Can glycated albumin assist in management of diabetes mellitus?

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Introduction

Diabetes mellitus can be described as a group of disorders in carbohydrate metabolism in which glucose is produced in excess amounts, leading to hyperglycemia. Diabetes usually occurs in three forms: type 1, type 2 and gestational. There are other forms of the syndrome, but they are quite rare.

Diabetes is prevalent around the world. According to the International Diabetes Federation, approximately 382 million people were estimated to have the disease in 2013. This prevalence is rapidly increasing, with the total number of affected individuals around the world expected to double by 2030 (1,2).

High blood glucose concentrations cause numerous types of negative effects, which can lead over time to tissue dysfunction, organ failure, and life-threatening health complications. For example, diabetes increases the risk of heart and kidney disease, stroke, blindness, peripheral blood vessel disease, peripheral neuropathy, foot ulcers and complications that require amputation of the lower extremities.

The concept of "prediabetes" has been coined to indicate fasting glucose levels that are higher than normal but not high enough to warrant a diagnosis of diabetes. Type 2 prediabetes increases the risk of cardiovascular disease, but its symptoms have not been clearly defined (3).

Many long-term effects of diabetes are due to protein glycation associated with the disease. Glycation, a non-enzymatic Maillard reaction, occurs when glucose molecules spontaneously react with the amine group of proteins, giving rise to stable ketoamines. The fraction of proteins that are glycated is elevated under hyperglycemic conditions, and these modified proteins cause chronic diseases as diabetes complications. Table 1 highlights several important proteins that can become extensively glycated under hyperglycemic conditions (4).

Some of these glycated proteins can be used as biomarkers to determine the degree of glycemia in individuals with diabetes or prediabetes. Glycated hemoglobin (HbA1c) is the recommended and most often used biomarker for assessing hyperglycemia in the clinic. Measuring levels of HbA1c provides a measure of glycemia valid for 2-3 months, the same as the lifespan of erythrocytes. Today, HbA1c is the "gold standard" for assessing glycemia during diabetes management, and since 2009 it has been recommended by both the American Diabetes Association (5) and the World Health Organization (6) as a diagnostic criterion for diabetes, with a diagnostic cut-off of >6.5% (48 mmol/mol).

Levels of HbA1c correlate linearly with average glucose concentration in most patients with diabetes (7). In some patients, however, levels of HbA1c are inadequate for determining the average glucose concentration and therefore for assessing glycemic state; in such patients, this biomarker is useless for predicting diabetes complications likely to develop. 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 (Table 2)(6).

Diabetes management, especially in early phases, requires frequent monitoring because significant changes can occur within 2-3 months. Such monitoring is particularly 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 and patients on hemodialysis (4,6).

For such patients for whom HbA1c levels cannot directly be used to assess glycemic state, Cohen et al.

Table 1. Proteins susceptible to glycation, particularly under hyperglycemic conditions.*

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 ref. (4).

Table 2. Factors influencing levels of HbA1c.*

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 ref. (6).

have proposed calculating the "glycation gap", defined as the difference between the measured level of HbA1c and the level predicted based on the amount of glycated albumin (8). More recently, Rodriguez-Segade et al. have shown that the combination of glycation gap and glycated albumin level provide a better indication of nephropathy risk, and may be more desirable for glycemic control (9).

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. In addition, 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. 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 as well as to clearly establish its clinical usefulness and reliability for predicting diabetes-related complications. Since then, numerous studies have tried to follow these recommendations and determine whether the level of glycated albumin can be useful in managing diabetes. Here we examine those efforts (6,10).

Colorimetric determination of glycated albumin

Recommended methods for determining glycated albumin are based on 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 (11), including albumin and all serum proteins, all of which are collectively known as fructosamine[1]. These methods work well with EDTA and heparin plasma samples, giving them an advantage over the HbA1c assay, which requires complete blood. However, all these techniques are complicated and require sophisticated equipment beyond the reach of many clinical laboratories. A much simpler and less expensive alternative is a colorimetric method for fructosamine determination known as the nitroblue-tetrazolium (NBT) reduction method (12). The method was automated soon after it was first described.

Despite its advantages over more complicated techniques, the NBT method does suffer from reduced specificity because the 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. Some assay manufacturers attempt to reduce this interference by incubating the reaction for slightly longer periods (10-15 min). However, this approach is not feasible with many laboratory analyzers, which lack the flexibility to program longer incubations. Some laboratories avoid the interference of the NBT assay by using alternative colorimetric methods based on 2-thiobarbituric acid (TBA) or phenylhydrazine.

Although the NBT method has been widely automated, it is still vulnerable to interference, the sources of which depend on the particular test. The following have been widely reported to interfere with this assay (13), though manufacturers' test inserts should be always be consulted for specifics:

1. EDTA and heparin plasma samples give lower fructosamine results than serum samples in the NBT colorimetric assay, so the same type of sample should always be used to monitor glycemia in a given patient.
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.

[1] Usually glycated protein or glycated albumin are referred to as "fructosamine" if determined colorimetrically, or as "glycated albumin" or "glycated serum proteins" if determined enzymatically (see next section).

4. Bilirubin has been shown to cause falsely elevated fructosamine results.
5. The NBT assay, like other colorimetric assays, is affected by changes in ambient temperature.

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 (14-17). The principle of the enzymatic assay is shown in Figure 1, and it can be performed with serum or plasma on virtually all biochemical analyzers. The commercial assays come supplied with ready-to-use reagents, which simultaneously allow determination of not only glycated albumin but also several other frequently used biomarkers, including glucose, cholesterol and triglycerides. These 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.

Although the tests from different manufacturers rely on slightly different methods, they all show 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 whether the results are expressed as a concentration (mmol/L or mmol/L) or as glycated albumin fraction (%GA). Determination of %GA also involves determination of total albumin.

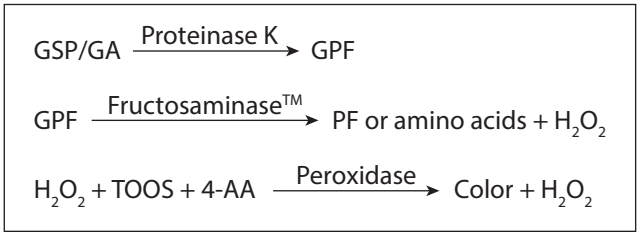

Figure 1. Principle of the Diazyme enzymatic assay to determine glycated serum albumin. Proteinase K digests serum proteins into low-molecular-weight glycated protein fragments (GPF), then a specific fructosaminase™ (microbial amadoriase) catalyzes the oxidative degradation of GPF Amadori product to 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 (14).

While the Lucica GA-L kit determines %GA, the Diazyme GlycoGap kit determines the concentration of glycated albumin in mmol/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 of the Clinical and Laboratory Standards Institute, the automated test shows the characteristics of a reference method, even though it has yet to be formally recognized as such.

Interference studies have reported various interferences, though these may vary from kit to kit, and the manufacturer's test insert should be consulted in all cases.

1. Since EDTA plasma samples, but not serum 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 fructosamine results.
3. As in the NBT colorimetric assay, bilirubin has been shown to cause falsely elevated fructosamine results.

Several common interfering substances in serum, such as ascorbic acid, bilirubin, glucose, triglycer-

ide, uric acid and hemoglobin, usually show ≤ 10% interference, though the manufacturer's insert for the particular test should be consulted.

Variability of glycated albumin and limitations as a biomarker of glycemia

Disorders in albumin metabolism affect levels of glycated albumin. Lower ratios of glycated albumin to blood glucose are observed in patients with nephrotic syndrome or hyperthyroidism and in patients on glucocorticoid therapy. These diseases involve elevated albumin metabolism. Conversely, higher ratios of glycated albumin to blood glucose are found in patients with liver cirrhosis and hyperthyroidism--conditions associated with reduced albumin metabolism.

Lower ratios of glycated albumin to blood glucose are observed in obese people; these ratios appear to reflect chronic micro-inflammation mediated by adiponectin released from fat cells, which leads to increased protein catabolism. Lower ratios of glycated albumin to glucose are also observed in smokers and in patients with hyperuricemia, hypertriglyceridemia, or alcohol-induced fatty liver disease associated with elevated levels of alanine aminotransferase (ALT)(10).

Albumin metabolism changes rapidly in children, and the levels of both albumin 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 hemoglobin levels on HbA1c levels (10). As a result, glycated albumin, not HbA1c, is used as an indicator of glycemic control in newborns with diabetes.

Biological variability of glycated albumin

Determination of glycated albumin in serum and plasma has become much easier since the development of automated enzymatic tests. The analytical coefficient of variation of 1.7% is much lower than the corresponding values of 2.8% for fructosamine and 2.4% for HbA1c published in 2013 (18). That study reported a within-subject coefficient of variation (CVW) of 2.1% and between-subject coefficient of variation (CVG) of 10.6% for glycated albumin; the corresponding values for albumin were 2.3% and 2.9%, and for fructosamine, 2.3% and 6.3%. This CVG of albumin (2.9%) is lower than the 4.2% reported in the Westgard biodatabase, while the CVGs for glycated albumin (10.6%) and fructosamine (6.3%) are higher than the corresponding values of 10.3% and 5.9% in the Westgard biodatabase. These comparisons indicate a high degree of individuality for both albumin and glycated albumin as biomarkers of glycemia. Some authors have suggested that because of the substantial between-subject variation of glycated albumin levels, the critical difference (CD) should be used instead of target values for monitoring glycemia (18). The estimated CD in this study was 7.5% for GA, 9% for albumin and 10% for fructosamine. This approach reduces between-subject variation, allowing enzymatic determination of glycated albumin to be recommended for clinical use in conjunction with determination of HbA1c for monitoring glycemic status (18).

A study in 2010 suggested that errors in determination of glycated albumin or HbA1c in patients with type 2 diabetes are on the order of 18% and can be attributed to between-subject variation (19). While the causes of this large variation are not entirely clear, variation in the erythrocyte lifespan, especially in diabetics, is likely to contribute, 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.

Conclusion

The available evidence points to glycated albumin levels as a useful marker for diabetes management, although much still needs to be learned about the mechanism of albumin glycation and about how this marker compares with the much better established HbA1c.

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