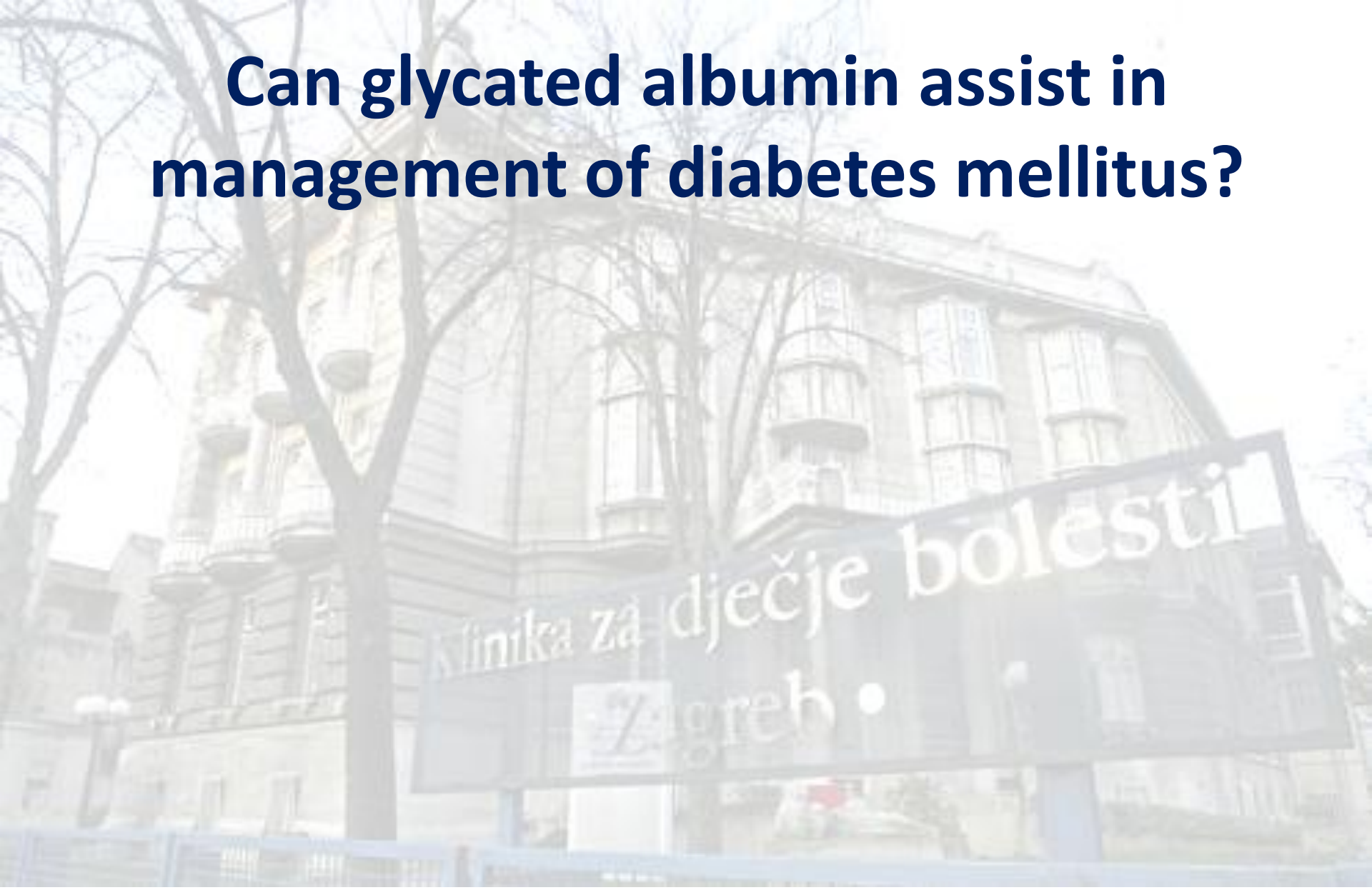
Can glycated albumin assist in management of diabetes mellitus?



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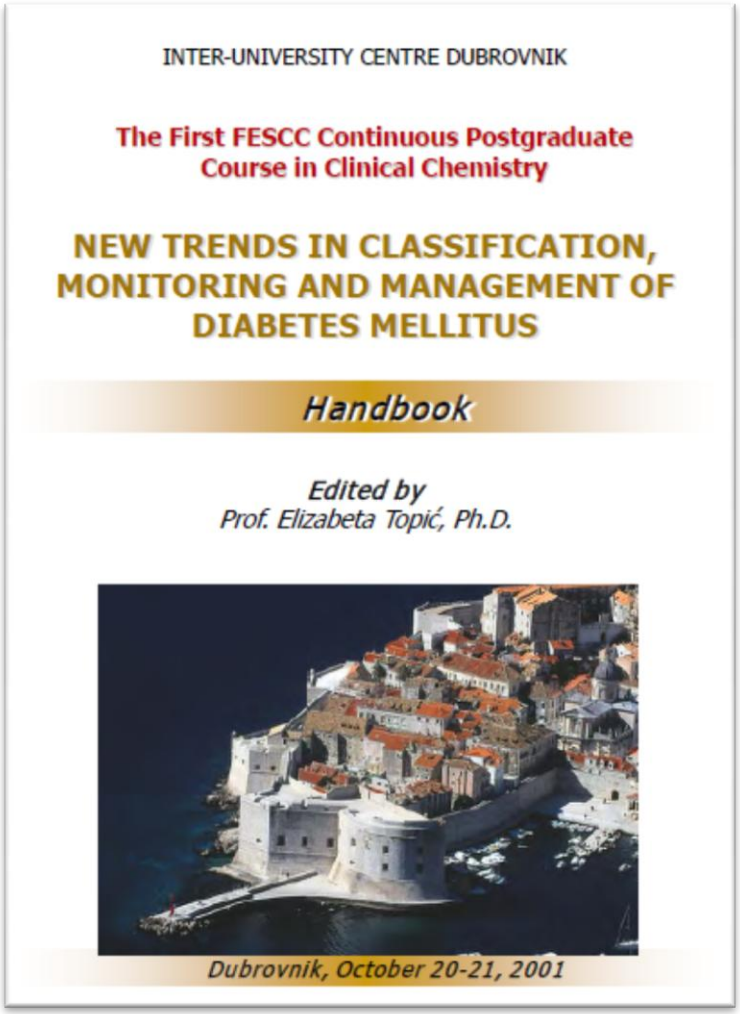
DIABETES MELLITUS
COURSE SUBJECT

2001
2014

WHEN WE PREPARED
PROGRAMME of THIS COURSE
IDEAS?...for topic of my lecture?
My pactice:
Patients with
Confimed diagnosis of diabetes and patients with no history of diabetes but with oneset high glucose concentration in blood

I was
READING HANDBOOK
FROM 1ST COURSE



What can I talking about ?

We all know that glycated hemoglobin (HbA1c) is the recommended and most often used biomarker for assessing hyperglycemia in the practice, but.....

My attention was caught the small paragraf about glycation proteins which isn't HbA1c....

Table of contents

6. MODERN ASPECTS OF LABORATORY DIAGNOSIS AND MONITORING OF DIABETES MELLITUS

Gábor L. Kovács

6.1. Introduction

Recently compiled data show that between 120 and 140 million people suffer from diabetes mellitus (DM) worldwide, and that this number may well double by the year 2025. Much of this increase will occur in developing countries and will be due to population aging, unhealthy diets, obesity and a sedentary lifestyle. By 2025, while most people with DM in developed countries will be aged 65 years or more, in developing countries most will be in the 45-64

6.8.2. Glycated serum proteins (GSP):

Because the turnover of human serum albumin is much shorter (half-life of 14–20 days) than that of hemoglobin (erythrocyte life span of 120 days), the degree of glycation of serum proteins (mostly albumin) provides an index of glycemia over a shorter period of time than does glycation of hemoglobin. Measurements of total GSP and glycated serum albumin (GSA) correlate well with one another and with measurements of HbA_{1c}. In situations where HbA_{1c} cannot be measured or may not be useful (e.g., hemolytic anemia), the GSP assay may be of value in the assessment of the treatment regimen. Several methods have been described that quantify either total GSP or total GSA. One of the most widely used is called the **fructosamine assay**. Values for GSP vary with changes in the synthesis or clearance of serum proteins that can occur with acute systemic illness or with liver disease. In addition, there is continuing debate as to whether fructosamine assays should be corrected for serum protein or serum albumin concentrations.

A single measurement of GSP provides an index of glycemic status over the preceding 1–2 weeks, while a single measurement of HbA_{1c} provides an index of glycemic status over a considerably longer period of time, 2–3 months. Measurement of GSP (including fructosamine) has been used to document relatively short-term changes (e.g., 1–2 weeks) in glycemic status, such as in diabetic pregnancy or after major changes in therapy. However, further studies are needed to determine if the test provides useful clinical information in these situations. Simultaneous measurements of GSP and HbA_{1c} might complement one another and provide more useful clinical information than measurement of HbA_{1c} alone.

Measurement of GSP, regardless of the specific assay method, should not be considered equivalent to measurement of HbA_{1c}, since it only indicates glycemic control over a short period of time. Therefore, GSP assays would have to be performed on a monthly basis to gather the same information as measured in HbA_{1c} three to four times a year. Unlike HbA_{1c}, GSP has not yet been shown to be related to the risk of the development or progression of chronic complications of DM.

And,
I decided to read out the recently published articles and present new insights into the glycated albumin and their role in management of diabetes mellitus today

Lecture have aim to give the answer:

✓Can glycated albumin assist in management of diabetes mellitus?

Goals of prezentation: W,W and W

- **What** is Glycated Albumin (GA)?
- **Why** we need another biomarker?
  - or
  - When (in which situation) we can use it?
- **Which** methods can we used for determined GA today?

1. What is glicated albumin (GA)?

Reminder

Glycation,
- a non-enzymatic Maillard reaction,
- occurs when glucose molecules spontaneously react with the amine group of proteins, giving rise to stable ketoamines.

Table: several important proteins in human that can become extensively glycated under hyperglycemic conditions

Modified proteins cause chronic diseases as diabetes complications.

Matrix proteins	**Enzymes**	**Plasma proteins**
Collagen	Cathepsin B	Albumin
Myelin	Lysozyme	Immunoglobulin
Fibronectin	Pancreatic ribose	Apo A-I, II
Fibrin	Copper/zinc SOD	Apo B
	Carbonate dehydratase	Apo C-I
Membrane proteins	β-N-acetyl hexominase	Apo E
Red cell Glu transport protein	Alcohol dehydrogenase	Haptoglobin
Red cell spectrin	Aldose reductase	Ferritin
Red cell membrane protein	Aldehyde reductase	Transferrin
Endothelial plasma membrane protein	Sorbitol dehydrogenase	α1-antitrypsin
	Na+/K+-ATPase	Plasminogen
Intracellular proteins		Plasminogen activator
Hemoglobin	**Hormones**	Fibrinogen
Crystallin	Thyroid hormone	Fibrin
Tubulin	Insulin	Antithrombin III
Calmodulin		β2-microglobulin
		Ceruloplasmin

*Adapted from Koga M, Kasayama S. Clinical impact of glycated albumin as another glycemic control marker. *Endocrine Journal* 2010;57:751-62.

1. What is glicated albumin (GA)?

- Some of these glycated proteins can be used as **biomarkers to determine the degree of glycemia** in individuals with diabetes or prediabetes.
- Today, **HbA1c is the "gold standard"** for assessing glycemia during diabetes management, and since 2009 it has been recommended by both the ADA and WHO as a diagnostic criterion for diabetes, with a diagnostic cut-off of >6.5% (48 mmol/mol).
- In some patients, **levels of HbA1c are inadequate for determining the average glucose concentration**

1. What is glicated albumin (GA)?

Lack of linear correlation between HbA1c levels and average glucose concentration arises because

numerous factors affect HbA1c levels,
including genetics, hematological factors and the

presence of certain comorbidities, such as hemoglobinopathy, certain anemias, and

disorders associated with shorter erythrocyte lifespan .

Process	Factors	Effect on HbA1c level
Erythropoiesis	iron, vitamin B12 deficiency, decreased erythropoiesis	increase
	erythropoietin administration, iron, vitamin B12, reticulocytosis, chronic liver disease	decrease
Hemoglobin modification	Genetic or chemical modifications of hemoglobin (hemoglobinopathies, HbF, methemoglobin)	increase or decrease
Glycation	alcoholism, chronic renal failure, decreased intra-erythrocyte pH	increase
	aspirin, vitamins C and E, certain hemoglobinopathies, increased intra-erythrocyte pH	decrease
	Genetic determinants	increase or decrease
Erythrocyte destruction	increased erythrocyte life span, e.g. due to splenectomy	increase
	decreased erythrocyte life span, e.g. due to hemoglobinopathies, splenomegaly, rheumatoid arthritis or drugs such as antiretrovirals, ribavirin and dapsone.	decrease
Assays	hyperbilirubinemia, carbamylated hemoglobin, alcoholism, high-dose aspirin, chronic opiate use	increase
	hemoglobinopathies	increase or decrease
	hypertriglyceridemia	decrease

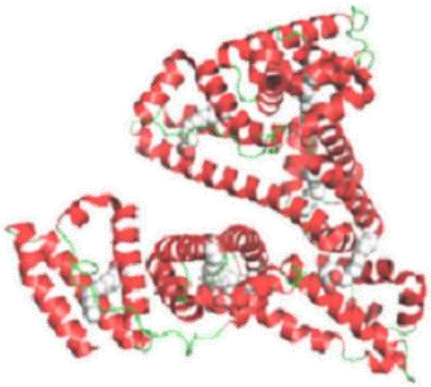
*Adapted from WHO. Use of glycated haemoglobin (HbA1c) in the diagnosis of diabetes mellitus: abbreviated report of a WHO consultation.

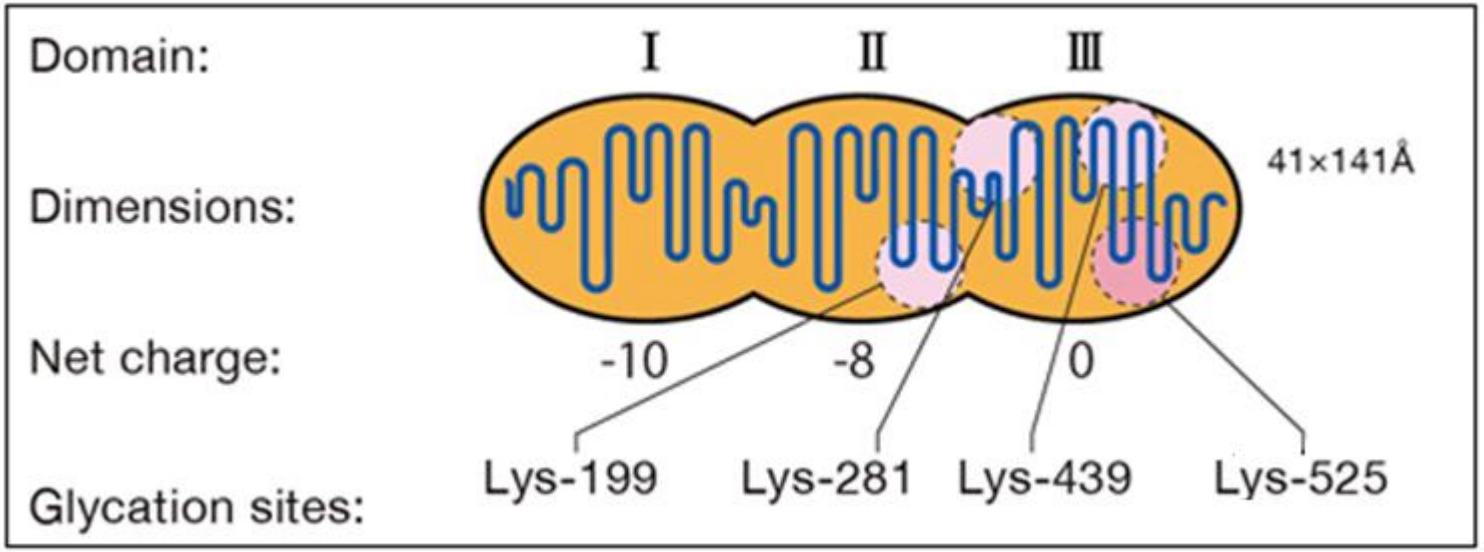
1. What is glicated albumin (GA)?

- The development of new biomarkers of hyperglycemia for cases when HbA1c levels are inadequate has been the subject of intense investigation over the last 5 years.
- One candidate biomarker is albumin, which accounts for approximately 60% of serum proteins and is present in the blood at concentrations of 30-50 g/L.
- This protein is predicted to be highly susceptible to glycation because it contains numerous arginine and lysine residues near its N- and C-termini.
- It persists for 2-3 weeks- once released into the circulation, making it potentially well-suited to be a biomarker that can detect short and mid-term changes.

1. What is glicated albumin (GA)?

Albumin (HSA)
- Most abundant protein in blood
- Produced in the liver
- 20 day lifespan
- Monomeric, 585 amino acids





2. Why we need another biomarker? or When (in which situation) we can use it?

Two reason/situation:

1. Numerous factors affect HbA1c levels

2. Life spine HbA1c in situation when frequent monitoring is required

2. Why we need another biomarker? or When (in which situation) we can use it?

- Diabetes management, especially in early phases, requires **frequent monitoring** because significant changes can occur within 2-3 months.
- Important for:
  - individuals on therapy to **treat prediabetes**
  - patients undergoing **new therapy** or a **change** in their current therapy
  - individuals on **intensive insulin therapy** during early stages of diabetes
  - **pregnant** women
  - patients on **hemodialysis.**

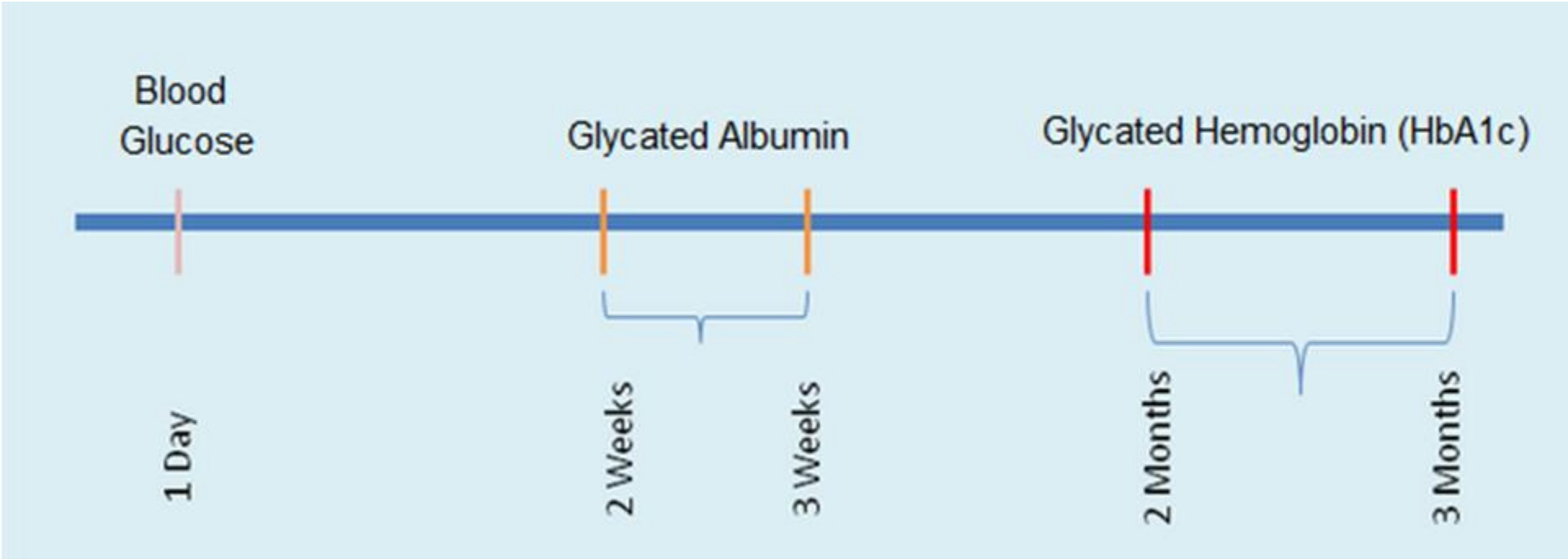
3. Which methods can we used for determined GA today?

- In order for glycated albumin to be measured routinely in the clinic, the American Diabetes Association in 2011 called for studies to develop
- a standardized method for its measurement
- clearly establish its clinical usefulness
- reliability for predicting diabetes-related complications
- Since then, numerous studies have tried to follow these recommendations and determine:

  Is the level of glycated albumin can be useful in managing diabetes

3. Which methods can we used for determined GA today?

Time differential of diabetic monitoring using blood glucose, glycated albumin and HbA1c



3. Which methods can we used for determined GA today?

Recommended methods for determining glycated albumin are:

affinity chromatography, ion-exchange chromatography and high-performance liquid chromatography (HPLC).

Recent research suggests that liquid chromatography-tandem mass spectrometry (LC-MS/MS) may be the "gold standard" method for quantitative determination of glycated proteins, including albumin and all serum proteins, of which are collectively known as fructosamine

....all these techniques are complicated and require sophisticated equipment which is not available for many clinical laboratories

3. Which methods can we used for determined GA today?

Colorimetric determination of glycated albumin

A much simpler and less expensive alternative is a colorimetric method for fructosamine determination known as the nitroblue-tetrazolium (NBT) reduction method. The method was automated soon after it was first described.

reduced specificity	NBT reacts with various endogenous reducing substances, including thiol groups, ascorbate, and NADH--the levels of all of which can vary from sample to sample.

2-thiobarbituric acid (TBA) or phenylhydrazine can instead NBT in colorimetric method

Interfering substances and sources of error

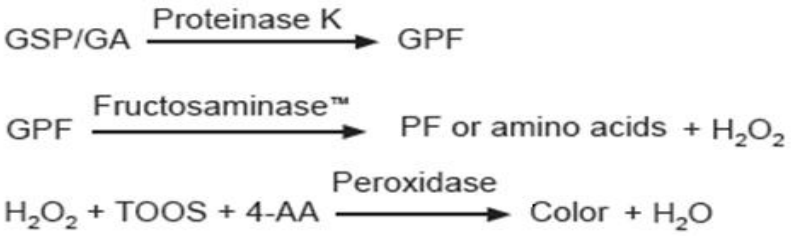
1. EDTA and heparin plasma - lower fructosamine results than serum samples in the NBT colorimetric assay, so the same type of sample should always be used to monitor glycemia
2. Urate, glutathione and vitamin C lead to artificially high fructosamine results.
3. Cysteine, methyldopa, dobesilate calcium, oxytetracycline and hemolysis can cause artificially low fructosamine results (all assays).
5. Bilirubin has been shown to cause falsely elevated fructosamine results.
6. The NBT assay, like other colorimetric assays, is affected by changes in ambient temperature.

3. Which methods can we used for determined GA today?

Enzymatic determination of glycated albumin

Recently a quite precise and automated enzymatic assay for determination of glycated albumin has been commercialized by Diazyme Laboratories, Asahi Kasei Pharma, and Randox Laboratories

Principle of enzymatic assay



Proteinase K digests serum proteins into low-molecular-weight glycated protein fragments (GPF), then a specific fructosaminase™ (microbial amadoriase) catalyzes the oxidative degradation of GPF Amadori product to yield a protein fragment (PF) or amino acids and H_2O_2. The H_2O_2 released is measured by a colorimetric Trinder end-point reaction. The absorbance at 546 nm is proportional to the concentration of glycated serum proteins (GSP) or glycated albumin

performed with serum or plasma on virtually all biochemical analyzers.

multiple determinations do not require multiple blood samples or a total blood sample, as is required for HbA1c determination.

Stability tests indicate that samples for the enzymatic assay can be stored for up to 2 weeks at 2-8 ºC or up to 4 weeks frozen.

3. Which methods can we used for determined GA today?

all enzymatic assay
good analytical characteristics and correlate well
with one another, as well as with HPLC-based methods.

The tests differ principally in **what enzyme** is used **and how results are expressed**: concentration (umol/L or mmol/L) or as glycated albumin fraction (%GA) Determination of %GA also involves determination of total albumin.

3. Which methods can we used for determined GA today?

While the Lucica GA-L kit determines %GA, the Diazyme GlycoGap kit determines the concentration of glycated albumin in umol/L, and the Randox kit determines the concentration in mmol/L.

The Lucica GA-L kit determines albumin using a bromcresol purple (BCP) method that is more specific than the bromocresol green (BCG) method most often used to determine albumin in clinical laboratories. Each assay manufacturer provides reference intervals for glycated albumin for diabetics and non-diabetics in the appropriate concentration units or %GA.

These enzymatic tests show extremely good reproducibility and specificity, correlating closely with glycated albumin levels determined by HPLC ($r > 0.98$). Based on the performance of these enzymatic assays, which according to the manufacturers is evaluated in compliance with guideline EP5-A (CLSI),

the automated test shows the characteristics of a reference method, although it is not yet confirmed from relevant institutions

3. Which methods can we used for determined GA today?

Interference studies

the manufacturer's test insert

1. EDTA plasma samples have been internally validated to show no matrix effects in enzymatic assays; serum should be separated from cells immediately after blood collection.
2. As in the NBT colorimetric assay, cysteine, methyldopa, dobesilate calcium, oxytetracycline and hemolysis can cause artificially low GA /GSP results.
3. As in the NBT colorimetric assay, bilirubin has been shown to cause falsely elevated GA /GSP results.

Several common interfering substances in serum, such as ascorbic acid, glucose, triglyceride, uric acid and hemoglobin, usually show ≤ 10% interference, though the manufacturer's insert for the particular test should be consulted.

3. Which methods can we used for determined GA today?

Variability of glycated albumin and limitations as a biomarker of glycemia

Disorders in albumin metabolism

nephrotic syndrome
Hyperthyroidism
glucocorticoid therapy

involve elevated albumin metabolism.

lower ratios of glycated albumin to blood glucose

liver cirrhosis and hyperthyroidism

involve reduced albumin metabolism.

higher ratios of glycated albumin to blood glucose

3. Which methods can we used for determined GA today?

Lower ratios of glycated albumin to glucose are also observed

obese people, smokers and in patients with hyperuricemia, hypertriglyceridemia, or alcohol-induced fatty liver disease associated with elevated levels of alanine aminotransferase

Albumin metabolism changes rapidly in **children**, and the levels of both albumin , fetal Hb and glucose in infants increase rapidly with age.

While these effects limit the reliability of glycated albumin as a biomarker of glycemia, they are still less severe than the significant influence of changes in fetal Hb levels on HbA1c levels .

As a result, glycated albumin, can use as an indicator of glycemic control in newborns with diabetes.

3. Which methods can we used for determined GA today?

Biological variability of glycated albumin

Determination of glycated albumin in serum and plasma has become much easier since the development of automated enzymatic tests with optimal analytical performance.

	Enzymatic assay GA	Albumin	Fructosamine	HbA1c
Analytical CV	**1.7%**		2.8%	2.4%
Within-subject CV (CVW)	2.1%	**2.3%**	2.3%	
Between-subject CV (CVG)	**10.6%**	**2.9%**	**6.3%**	
the Westgard biodatabase	10.3%	4.2%	5.9%	
Critical difference (CD)	7.5%	9%	10%	

Montagnana M, Paleari R, Danese E, Salvagno GL, Lippi G, Guidi GC, Mosca A. Evaluation of biological variation of glycated albumin (GA) and fructosamine in healthy subjects. Clin Chim Acta 2013; 423:1–4.

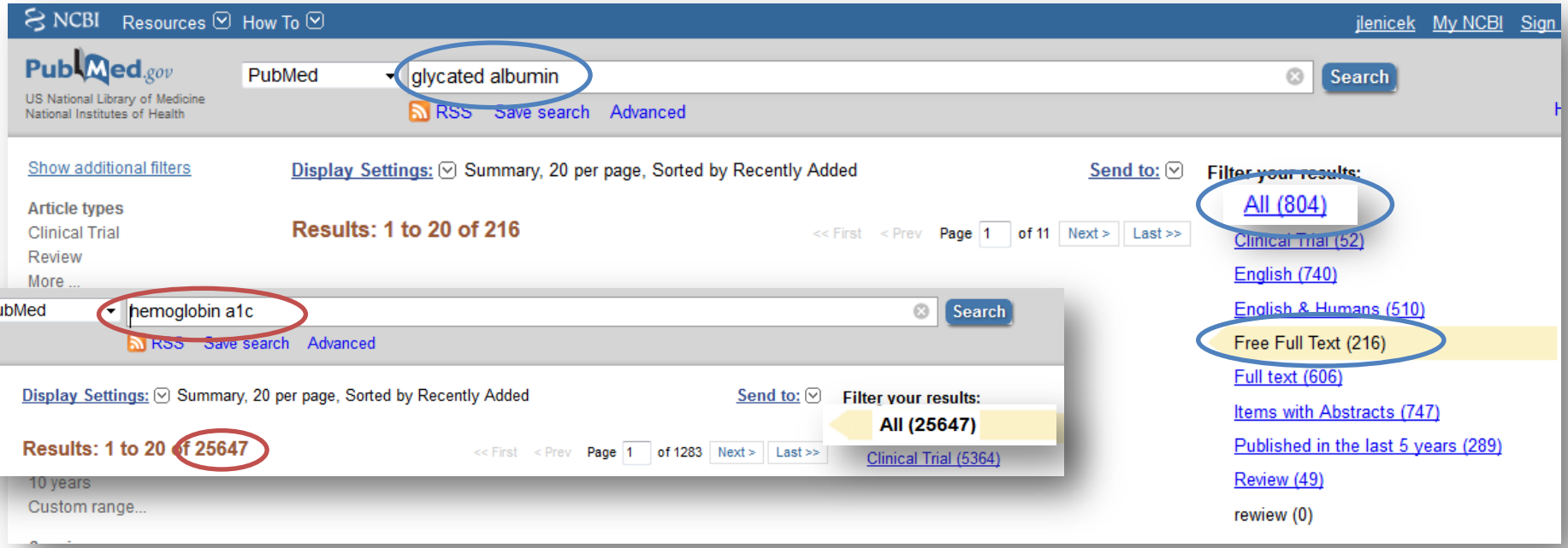
These comparisons indicate a high degree of individuality .

Some authors have suggested : significant CV between-subject variation of glycated albumin levels, the critical difference (CD) should be used instead of target values for monitoring glycemia

3. Which methods can we used for determined GA today?

While the causes of this large variation are not complitly clear, variation in the erythrocyte lifespan, especially in diabetics as is variation in albumin half-life due to glycation, the authors of that study strongly recommended monitoring diabetes using a combination of two or more glycemia biomarkers, in order to obtain more reliable information about glycemic state.

Conclusions



Although **far less published results** of studies on the evidence of pathological mechanisms and clinical utility of **GA compared to HbA1c (**approximately 32 times less **)** ,

there is **growing evidence to show that the GA is a useful marker in their management of diabetes.**

Finally: thanks to our first results

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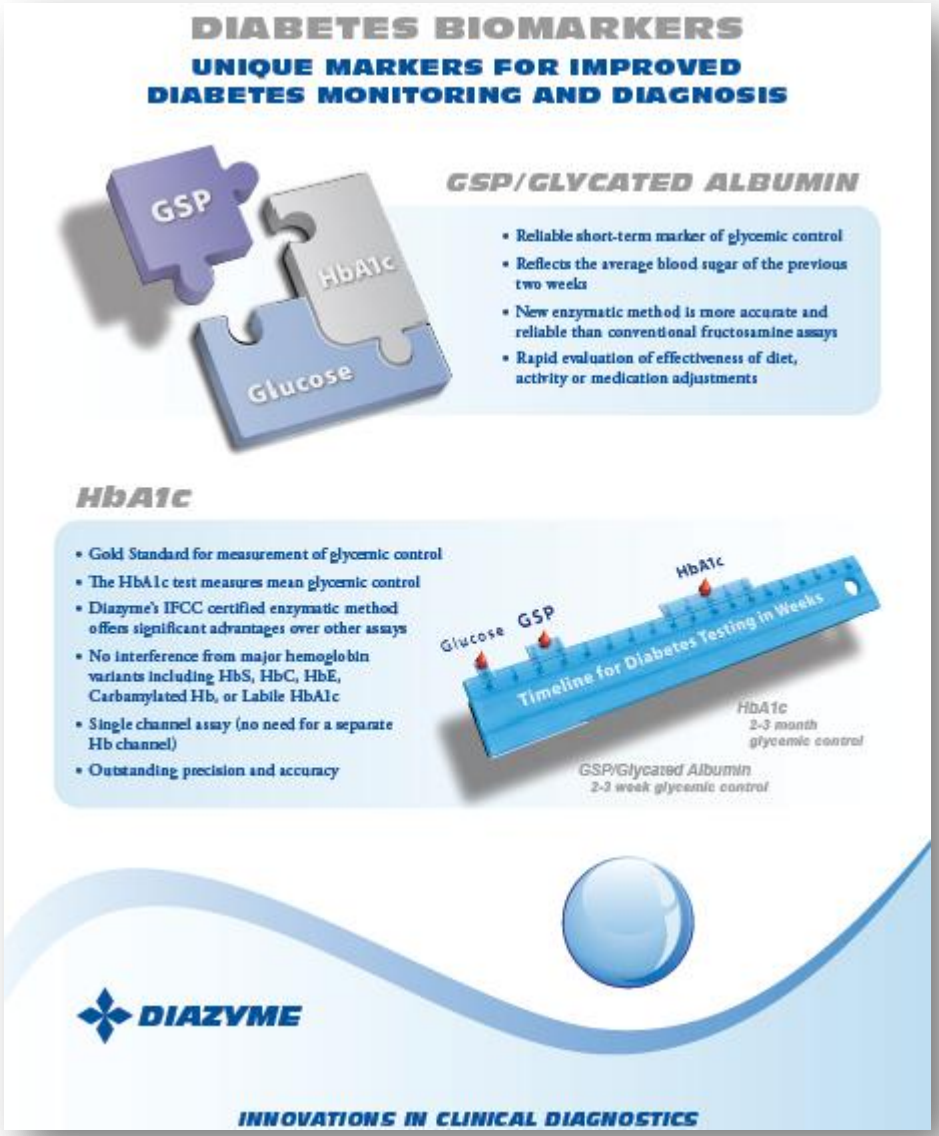


Finally: thanks to our first results

Klinika za dječje bolesti Zagreb
Children's Hospital Zagreb

and

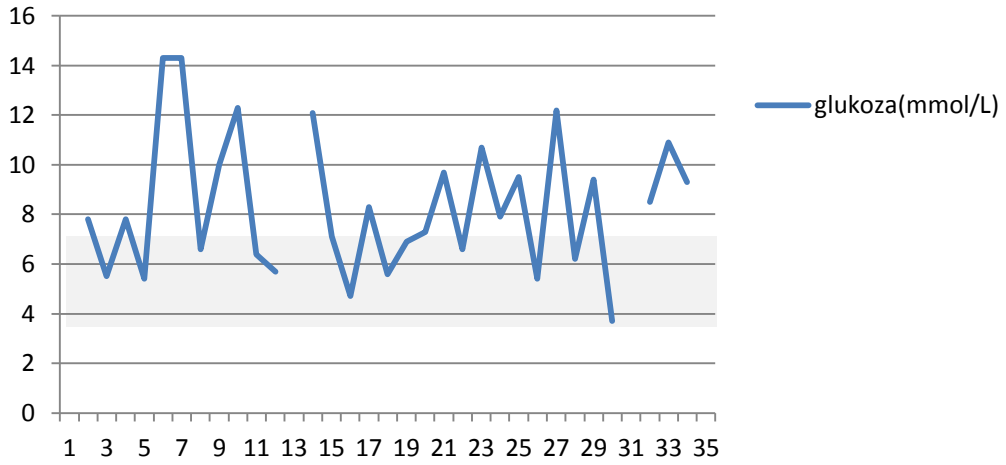
KBCRI
KLINIČKI BOLNIČKI CENTAR RIJEKA
Univesity Hospital Centar Rijeka, Croatia

Tim: J. Lenicek Krleza; A. Grzunov; L.Bilic-Zulle; D. Antoncic; S. Hrabric-Vlah, E. Fisisc

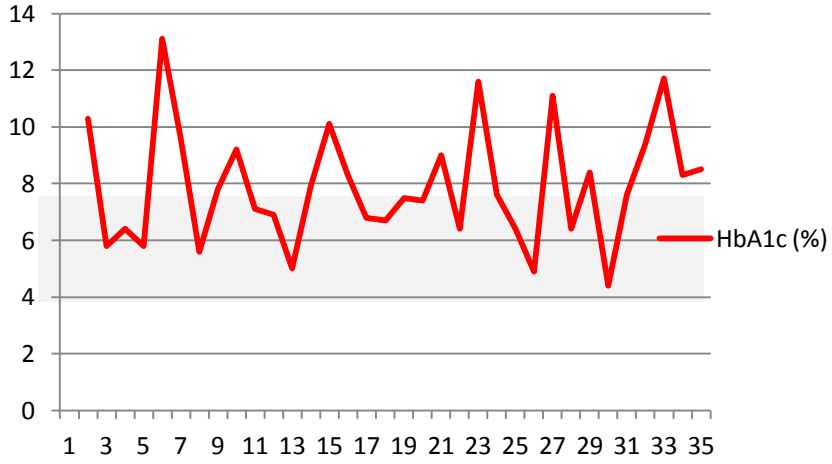
Mini project: Comparsion of Glycated Albumin and HbA1c in Type 2 Diabetic Patients on Hemodialysis.

In process

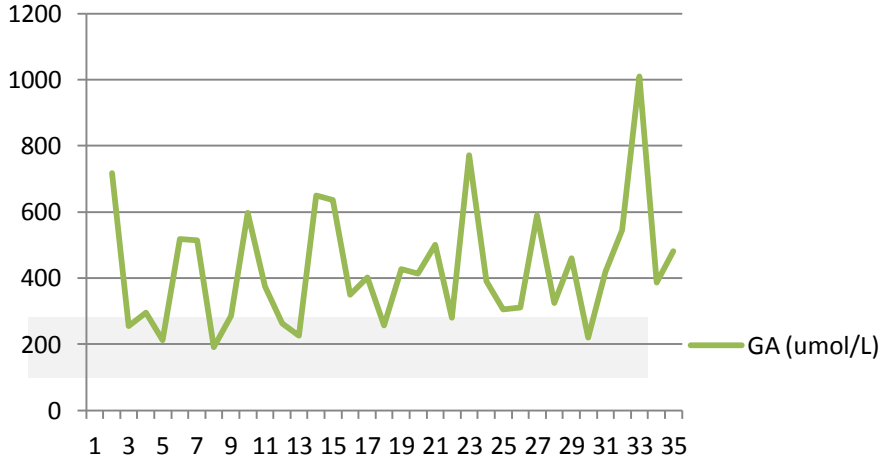
Glukoza(mmol/L)



HbA1c (%)



GA (umol/L)



Thank you for your attention