acute pancreatitis is most often triggered by overpressure in the common pancreatic and bile ducts (in cholelithiasis) and alcoholism.

The breakdown of pancreatic acinar cells leads to the spillage of their components into the blood. We can then demonstrate a high catalytic concentration of pancreatic enzymes, especially α -amylase and pancreatic lipase.

α -amylase

α -amylase hydrolyzes α -1-4-glycoside bond; The pH optimum of α -amylase is between 7.0-7.2. It occurs in the body in two forms - as a salivary and pancreatic isoenzyme according to organ origin. Both isoforms of AMS differ from each other by the sugar component and can be distinguished electrophoretically or by precipitation using a special lectin or antibody. α -amylase is formed in the acinar cells of the pancreas and accumulates in zymogenic granules. It enters the intestinal lumen in the form of pancreatic secretion (pancreatic juice) along with other digestive enzymes. Under physiological conditions, the enzyme molecule is not absorbed by the intestinal surface and the serum level is low, corresponding to the activity of the enzyme released into the circulation directly from the glandular cells, resp. lymphatic drainage. The molecular weight of α -amylase is 55,000. A-Amylase is eliminated from the circulation in the kidneys by glomerular filtration.



3-D Structure of Alpha-amylase

The macro-form of the enzyme (macroamylase) is formed by the binding of the enzyme to certain blood serum proteins, especially immunoglobulins, circulating immunocomplexes or other glycoproteins. The macro-form of the enzyme has a significantly higher molecular weight (from 150,000 to 2,000,000) and is therefore not eliminated by glomerular filtration. For clinical diagnosis, serum and urinary α -amylase levels are determined and the amylase / creatinine clearance index is calculated.

In the laboratory, protein concentration can be determined by immunological techniques or enzyme catalytic concentration using specific substrates. The presence of inhibitors in serum and the formation of enzyme macro-forms should be considered when determining both mass and catalytic enzyme concentrations. The commonly used determination of α -amylase activity is based on the cleavage of a chromogenic substrate. Older processes that used derivatives of the natural substrate, starch, were difficult to standardize and are no longer used. Current synthetic substrates are derived from maltose, as chromogen is the most commonly used 4-nitrophenyl phosphate. The determination of α -amylase isoenzymes is made possible by the inhibition of one of the two isoenzymes by a specific monoclonal antibody.

In the most common arrangement, the salivary isoenzyme amylase is first inhibited by the antibody in the sample. The reaction is started by adding a substrate protected by ethylidene - glucose heptamer, at one end of which a chromogen is bound and at the other end of ethylidene. Amylase from the sample hydrolyzes the oligoglucoside chain. The color nitrophenol is then released by the action of another component of the reaction mixture, glucosidase. Because α -glucosidase can only cleave end-chain glucose and does not recognize ethylidene-protected glucose, a substrate that has not been hydrolyzed by amylase is protected from glucosidase.

Reference values

S-AMS total serum amylase **0,30-1,67 μ kat/l**
U-AMS total amylase in urine **< 7,67 μ kat/l**
S-pAMS pancreatic amylase in serum **0,22-0,88 μ kat/l**
U-pAMS pancreatic amylase in urine **< 5,83 μ kat/l**
macroamylase undetectable

Interpretation of findings

From a practical point of view, an increase in serum α -amylase activity is an important finding. It can be caused by:

- **increased amylase** release from damaged pancreatic or salivary gland cells, or
- by **reducing glomerular filtration**, where this small protein is lost to the primitive urine to a lesser extent than usual.

Hyperamylasia with damage to the pancreas or salivary glands is accompanied by an increase in urinary amylase activity during normal renal function; however, it is necessary to take into account that it appears in the urine with a delay of several hours. In this case, it remains to distinguish whether amylase comes from the pancreas or salivary glands. If a decision cannot be made on the basis of the clinical picture, the determination of isoenzymes will give an answer.

Decreased glomerular filtration of amylase is most often due to renal insufficiency. In this case, hyperamylasemia will be accompanied by low urinary concentration and amylase activity. Another, much rarer cause of decreased renal amylase clearance is macroamylasemia.

Lipase

A newer and more specific marker of pancreatic damage is lipase.

Lipase (triacylglycerol acyl hydrolase, EC 3.1.1.3) is a glycoprotein with 420,449 amino acid residues and a molecular weight of 46,000-56,000 for pancreatic lipase and 32,000-39,000 for serum lipase.

It is a hydrolytic enzyme that breaks down triacylglycerols with fatty acids with a chain longer than 12 carbons, in the presence of bile acids it breaks down fat into monoacylglycerols and diacylglycerols. Fatty acids at the sn-1 and sn-3 positions are cleaved predilection. Like α -amylase, lipase is produced by pancreatic glandular cells and secreted into the intestinal lumen in pancreatic juice. The concentration gradient between pancreatic tissue and serum lipase is about 20,000: 1.

The determination of lipase activity involves various procedures:

- enzymatic cleavage of the natural substrate;
- enzymatic breakdown of chromogenic and fluorogenic substrates;
- immunological methods (ELISA, latex agglutination).

Nephelometric and turbidimetric procedures based on the cleavage of the natural substrate triacylglycerol are most often used. Most lipase enzyme assay kits also contain co-lipase. The turbidimetric determination of lipase activity is based on the clarification of the oil emulsion by the action of lipolytic activity. However, this process can also be affected by other components of the serum, such as the so-called clarification factor pseudolipase. These are most often circulating IgM-type immunocomplexes. For the differential determination of serum pancreatic lipase in addition to pseudolipase using a standard turbidimetric procedure, a procedure based on the inactivation of pseudolipase with β -mercaptoethanol is developed, which leads to the dissociation of IgM complexes. Newer chromogenic assays are based on an enzyme cascade of lipase that cleaves 1,2-diacylglycerol, glycerol kinase, glycerol-3-phosphate oxidase, and peroxidase with a chromogenic product. A completely new type of technique for the determination of pancreatic lipase is based on changing the conductivity of the solution by releasing fatty acids from the substrate - trioleate; it is detected by an acoustic sensor and the measured value is the frequency response.

Normal values

up to 1 μ kat / l

An **increase in pancreatic lipase** concentration is a more specific sign of acute pancreatitis than α -amylase. Its serum levels remain elevated for about two weeks after the acute event. It does not increase significantly in renal diseases.

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