Glycated proteins

A direct consequence of elevated blood sugar levels is its intense protein binding, which is the essence of **non-enzymatic glycation.** The glycation process is conditioned by the presence of free amino groups in the protein molecule.

The rate of glycation is mainly affected by the concentration of glucose and its duration of action. It also occurs in healthy individuals. Glycation takes place on all serum proteins and leads to the formation of glycated derivatives. Both soluble and structural proteins are glycated.

Non-enzymatic glycation takes place in several phases of the so-called **Maillard reaction** .

- The sequence of glycation reactions is initiated by the aldehyde group of the reducing sugar, which binds to the amino group of the protein. A **labile aldimine** of type **Schiff base** is created (*early glycation products*). Schiff's formation is quick, reaching equilibrium within hours. This reaction is reversible.
- Schiff's base then slowly, within a few days, undergoes chemical rearrangement. A more stable *glycation transition product* is formed, the so-called **Amadori product**, which has the character of a **ketoamine**. This produces a keto group on the second carbon of the sugar, which is characteristic of fructose. The concentration of **fructosamines** formed can be determined by reaction with nitrotetrazolium blue (see below). Amadori's products are also **somewhat reversible**, although the balance is shifted considerably in the direction of their formation. Steady state is reached in a few weeks. The amount of ketoamines can be reduced by normalizing glycemia.
- Aldose, which is two carbons shorter than the original protein-bound sugar, can also be cleaved from Schiff's base. A di-carbon residue remains attached to the protein, which is further oxidized and reacts with another amino group of the protein. The chain of other reactions results in the formation of highly reactive glyoxal-type dialdehydes.
- Amadori products are reactive substances. In weeks and months, regardless of the presence of glucose, Amadori products are formed by reactions mainly with long-lived proteins (eg collagen, elastin, nerve myelin) **advanced glycation products** (*advanced glycation end-products*, **AGE**). These processes are **irreversible**.

Non-enzymatic glycation in long-term hyperglycemia is one of the causes of tissue damage in some organs in patients with diabetes mellitus.

The determination of Amadori-type glycation products is a suitable indicator of long-term glucose concentration and provides **indirect information about the course of glycaemia over a period of time that corresponds to the biological half-life of the protein**. **Glycated hemoglobin** and **glycated proteins** are commonly determined.

The determination of glycated hemoglobin and glycated proteins is used to control diabetes mellitus and to diagnose persistent hyperglycemia. Elevated glycated derivatives indicate that elevated blood glucose levels have prevailed in the patient in recent weeks and that diabetes mellitus has not been adequately controlled.

Glycated hemoglobin

Glycated hemoglobin (**HbA_{1c}**) is considered **the best way to control glucose levels in diabetics**. Glycated hemoglobin concentration indicates blood glucose values in the previous 2-3 months (erythrocyte lifespan). It is determined by chromatographic or immunochemical methods.

Evaluation of glycated hemoglobin concentration (HbA_{1c}) in diabetics

Compensated DM	≤ 45 mmol/mol
The need for a change in therapy	≥ 53 až 70 mmol/mol

Glycated hemoglobin concentration refers to total hemoglobin concentration and is expressed in **mmol HbA_{1c} per mole of total hemoglobin**. You can also find a percentage expression HbA_{1c} of total hemoglobin (1 % ≙ 10 mmol/mol). The assessment of glycated hemoglobin concentration in the table above is indicative. Target concentrations vary according to the risk of hyper-/hypoglycemia in a particular patient.[1]

The glycated hemoglobin assay can also be used to screen for diabetes mellitus. At concentration HbA_{1c} over 39 mmol/mol we suspect diabetes mellitus[2]. Je-li koncentrace HbA_{1c} vyšší než 48 mmol/mol, můžeme diagnózu diabetu mellitu považovat za potvrzenou.[3]

Evaluation of glycated hemoglobin concentration (HbA_{1c}) in diabetes mellitus screening

Physiological values	< 39 mmol/mol
Suspicion of diabetes mellitus	39–48 mmol/mol
Diabetes mellitus	≥ 48 mmol/mol

Fructosamines

Glycated proteins or **fructosamines** have a shorter half-life and their levels reflect the average glucose concentration for the period of 2-3 weeks before examination. Their main component is glycated albumin. Hypoproteinemia may falsely reduce the results. Today, determination of fructosamine concentrations is not a routine test in diabetics.

Evaluation of glycated protein concentration (S-glycated proteins)

Physiological values:	205–285 µmol/l
Good compensation DM:	285–320 µmol/l
Satisfactory compensation DM:	321–370 µmol/l
Bad compensation DM:	nad 370 µmol/l

Principle of determination of glycated proteins (fructosamine)

Examination of glycated proteins uses the reducing properties of fructosamine in an alkaline environment. In the presence of carbonate buffer, fructosamine rearranges to its tautomer - eneaminol, reacts with nitrotetrazolium blue (NBT). During the reduction, the heterocyclic rings of NBT are opened and colored formazan is formed. The rate of formazan formation is directly proportional to the fructosamine concentration.

Like any method that utilizes the reducing properties of some serum components, this test is non-specific. It therefore begins with a few minutes of incubation to remove the effect of fast-reacting reducing agents. In commercial kits, the agent includes the enzyme uricase, which eliminates the reduction of NBT by uric acid.

Links

Reference

1. KAREN, Igor – SVAČINA, Štěpán. *Diabetes mellitus : recommended diagnostic and therapeutic procedures for general practitioners 2018.* - edition. Society of General Medicine ČLS JEP, 2018. ISBN 9788086998992.
2. -. *Recommended procedure for the treatment of type 2 diabetes mellitus*. 2016. Available from <http://www.diab.cz/dokumenty/doporuceni_DM_2015-2.pdf>.
3. -. *Diabetes mellitus - laboratory diagnosis and monitoring of patients' condition*. 2015. Available from <http://www.cskb.cz/res/file/doporuceni/DM/DM_dop_201601.pdf>.