Glycated Albumin and Diabetes Monitoring Research Update

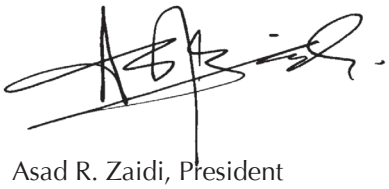
EPINEX DIAGNOSTICS, INC.

Corporate Information Series - 6

Foreword

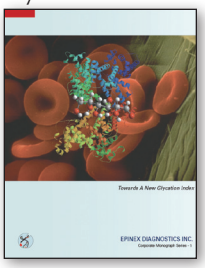
This is the sixth in a series of corporate informational documents that we hope will provide critical data for our partners, collaborators, supporters, doctors, educators, and investors concerned about the growing worldwide epidemic of diabetes, and the problems and opportunities it presents to the healthcare industry. This educational presentation was prepared by our Director of Corporate Communications, Dr. David Trasoff. The report presents a summary of recent research that validates the significance of glycated albumin as marker for diabetes, in applications that range from causation of complications to large scale diagnosis. It highlights recent work that promises to make a rapid test for glycated albumin a workable reality in the near future. The report also outlines recent studies at the cutting edge of diabetes control, and the ways that GA can work synergistically with the most effective methods of diabetes control currently advocated.

I sincerely hope that readers find this document helpful. We continue to strive to disseminate information that will contribute to stemming the tide of diabetes.



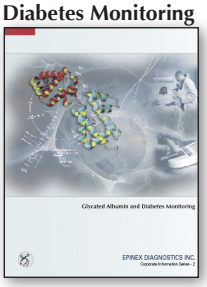
Asad R. Zaidi, President
Epinex Diagnostics, Inc.

Toward a New Glycation Index



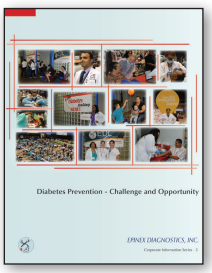
Understanding Diabetes

Glycated Albumin and Diabetes Monitoring



Diabetes in the Middle East

Diabetes Prevention: Challenge and Opportunity



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Scientific Advisory Board

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Recent Research in Glycated Albumin

The following update to the Company's 2008 report will survey and highlight significant recent research on glycated albumin in several areas:

1) the basic science behind the glycation of Human Serum Albumin;
2) the increasingly close association of GA with a range of diabetes complications, including causal implications;
3) recent results that have been obtained using GA to monitor and control diabetes in a patient population;
4) the prospect of using GA as a full diagnostic and screening tool for diabetes;
5) advances in sensor technology for glycated proteins; and
6) recent progress in the Monthly Care Paradigm for diabetes care.

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Introduction

Why isn't there a simple, inexpensive test that can immediately tell someone his or her risk for developing diabetes? This goal has been achieved for many other common health situations, beginning with the pregnancy test and now available across a wide range of conditions. There is a clear unmet need for such a diabetes test, something with the potential to help the world address what many believe is the major healthcare issue of our time.

Beginning a decade ago, Epinex Diagnostics suggested that glycated albumin (GA), a marker for intermediate glycation and at that time an obscure test available only through a very few clinical laboratories, had the scientific potential to be the sought-after superior test for diabetes. The company began its long and difficult journey to create a convenient rapid test for GA, a process now on the verge of full realization.

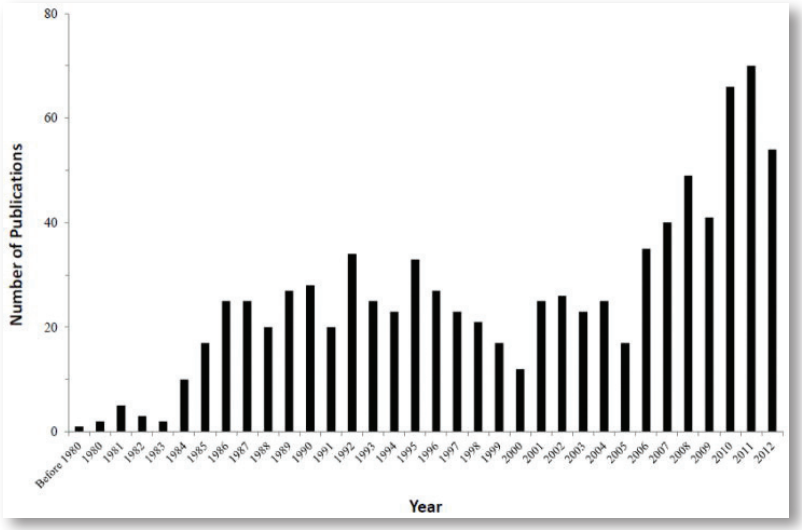
In 2008 Epinex Diagnostics published Glycated Albumin and Diabetes Monitoring, the second document in the company's Corporate Information Series, a series of review articles and position papers designed to stimulate discussion about the diabetes epidemic. This report has been downloaded from the Company web site thousands of times, and has played a significant role in contributing to a broader understanding of the role that glycation of serum albumin plays in the onset of diabetes complications, as well as the potential utility of using measurement of glycated albumin (GA) as a means of monitoring diabetes.

2008 also saw the publication in the **Journal of Diabetes Science and Technology** of *A Review Of Glycated Albumin As An Intermediate Glycation Index For Controlling Diabetes,* a seminal article written by Dr. Vern Roohk, a founding member of the Epinex Diagnostics Scientific Advisory Board, with Asad Zaidi, CEO of Epinex Diagnostics. The article presented a rigorous review of GA measurement methodologies, linkages to diabetes complications, and the results of a survey of endocrinologists conducted by Epinex that validated support for GA as a needed test for glycemic control. This article, which has been cited 74 times to date in other journal articles, has played a significant role in moving the dialogue about glycated albumin forward in the scientific community.

In the seven years since both publications, interest in and exploration of the utility of GA for diabetes monitoring has increased exponentially. The articles and findings reviewed here are just a representative sample of the large number of publications on the utility of glycated albumin that have appeared in the last few years. A review of PubMed, the online database for biomedical-related scientific publications, shows that while 67 articles referring GA were published between 2000 and 2004, almost 500 were published between 2005 and 2009, and another 350 since 2010.

Glycated albumin is now a mature research subject, no longer considered a fringe area of possible potential interest for diabetes monitoring and control. Now this potential is being realized, GA is being brought into the mainstream, and the early predictions of its utility are being borne out. Most of the ideas put forward by Epinex regarding GA, its role in diabetes and its potential have been validated, and GA testing is even coming into limited use as a diagnostic and screening tool. However, the GA test remains limited to the clinical laboratory, which imposes a continuing restriction on the broader application of glycated albumin.

An easily available glycated albumin test could have a broad and beneficial impact across the spectrum of diabetes care, from screening and diagnosis to treatment. A simple, convenient and inexpensive rapid test, available at the point-of-care and as a hand held device for home use, could be the means to translate the promise of glycated albumin technology into improving health outcomes and making real progress against the tide of the diabetes epidemic. From its founding to the present, Epinex Diagnostics is fully committed to achieving this goal.



Number of journal articles published about glycated albumin on a yearly basis: 1980-2012

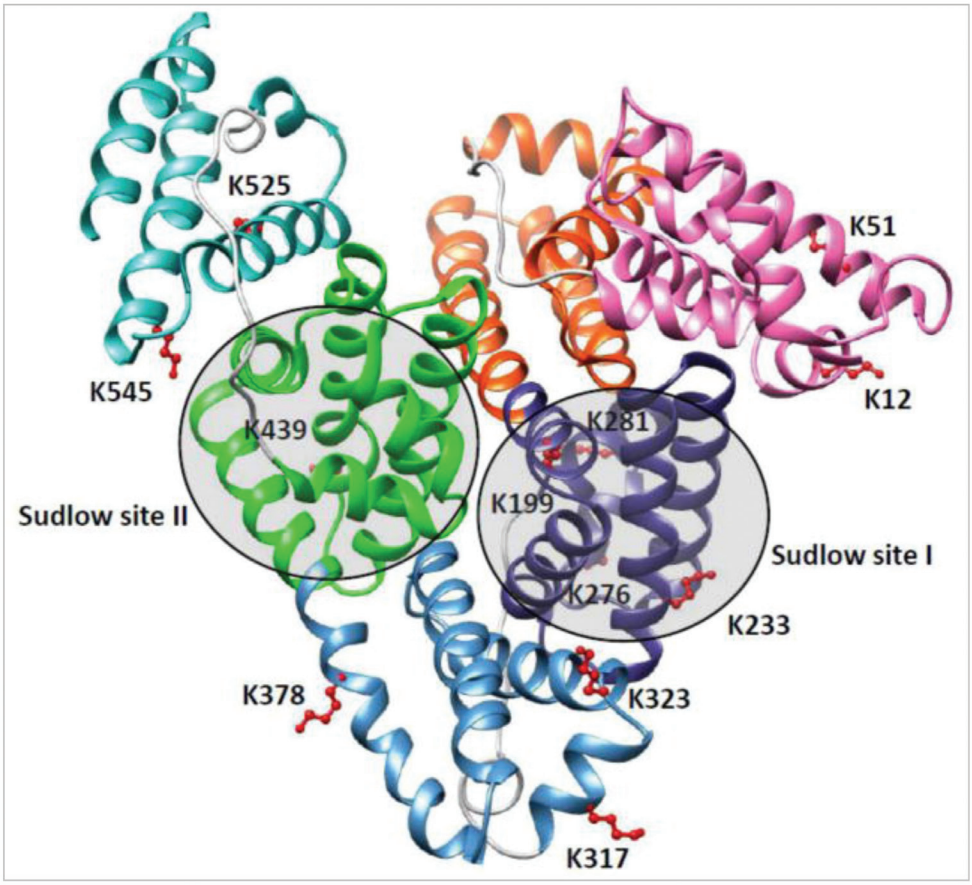
Basic Science of Albumin Glycation

Rondeau and Bourdon have identified as many as 29 potential albumin glycation sites, although lysine-525 is depicted as the predominant site of the non-enzymatic glycosylation of human serum albumin in vivo. Non-enzymatic adduction of glucose at this residue accounts for 30% of the overall glycation. They note that glycoxidation of albumin in both in vitro and in vivo models is associated with important structural modifications. In particular, tertiary structure conformation, probed by tryptophan fluorescence, is significantly affected by glycation. The antioxidant properties of albumin are negatively affected by glycation as well as the age of the molecule (which often go together), and are correlated to the changes in the structural conformation of the molecule. The authors describe a number of different pathways by which the advanced glycation endproducts that form from glycated albumin are implicated in diabetic renal insufficiency, vascular complications, retinopathy and immunogenic responses. The authors conclude that:

> "In contrast to HbA1C, glycated albumin level is associated with peripheral vascular calcification and arterial stiffening , which are two of the most common diabetic complications. Recent clinical studies have suggested glycated albumin as the ideal marker of glycemic control in numerous physiopathological states, including hemodialysis patients, gastrectomized subjects, or for gestational diabetes. All these data support the utility of glycated albumin in the detection of short-term changes in glycemic control." (Rondeau 2011)

Ueda and Matsumoto reported new research on the tertiary structure of the glycation sites on human serum albumin. Their review discusses how the glycation sites of lysine residues located inside of the HSA (human serum albumin) molecule are modified with glucose, and directly compares rates of glycation between GA and HbA1c using human blood. (Ueda 2015).

Anguizola conducted a review of structural studies of glycated HSA, both in vivo and in vitro, along with data on the rate and thermodynamics of HSA glycation. In addition, this review considers various studies that have investigated the effects of glycation on the binding of HSA with drugs, fatty acids and other solutes and the potential clinical significance of these effects (Anguizola 2013).



The crystal structure of HSA. The locations of the main drug binding sites in this protein (i.e., Sudlow sites I and II) are shown, as well as the locations for several lysines that have often been reported to take part in glycation. This structure was generated using Protein Data Bank (PDB) file ID: 1AO6. (Anguizola 2013)

Causal relationship of GA to diabetes complications

Scientists from the School of Mechanical and Aerospace Engineering at Oklahoma State University studied the combined effects of physiologically relevant disturbed wall shear stress and glycated albumin on endothelial cell functions associated with inflammation, thrombosis and cytoskeletal dynamics. Their data suggest a pathway for irreversibly glycated albumin (advanced glycation end-products (AGEs)) to promote cardiovascular diseases (CVD) by enhancing endothelial cell inflammatory and thrombotic responses, and through the deterioration of the cytoskeletal organization (Maria 2014).

In general, the presence of irreversibly glycated albumin promoted CVD development, suggesting that under diabetic conditions, platelets and endothelial cells can negatively feedback on each other, likely via enhanced adhesion, to elicit a reduced response associated with CVD progression (Rubenstein 2014).

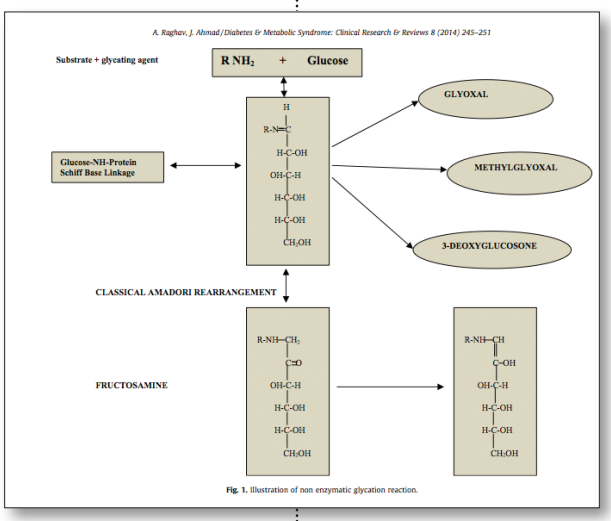


Fig. 1. Illustration of non enzymatic glycation reaction.

Review Studies

The accumulation of recently published research on glycated albumin and its relationship to a broad range of diabetes-related complications has prompted the publication of a number of reviews that summarize the utility of glycated albumin as a marker for diabetes.

In 2011, Rondeau and Bourdon noted that, "Numerous studies have reported glycated albumin as an alternative marker for glycemic control. First of all, it has been shown that glycated albumin is strongly involved in the development of major diabetes complications, including nephropathy, retinopathy and Alzheimer's disease (Rondeau 2011)."

Kim and Lee reviewed the roles of glycated albumin as intermediate glycation index and pathogenic protein in 2012. Their review evaluates the potential of GA as a glycemic index for diagnosing and managing diabetes, as well as for predicting diabetic complications. It also investigates its role as a pathogenic protein affecting the worsening of diabetes and occurrence of diabetic complications (Kim KJ 2012).

In Zheng (2012), the clinical aspects of GA were discussed, including a comparison of GA with other glycated proteins, the utility and limitations of GA as a glycemic index, its influence on the therapeutic effects of hypoglycemic agents, its correlations with vascular complications, and its potential role in pathogenesis, specifically in diabetic patients with chronic kidney disease (CKD).

The review notes that rapid changes in GA values in response to glucose concentrations are beneficial in diabetic patients with fluctuating glucose levels, such as those with postprandial hyperglycemia, great glucose variability, or abrupt changes in glycemic status over a very short period of time and concludes that GA may be a more useful marker of glycemic status than HbA1c, especially in patients with conditions, such as variant hemoglobinopathies, iron deficiency anemia, and pregnancy.

Zheng also notes that in addition to reflecting blood glucose values, GA has also been found to be a surrogate marker for vascular complications. GA is associated with vascular calcification in diabetic patients on dialysis. Increased GA levels may also be associated with diminished immune function, increased oxidative stress, disturbed cholesterol homeostasis, impaired endothelial function, and proinflammatory responses, suggesting that GA may play a role in the pathogenesis of atherosclerosis.

Raghav and Ahmad published a 2014 review that included an overview of biochemical mechanisms by which GA causes range of diabetes complications. They state that glycation of human serum albumin leads to several pathological events such as diabetic nephropathy, neuropathy, retinopathy and cardiovascular complications. Glycated albumin can be used to determine glycemic control due to a shorter half-life than erythrocytes, which makes it an alternate reliable disease marker in diabetes.

They conclude that "human serum albumin imposed glycation can serve as future tool not only for diagnosing diabetes but also for its potential in assessment of diabetes-associated complications" and suggest that GA will be the new gold standard future diagnostic marker in diabetes-associated complications (Raghav 2014).

A 2015 review notes the conditions that can interfere with the reliability of HbA1c measurements and discusses the relative value of HbA1c, glycated albumin, and fructosamine, in prediabetes and diabetes diagnosis, evaluation of glucose variability, and complications risk prediction. The study concludes that glycated albumin may be beneficial to monitor rapid metabolic alterations or changes in diabetes treatment and presents a novel molecular role for albumin "by which glycated albumin contributes to glucose intolerance development and thus to progression to diabetes, besides the role of glycated albumin as a pro-atherogenic factor (Ribeiro 2015)."

Superiority of Glycated Albumin for Diabetes Monitoring

Keypoint

Numerous studies conducted in the past 8 years has confirmed that glycated albumin is equivalent or superior to HbA1c as a marker for monitoring diabetes.

The combination of patient-administered multiple daily blood glucose tests (SMBG) and two to four HbA1c tests per year has remained the standard prescription for diabetes monitoring and control for the past several decades, even though these recommendations have not proven effective for large numbers of type 2 diabetics. Both methodologies have come under increasing criticism in recent published studies.

The last five to eight years have seen a flood of studies that compare glycated albumin to the previous standards for monitoring diabetes, such as fasting glucose and, especially, hemoglobin A1c (HbA1c). These studies have confirmed that (1) GA testing is equivalent in basic accuracy to HbA1c in terms of standard measurement of hyperglycemia, (2) GA testing can provide a faster and more accurate indication of changes in overall glycation levels and (3) GA is a more accurate indicator than A1c in a number of circumstances where a patient's physiological condition can interfere with red blood cell or hemoglobin metabolism.

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

(Krleza 2014)

Factors That Impact HbA1c Accuracy

Physiological factors that influence the accuracy of HbA1c levels were summarized in a review by Krleza (2014)

Koga (2014) also lists hematological disorders that can affect the accuracy of HbA1c measurement:

Hemolytic anemia
Iron deficiency anemia
Pregnancy
Liver cirrhosis (chronic liver disease)
Chronic kidney disease (renalanemia)
Variant hemoglobin
Neonatal diabetes mellitus, hereditary persistence of fetal hemoglobin

A general methodological review by Heinemann and Freckmann addresses multiple causes of variability in found in HbA1c measurement systems, as well as discrepancies in HbA1c levels caused by deviations of red blood cell physiology from the norm. The review also catalogues the common and widespread causes of the unreliability of blood glucose readings from patients (such as patient denial, deliberate misreporting, etc.). Together these factors lead to diabetics not being monitored and treated effectively (Heinemann 2015). A better methodology would reduce or eliminate patient test bias as well as potentially unreliable HbA1c readings. In a companion piece to Heinemann, Smith and Cohen present evidence that the relationship between HbA1c and mean blood glucose is influenced by variation in red blood cell survival even in the hematologically normal (Smith 2015). This finding introduces another potentially complicating factor in depending on HbA1c as a means to monitor blood glucose control.

On the most fundamental physiological level, the difference in the respective metabolism of albumin and hemoglobin suggests that monitoring albumin (turnover time of 2-3 weeks) versus monitoring hemoglobin (turnover time of 2-3 months) should more rapidly reflect patient circumstances. If the diabetic patient is receiving treatment, any improvement should be reflected first in decreasing levels of GA, and only later by HbA1c. Many recent clinical studies and meta-studies confirm this supposition.

In a study of emerging trends in optical sensing of glycemic markers for diabetes monitoring, Pandey et. al. note that there is a clinical need for biomarker measurements that are more sensitive than HbA1c to shorter-term alteration in average blood-glucose levels.

> While further clinical studies are necessary to define which biomarker works best for specific classes of patients (since it is clear that none of the markers can provide the complete picture in all diabetic patients), measurement of glycated albumin has the potential to provide more accurate results in patients with certain hemoglobin variants and in patients whose RBC lifespans are altered, and to help implement therapy by providing a shorter term response. In the light of the growing recognition of the limitations of HbA1c and

the lack of clinically available methods for glycated albumin estimation, development of robust, sensitive glycated albumin assays is extremely desirable in order to fill the gap in the present diagnostic landscape (Pandey 2015).

A 2014 study by Parrinello and Selvin of the Johns Hopkins Bloomberg School of Public Health referenced the growing literature linking nontraditional markers such as GA and 1,5-anhydroglucitol (1,5-AG), with microvascular and macrovascular complications and mentioned that GA has been shown to improve identification of persons with diabetes. The authors note that, "(e)xpanded use of these tests has the potential to improve diabetes care as these measures may overcome limitations of HbA1c in certain patients, complement traditional measures by providing additional information on shorter-term glycemic control, and improve risk stratification for diabetes and its complications."

A 2015 study by clinicians based at the Universities of Verona and Parma restates that while the current diagnostic and prognostic strategies in diabetes are mainly based on two tests, plasma (or capillary) glucose and glycated hemoglobin (HbA1c), these measures are not foolproof, and their clinical usefulness is biased by a number of clinical and analytical factors. They confirm that "the introduction of other indices of glucose homeostasis in clinical practice such as fructosamine and glycated albumin (GA) may be regarded as an attractive alternative, especially in patients in whom the measurement of HbA1c may be biased or even unreliable. These include patients with rapid changes of glucose homeostasis and larger glycemic excursions, and patients with red blood cell disorders and renal disease." They state further that "according to available evidence, the overall diagnostic efficiency of GA seems superior to that of fructosamine throughout a broad range of clinical settings. The current method for measuring GA is also better standardized and less vulnerable to preanalytical variables than those used for assessing fructosamine." (Danese 2015)

In his review article of the clinical usefulness of GA testing, Koga (2014) finds that glycated albumin is a superior marker for both positive and negative changes in glycemic control. Where there was rapid improvement of glycemic control it was possible to judge the treatment effect by measuring GA during a short period (approximately 2 weeks after treatment), which would provide an estimate of HbA1c levels 12 weeks later. In the opposite circumstance, when glycemic control deteriorated rapidly, an increase in GA preceded that in HbA1c, making it possible to detect a deterioration of glycemic control early by measuring GA. In patients who had a relapse after discharge from the hospital, increased GA levels were observed, whereas HbA1c levels remained low.

In summary, Koga found that glycated albumin more accurately reflects changes in plasma glucose during the short term and postprandial plasma glucose. GA also reflects glycemic control in patients with hematologic disorders although GA does not reflect glycemic control in patients with disorder of albumin metabolism. He concludes that GA is a glycemic control indicator that overcomes most of the disadvantages of HbA1c, and could be therefore expected to replace HbA1c as the standard glycemic control indicator in the near future (Koga 2014).

Paradoxical Results With HbA1c

For some patients, evaluating the effectiveness of diabetes treatment using HbA1c can lead to paradoxical results. A 2015 study observed that for some patients, there was a discrepancy between changes in GA and HbA1c. When diabetes treatment was started, added, or changed (intensification of treatment) in patients with poor glycemic control, although GA (glycated albumin) decreased within a few weeks, HbA1c increased in some patients. The study concluded, "it is preferable to evaluate glycemic control by fasting plasma glucose, GA and fructosamine in such situations (Koga 2015)."

Extension of glycated albumin to type 1 diabetes

Diabetes researchers are finding that glycated albumin is a useful marker to monitor aspects of type 1 diabetes, especially where it can be beneficial to follow short term changes in glycemic levels.

A Korean study investigated the association of GA and GA/HbA1c ratio with the levels of fasting C-peptide and fasting plasma glucose in type 1 and type 2 pediatric diabetes. 50 total cases were included: 30 type 1 diabetes mellitus (T1DM) cases and 20 type 2 diabetes mellitus (T2DM). The relationships among HbA1c, GA, and the GA/HbA1c ratio as well as fasting glucose and fasting C-peptide were analyzed. The authors concluded that GA seems to more accurately reflect fasting plasma glucose level than HbA1c and that the GA/HbA1c ratio appears to reflect insulin secretory function (Lee 2013).

Use of glycated albumin to monitor diabetes therapy

Glycated albumin has proven to be an effective means to monitor diabetes therapy in a variety of contexts, especially when compared to the existing standard using HbA1c results. A 2012 study confirmed that the reduction in glycated albumin 3 weeks after the initiation of treatment corresponded with the reduction in HbA1c 3 months after starting treatment in both the group treated with an oral hypoglycaemic agent and the insulin-treated group of Korean patients with Type 2 diabetes (Won 2012). A 2013 study evaluated whether GA is useful for early detection of deterioration of glycemic control

state after discharge from educational admission. This study followed 21 diabetics for at least 10 weeks after discharge from educational admission. GA was found to be useful for early detection of deterioration of glycemic control state after discharge from educational admission (Murai 2013).

GA is now being used routinely as a clinical tool to study the effectiveness of drug treatments for type 2 diabetes under a variety of clinical conditions. A recent study in Japan that followed the effectiveness of metformin therapy, the most commonly used treatment for early stage diabetes, demonstrated that GA measurement has become a useful and accepted tool to monitor the effectiveness of treatment for subjects with newly diagnosed diabetes (Sumitani 2015). Researchers are using GA as a means to follow the course of treatment with drugs such as sitagliptin (Shima 2014) and vildagliptin (Ito 2013) in order to evaluate their effectiveness in patients with different types of complications. With regard specifically to sitagliptin, a drug treatment coming into increasing use as a treatment for type 2 diabetes, Shima et al found that:

> In the present study, the percent reduction from baseline was significantly greater for GA than HbA1c at most observation time points...The efficacy of sitagliptin was significantly greater at week 4 and week 12 when assessed with GA than when assessed with HbA1c...Access to this information will help clinicians to decide whether to continue therapy in such patients. These data suggest that GA is superior to HbA1c for evaluating antidiabetic drug efficacy in patients with type 2 diabetes.

Table 2. Efficacy of Sitagliptin Therapy at Week 4 and Week 12 Assessed by HbA1c and GA

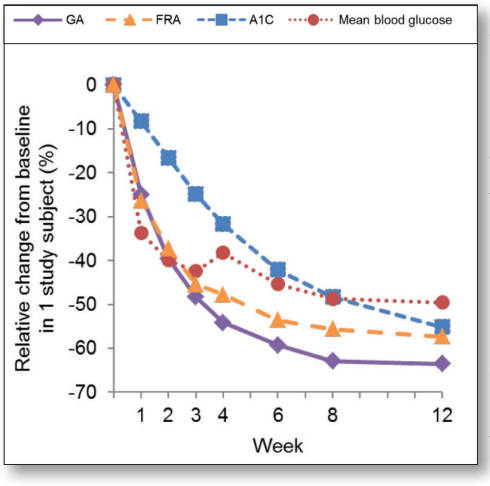
Time of assessment		Week 4	Week 12
Assessed with		Patients deemed to have effective response to therapy	
	HbA1c	25 (37.3%)	48 (71.6%)
	GA	56 (83.6%)	65 (97.0%)
HbA1c vs.GA		p<0.01	p<0.01

A unique element of a study design by Desouza (2015) was selection of a study population with changing, rather than stable, glycemia, which permitted the investigators to assess how the different glucose indices reflected patients' response to therapy. This study highlighted the limitations of both A1C and SMBG and suggests the potential of GA as an intermediate glycemic index to assess the early impact of diabetes management choices. The results demonstrated that:

1) GA (the primary focus of the study) and fructosamine both accurately reflected changes in mean blood glucose values not detected by A1C.

2) GA at 4 weeks predicted A1C at 12 weeks in the population studied.

The study highlights the limitations of SMBG as a marker of glycemic control and the overall unreliability of blood glucose values for therapeutic decision-making for non-insulin patients. It supports the ability of GA to predict short-term therapeutic responses. GA proved more useful than A1C in detecting early response to insulin therapy.



Use of glycated albumin to monitor non-diabetes therapies

Glycated albumin is being investigated as a useful marker in several non-diabetes related conditions, mostly relating to platelet metabolism.

A study of patients who had experienced a minor ischemic stroke and were being treated with antiplatelet therapy to counteract the risk of a recurrent stroke investigated monitoring GA as a predictor of a patient's response to the therapy. The authors report that their study has suggested that using GA as a biomarker would be a practical, easy to operate,and economical method to predict a patient's response to antiplatelet therapy, versus using a complex method of genetic screening to determine which therapy may be more effective (Li 2015).

In hepatitis B virus- (HBV-) positive patients, the relationship between the metabolic variables and histological degree of liver fibrosis was investigated by Enomoto. A total of 176 HBV-positive patients were assessed in whom the ratios of glycated albumin-to-glycated hemoglobin (GA/HbA1c) were calculated in order to investigate the relationship with the degree of liver fibrosis. The GA/HbA1c ratio increased in association with the severity of fibrosis. The GA/HbA1c ratios were inversely correlated with four variables of liver function: the prothrombin time (PT) percentage, platelet count, albumin value, and cholinesterase value. The GA/HbA1c ratio was positively correlated with two well-known markers of liver fibrosis, FIB-4 and the AST-to-platelet ratio index (APRI). Furthermore, the GA/HbA1c showed better correlations with two variables of liver function (PT percentage and cholinesterase value) than did FIB-4 and with all four variables than did the APRI. The authors conclude that the GA/HbA1c ratio is associated with the degree of liver fibrosis in HBV-positive patients (Enomoto 2014).

Relationship of Glycated Albumin to Diabetes Complications

Keypoint

Glycated albumin has been linked as a superior indicator of several of the most common complications of diabetes, including chronic kidney disease, cardiovascular complications, retinopathy and neuropathy. Ongoing research now links glycated albumin as a direct causal agent of complications as well.

Acceptance of GA as a biomarker for assessing the risk of diabetes complications has been reinforced by current research studies ranging from large-scale retrospective analysis of at-risk populations to smaller studies that focus on a particular relationship of GA to a specific medical condition.

General Studies

A study conducted at the Johns Hopkins Bloomberg School of Public Health and published in **Lancet** examined whether markers of short-term (2-4 weeks) glycemic control such as glycated albumin and fructosamine would add complementary prognostic information to HbA1c, the standard measure by which to monitor long-term (2-3 months) glucose control in people with diabetes. The study aimed to clarify the performance of fructosamine and glycated albumin measurements for identifying people at risk of incident diabetes or diabetic complications.

Researchers measured glycated albumin and fructosamine in blood samples from 11,348 adults without diabetes and 958 adults diagnosed with diabetes mellitus (both type 1 and 2) from the 1990-92 Atherosclerosis Risk in Communities (ARIC) study. They assessed the associations of fructosamine and glycated albumin with risk of incident diabetes, retinopathy, and risk of incident chronic kidney disease (CKD), during two decades of follow-up. They compared these associations with those of HbA1c with incident diabetes, retinopathy, and CKD.

The study found that fructosamine and glycated albumin were strongly associated with retinopathy and that prediction of incident CKD by fructosamine and glycated albumin was nearly as strong as by HbA1c. They concluded that fructosamine and glycated albumin were strongly associated with incident diabetes and its microvascular complications, with prognostic value comparable to HbA1c. (Selvin 2014)

Chronic Kidney Disease

Evidence continues to accumulate that glycated albumin is a more reliable indicator than HbA1c for diabetes patients with chronic kidney disease. A 2015 study in Nephrology (Kim 2015) extended this view to cover diabetics who are still pre-dialysis as well as those already on dialysis. The results demonstrated that "HbA1c significantly underestimated

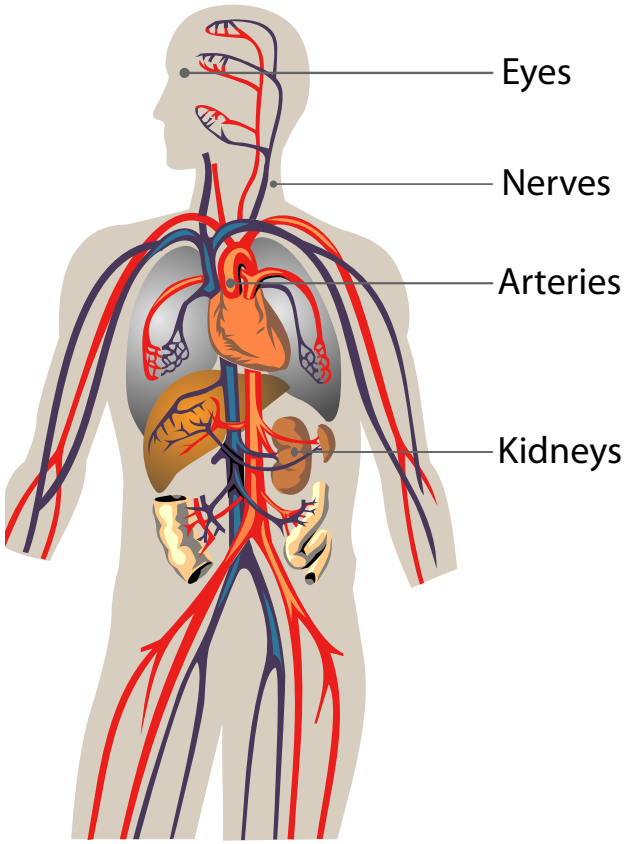


Table 1
The roles of glycated albumin in diabetic patients with chronic kidney disease.

Clinical significance	
As a glycemic index in diabetic patients with CKD	Could be better than HbA1c in those: With anemia Undergoing erythropoietin treatment Undergoing hemodialysis With large fluctuations in glucose levels Limited use in those with: Liver cirrhosis Nephrotic-range proteinuria Inadequate evidence: Early stages of CKD Patients on peritoneal dialysis
As a prognostic factor in diabetic patients with CKD	Could be better than HbA1c in: Predicting survival in dialysis patients Could be involve in pathogenesis of: Cardiovascular complications Diabetic nephropathy Contrast-induced acute kidney injury Inadequate evidence: Early and moderate stages of CKD or non-dialysis patients Microvasculopathy
Screening of diabetes in non-diabetic patients with CKD	Inadequate evidence

(Zheng 2012)

glycemic control, whereas GA more accurately reflected glycemic control in diabetic patients with pre-dialysis CKD."

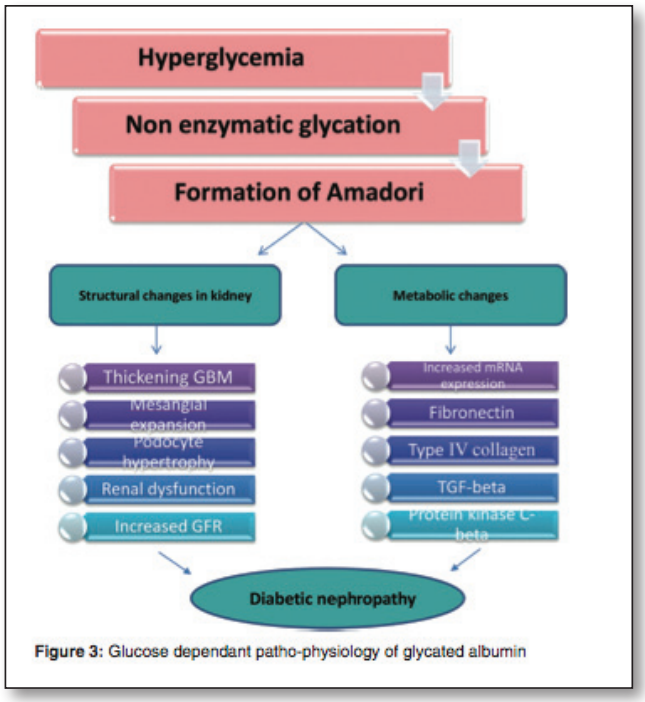


Figure 3: Glucose dependant patho-physiology of glycated albumin

(Neelofar 2015)

Hasslacher notes that earlier studies have established that glycated albumin (GA) represents a better marker of glycemic control than HbA1c in patients with renal failure. Elevated GA values are seen to be predictive for the development of cardiovascular complications, hospitalization and death in diabetic patients with renal insufficiency. This report extends the potential range of GA by studying GA as a marker of glycemic control and predictor of vascular complications in type 2 diabetic patients with normal or moderately impaired renal function.

In order to provide objective evidence about GA in the routine clinical context that may translate into a better management of this group of patients, a 5-year cohort study of type 2 diabetes patients was conducted with a focus on (a) the relationship between GA and HbA1c in patients with normal and reduced renal function and (b) the GA value as predictor of macro- and microvascular complications (Hasslacher 2014).

The study concluded that GA is a particularly good predictor of renal events.

A study in Cairo (Sany 2013) compared plasma glucose, glycated hemoglobin and glycated albumin of hemodialysis patients. HbA1c levels in these patients fluctuated in response to dialysis related treatment, such as erythropoietin, while plasma glucose and GA levels remained steady. It was also observed "Categorization of glycemic control into arbitrary quartiles by GA level led to better glycemic control in a significantly higher proportion of hemodialysis patients with diabetes than those assessed by HA1c." The authors conclude that:

> GA provides a significantly better measure to estimate glycemic control in hemodialysis patients with diabetes and that the assessment of glycemic control by HbA1c in these patients might lead to likely underestimation as a result of the increasing proportion of young erythrocyte[s] [as a result of] the use of erythropoietin.

A 2103 study of diabetic nephropathy conducted in India confirmed that a very high percentage of patients suffering from end-stage renal disease (ESRD) also have type 2 diabetes. The pathological mechanism of elevated urinary albumin excretion is caused by protein glycation, with advanced glycated end products and their deposition, resulting in hypertrophy of glomerular and renal systems. This in turn leads to the leakage of low molecular weight proteins such as albumin. The continuous persistent leakage of these proteins into urine results in overt diabetic nephropathy, which results in the gradual development of ESRD and cardiovascular complications.

This study was carried out to evaluate microalbuminuria in relation to GA and duration of diabetes. Microalbuminuria and GA were measured as risk markers of renal damage and glycemic control respectively. The results identified that the risk of microalbuminuria increased with a poor glycemic control. The authors conclude that a persistent increase in GA and microalbuminuria may be considered as risk markers in diabetic nephropathy. They suggest that a regular screening for microalbuminuria and estimation of GA can help in clinical management to prevent complications.

Having found that increased levels of GA and microalbuminuria reflected a quicker response to short–term changes in diabetes treatment, the authors propose that a regular screening of GA and microalbuminuria should be performed every one month, in addition to the estimation of HbA1C, and that a test which is based on GA can provide a stable monthly index of glycemic control (Kondaveeti 2013).

Noting that, "glycated albumin (GA) is considered a more reliable marker than glycated hemoglobin (HbA1c) for monitoring glycemic control, particularly in diabetic hemodialysis patients," Isshiki et al investigated the associations of GA, HbA1c, and random serum glucose levels with survival. GA was found to be a significant predictor for mortality, whereas HbA1c and random glucose levels were not predictors for mortality. GA predicted the risk of all-cause and cardiovascular mortality in diabetic hemodialysis patients (Isshiki 2014).

Cardiovascular Risk and Stroke

A 2015 study from the Johns Hopkins Bloomberg School of Public Health followed over 11,000 participants in the long-

term community-based Atherosclerosis Risk in Communities (ARIC) Study for two decades. The authors evaluated associations of glycated albumin with risk of coronary heart disease, ischemic stroke, heart failure and mortality, and compared associations to those observed for HbA1c. They concluded that glycated albumin was associated with vascular outcomes and mortality and that these associations were similar to those observed for HbA1c. (Selvin 2015)

A more specific study investigated the associations of two nontraditional glycemic markers, glycated albumin (GA) and 1,5-anhydroglucitol (1,5-AG), as well as glycated hemoglobin A1c (HbA1c) with coronary artery disease (CAD). Analysis of 272 subjects demonstrated that GA was more closely correlated with CAD than HbA1c and 1,5-AG in a Chinese population with high risk of CAD (Ma 2015).

Noting that GA is already more strongly correlated with microvascular conditions than HbA1c, and an increase in serum GA is also associated with the presence and severity of CAD in type 2 DM, Norimatsu investigated the associations between serum levels of GA or HbA1c and the presence of CAD in patients who underwent coronary computed tomography angiography (CTA), and considered whether GA is more useful than HbA1c for predicting the presence of CAD.

The group identified as having CAD showed a significantly higher GA level. In particular, GA was identified as the only significant independent variable for predicting the presence of CAD in the DM group. Norimatsu concludes that a GA-based target therapy may be more effective for reducing the complications of arterial sclerosis than HbA1c target therapy (Norimatsu 2015).

Studies confirming that GA was superior to HbA1c as a predictor of the presence of CAD were also carried out by Furusyo (2013) on 1575 Japanese subjects aged 26 to 78 years, by Song (2012) for 218 Korean subjects and by Ma (2015) in a Chinese population (272 subjects) with high risk of CAD. Ma also noted that "(a)s a predominant early Amadori-type glycation protein in the circulation of patients with diabetes, GA plays a pivotal role in the physiological mechanisms of diabetic atherosclerosis.

Shen (2012) also found that serum GA levels were higher in diabetic patients with significant CAD than in those without but that HbA1c was similar in the two groups. GA, but not HbA1c, was independently associated with significant CAD. The study concluded that serum GA level is a better indicator than HbA1c for evaluating the presence and severity of CAD and predicting major adverse cardiac events in patients with type 2 diabetes.

A 2013 study in Japan assessed the possible correlation of GA with the presence of carotid plaque to evaluate the potential clinical usefulness of GA for predicting atherosclerotic cardiovascular complications in patients with type 2 diabetes. 236 patients with type 2 diabetes were assessed for glycemic control and the presence of carotid plaque.

In patients with carotid plaque GA was higher than those without carotid plaque. In contrast, neither fasting plasma glucose nor glycated hemoglobin was significantly different between the groups. The authors conclude that:

> The positive correlation of serum GA with the presence of carotid plaque in type 2 diabetes suggests that GA will serve as a useful clinical marker for predicting diabetic cardiovascular complications (Sato 2013).

A Korean study in 2012 also suggests that glycated albumin is not only a useful glycemic index but also might be an atherogenic protein in the development of diabetic atherosclerosis (Song 2012).

Retinopathy

A five-year retrospective longitudinal study published in 2014 followed 359 subjects in China. Its aim was to assess the predictive value of glycated albumin (GA) and other risk factors on a progression of diabetic retinopathy (DR). The study confirmed that poor glycemic control, glycated albumin, and impaired renal function predicted DR progression in patients with type 2 diabetes.

Taking into account the growing evidence that GA is a precursor of AGEs, which also correlates with enhanced oxidative stress and endothelial injuries leading to DR, the authors investigated whether using GA as a serum glycemic marker might provide important clinical values for the prediction of people at risk for microvascular conditions. They found that mean GA and mean HbA1c presented similar data for DR progression, which indicated that these two glycemic indices had similar predictive values for DR progression. They concluded that GA might be a valuable glycemic parameter in predicting DR progression (Pan 2014).

A Japanese study investigated whether both indices of chronic glycemia, GA and HbA1c, are differently influenced by diabetic duration and diabetic vascular complications. Annual mean levels of HbA1c, GA, and the GA/HbA1c ratio were determined, and the associations of these values with diabetes duration, diabetic retinopathy, and diabetic nephropathy were analyzed.

These results indicate that GA, rather than HbA1c, reflects diabetic retinopathy in patients with type 2 diabetes mellitus (Morita 2013).

Glycated Albumin For Diabetes Diagnosis

Keypoint

Several recent studies are pointing towards the utility of glycated albumin as a tool for diagnosis of diabetes, with advantages over both HbA1c and conventional diagnosis methodologies.

A cross-sectional, community-based population study of 908 non-diabetic Japanese residents found that GA and HbA1c were comparable methods of diagnostic screening for diabetes and prediabetes, as confirmed through traditional testing methods such as fasting plasma glucose (FPG) and the oral glucose tolerance test (OGTT).

Quoting at length from this study: "The American Diabetes Association and the World Health Organization (WHO) have proposed the measurement of glycated hemoglobin (HbA1c) as a diagnostic tool. As the average lifespan of erythrocytes is approximately 120 days, an HbA1c level reflects a glycemic control state over the prior several months. The advantages of HbA1c are that it does not require special preparation and that it can be measured at any time of day. The disadvantages are that the assay requires whole blood, and values are affected by a shortened red blood lifespan as observed in patients with anemia, hepatic cirrhosis, and hemoglobinopathies. Under such conditions HbA1c may be inaccurate and unsuitable as a marker of glycemic control.

As the half-life of serum albumin (about 17 days) is considerably shorter than that of erythrocytes, serum glycated albumin (GA) reflects a shorter-term glycemic control (about 2–3 weeks) as compared with HbA1c. GA has more rapid and greater changes than HbA1c. Therefore GA may be more useful to document treatment effects when initiating or changing medications for glucose control. GA has also been shown to be accurate for assessing glycemic control in patients with anemia and hemoglobinopathies, as well as those on hemodialysis. Moreover GA may have an important nephropathogenic role that might be therapeutically addressed independently of glycemic status. Moreover we have reported a relationship between GA levels and subclinical atherosclerosis. Other investigators have reported that GA may serve as a better indicator of glycemic fluctuations and has a better correlation with the severity of cardiovascular disease than HbA1c." (Ikezaki 2015)

Since GA can be measured at any time of day without fasting, the authors state that it is suitable for screening in general practice. This advantage has implications for the early identification and treatment of undiagnosed diabetes. Ikezaki concludes that therefore GA measurement may be an appropriate option for detecting undiagnosed diabetes.

In another diagnostic-related study, 852 Korean subjects were tested to determine a GA cut-off value to diagnose pre-diabetes and diabetes in Korean adults. In addition, the study compared the performance of GA for the diagnosis of diabetes with that of glycated hemoglobin (A1c) and found that the combined measurement of FPG and GA may detect diabetes earlier than the measurement of FPG and A1c. The study found that the optimal cutoff for using GA as a diagnostic measure was lower than that proposed for Japanese and Chinese populations.

The study further noted several additional disadvantages for HBA1c measurement has in addition to the known issues involving RBC morphology and metabolism First, HBA1c concentrations may vary with subjects' race or ethnicity. Second, it is still uncertain whether the diagnostic criteria apply equally well to children or adolescents with type 2 diabetes (Hwang 2014).

The Glycated Albumin / HbA1c Ratio

The calculation of the GA/HbA1c ratio provides another increasingly important use of glycated albumin as a diagnostic tool. Perhaps the most critical is in the diagnosis of fulminant type 1 diabetes, an uncommon but extremely serious condition that can occur during pregnancy. Fulminant type 1 diabetes mellitus (FT1DM) develops as a result of very rapid and almost complete destruction of pancreatic β cells. This condition is often fatal if undetected. A 2013 study by Koga confirms that an elevated ratio of glycated albumin (GA) to HbA1c (GA/HbA1c ratio) is a valid indicator of the onset of this deadly condition at any stage of pregnancy.

The following table summarizes diabetes-related conditions that for which the GA/HbA1c ratio can provide critical information (Koga 2014):

Table 3
Diseases and conditions with high/low GA/HbA1c ratios.

Diseases with high GA/HbA1c ratios	*Diseases with low GA/HbA1c ratios*
Rapid deterioration of glycemic control	Rapid improvement of glycemic control
At the time of onset of fulminant type 1 diabetes mellitus	Iron deficiency anemia
	Pregnancy
At the time of onset of acute-onset type 1 diabetes mellitus	Nephrotic syndrome
	Hyperthyroidism
Hemolytic anemia	Administration of glucocorticoids
During treatment of iron or erythropoietin	Cushing's syndrome
Liver cirrhosis	Obesity, smoking
Chronic kidney disease (renal anemia)	Hyperuricemia, hypertriglyceridemia
Neonates/infants, neonatal diabetes mellitus	Nonalcoholic fatty liver (NAFLD) with high ALT[a] levels
Hereditary persistence of fetal hemoglobin (HPFH)	
Hypothyroidism	Administration of drug for postprandial plasma glucose
Adrenal insufficiency	
Emaciation	Variant hemoglobin
Postprandial hyperglycemia/large glycemic excursion	*Diseases with high or low GA/HbA1c ratios*
Type 1 diabetes mellitus	Variant hemoglobin
Postgastrectomy	Diabetic nephropathy stage 4
	Nonalcoholic steatohepatitis (NASH)

[a] ALT: alanine aminotransferase.

Advances In Screening With Glycated Albumin

Keypoint

In a landmark development in diabetes screening, in 2009 the Japanese Red Cross Society introduced universal screening for glycated albumin, using a clinical lab-based test for GA. As of 2010 more than 3 million blood donors had been screened. Subsequent additional studies are confirming that GA is a valid marker to screen for both prediabetes and diabetes.

Highlights of Japanese Red Cross Study

When blood donors are informed of their GA levels:

- **More than 40% of pre–diabetic and diabetic donors showed decreased GA levels on the second testing date.**
- **More than 60% of normal–high donors showed decreased GA levels on second testing date.**

In 2009 the Japanese Red Cross Society introduced measurement of glycated albumin (GA) for all blood donors as a glycemic control marker, to help identify individuals with diabetes or those predisposed to the disease. Altogether GA was measured in 3.14 million blood donors who donated between April 2009 and March 2010. GA levels were analyzed by sex and age. All donors were notified of their GA levels. For repeat donors, a comparison was made between the GA levels at the first and second donations to verify the GA change after notification.

The analysis found that mean GA levels of both sexes increased with age and reached the same level of 14.8% in their 60s. The percentage of donors with prediabetes/diabetes (GA ‡16.5%) was 2.8% in males and 2.3% in females.

A total of 973,160 donors made more than one donation during this period, and a comparison was made between GA levels at their first donation and those at their second donation. Following notification of high GA levels, in 42.4% of donors identified as potentially prediabetic GA levels decreased to the normal range at a second blood donation. It remains to be elucidated whether the decrease in GA is a true effect of GA notification (Araki 2012).

The study concludes that donor blood screening for GA represents an effective measure to identify people at risk of diabetes. The decrease in the GA level after GA notification might indicate the potential usefulness of this strategy to improve glycemic control among people with high GA.

In a follow up to the decision of the Japan Red Cross Society to screen all blood donors in Japan for glycated albumin levels, Ikezaki conducted a cross-sectional study of a community-based population study of 908 non-diabetic Japanese residents. The results reinforce the indication that the measurement of glycated albumin may serve as a useful screening test for diabetes in a general Japanese population (Ikezaki 2015).

Evaluations of GA as a tool for screening for diabetes and prediabetes are ongoing. In 2015, Hsu et.al. conducted a study "to determine the universality and the clinical utility of GA in screening for diabetes mellitus." The study reported on 2192 residents of Northern Taiwan, of whom 54.2% had previously been diagnosed and treated for diabetes. The authors concluded that

- Glycated Albumin is a novel stable marker for mean blood glucose over 2–4 weeks
- GA can be used alternative marker for diagnosis of diabetes and prediabetes.
- GA values are comparable all over the world.

Prediabetes

Prediabetes is understood as a pre-clinical stage of increased risk for overt diabetes mellitus (DM) and cardiovascular disease. This prediabetic stage can be reversible to normal glucose tolerance. Therefore, medical intervention strategies at this stage are important in preventing the full onset of diabetes and its complications. Although both glycated albumin and HbA1c are indices capable of assessing glucose exposure, a Korean study found that glycated albumin has more potential for assessing insulin secretory dysfunction and glycemic fluctuation than HbA1c. The study enrolled 1379 subjects not diagnosed with diabetes. This study found that GA could be a better indicator for screening impending diabetes and assessing beta cell dysfunction in pre-diabetic period subjects (Hong 2014).

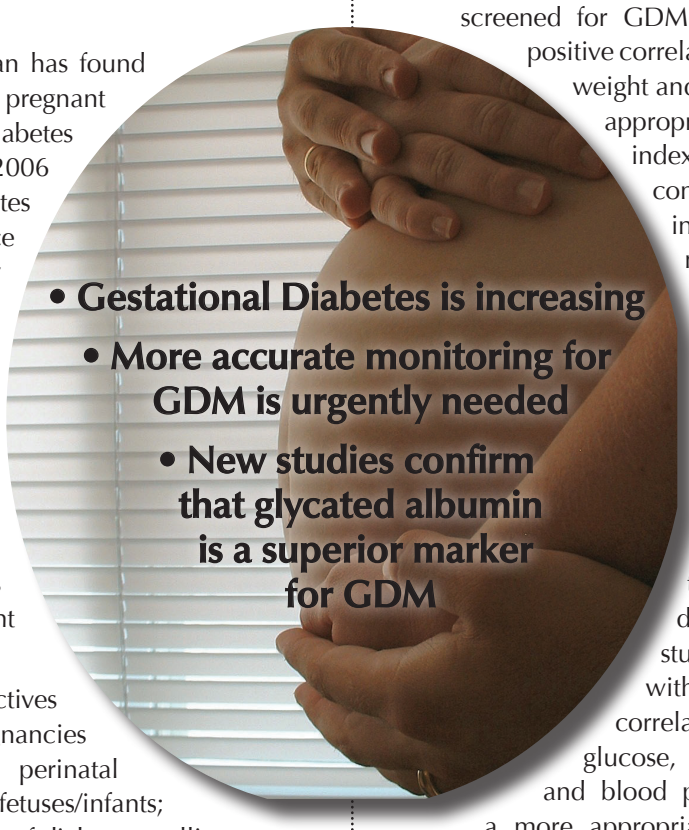
Gestational Diabetes

Keypoint

A point-of-care test for glycated albumin as a diagnostic tool for Gestational Diabetes was first advocated in 1999, and clinicians have called for development of a widely available test. New studies validate the utility of GA testing for gestational diabetes.

The prevalence of gestational diabetes appears to be broadly on the increase, following the overall trend of the diabetes epidemic as a whole. Several recent studies continue to confirm the importance of glycated albumin as a tool to diagnose and control gestational diabetes.

A 2012 study conducted in Japan has found that an increasing number of pregnant women have gestational diabetes mellitus (GDM). While a 2006 screening for Gestational Diabetes Mellitus estimated the prevalence of GDM at 2.92%, the new study of 676 women demonstrated that the prevalence of GDM among pregnant women in Japan needed to be recalculated to 12.08%. The study found that more than 10 percent of pregnant women have hyperglycemic disorders, and diabetic pregnancy has become one of the most important complications in Japan.



The study restated the objectives of managing diabetic pregnancies as follows: 1) prevention of perinatal complications in mothers and fetuses/infants; 2) prevention of the development of diabetes mellitus and metabolic syndrome from GDM; 3) Prevention of the development of diabetes mellitus and metabolic syndrome of the neonates in their future. The authors state,

> To meet these objectives , universal screening for GDM, (and)strict glycemic control during pregnancy...are of prime importance. Therefore, stricter glycemic control than that in non-pregnant women is necessary to prevent perinatal complications. Markers that more precisely reflect variations in blood glucose levels during pregnancy are thus urgently needed.

They conclude that compared to HbA1c, glycated albumin (GA) reflects blood glucose profiles during a more recent period and that GA is being increasingly used as a marker of blood glucose profiles for the past two to four weeks. The study confirmed that GA appears to be a useful marker for pregnant women, since it can be measured easily and changes rapidly and markedly (Hiramatsu 2012).

In a 2105 study, Li et al. aimed to assess the clinical utility of GA in the management of GDM by monitoring GA levels and evaluating the association between glycemic control and birth weight with glycated albumin in 2,118 Chinese women screened for GDM. They found that there was a positive correlation between GA levels and birth weight and concluded that GA might be an appropriate and conveniently measured index that can detect poor glycemic control and predict birth weights in GDM women. The authors noted that "Markers that more accurately reflect variations in blood glucose levels and mean glycemic status for short-term in GDM women are urgently required."

The study noted that HbA1c could be a flawed indicator of blood glucose control in a short-term period and not appropriate during pregnancy. A previous study cited showed that compared with HbA1c, GA is more closely correlated with fasting and postprandial glucose, regardless of insulin resistance and blood pressure, and that GA is likely a more appropriate index for evaluating blood glucose in GDM women.

> Few studies have assessed the validity of GA in GDM management. The primary utility of GA is to detect approximately 80% of subjects with poor glycemic control, to positively affect GDM management and to permit the early identification of subjects who are at imminent risk of disease development, and who can then be referred for further evaluation and appropriate management.
>
> The regular monitoring of GA of these women (once/3–4 weeks) helps to reduce the frequency of SMBG, thereby lowering healthcare costs, and increasing patient treatment compliance (Li et al. 2015).

Keypoint

There are many areas where a better picture of current and recent glycemic status would be extremely beneficial in making decisions regarding best practices for medical care and treatment.

The utility of GA testing has become increasingly evident in connection with diabetes monitoring as research has confirmed that it provides a more current picture of recent glycemic status than earlier testing protocols such as HbA1c. This advantage is inherent in the nature of the metabolic cycle of albumin versus that of hemoglobin. In addition, research conclusively demonstrates that GA provides a more accurate picture across a broader range of individuals and is less affected by potential underlying medical conditions than the HbA1c test. Many studies now also show that GA provides a better reflection of response to ongoing treatment.

A sample of areas where the availability and application of a convenient point-of-care rapid test for GA could have a positive impact includes:

- Cardiovascular surgery
- Insulin Biosimilars
- Emergency care

Cardiovascular surgery

Recent research has established that the hemoglobin A1c test is a useful predictor of the outcome of cardiovascular surgery. A survey of eleven studies concluded that elevated HbA1c is a strong predictor of mortality and morbidity after coronary artery bypass surgery irrespective of previous diabetic status (Tennyson 2013).

- Four studies found significant increases in early and late mortality at higher HbA1c levels.
- One study demonstrated that 30-day survival outcomes were significantly worse in patients with previously undiagnosed diabetes and elevated HbA1c compared with those with good control.
- Three studies identified a significant increase in infectious complications in patients with poorly controlled HbA1c.
- Elevated HbA1c vs normal HbA1c was associated with prolonged stay in hospital and in intensive care unit (ICU) in patients irrespective of previous diabetic status.

A similar 2015 study in Sweden of over 6,000 patients with type 2 diabetes who underwent coronary artery bypass grafting (CABG) also validated the association between preoperative hemoglobin A1c (HbA1c) levels and long-term mortality after surgery (Kuhl 2015).

While HbA1c can show a picture of long-term glycemia, a preoperative glycated albumin test would provide valuable information about glycemic control in the immediate preoperative period. Medical procedures could be better tailored to mitigate the potential for negative outcomes.

Insulin Biosimilars

Biosimilar insulins are coming into the world marketplace. A recent article pointed out that "[m]any branded analog insulins will lose patent protection during the next few years. In response, a number of companies are developing biosimilar insulins, which have the potential to lower healthcare costs and increase patient access (Edelman 2014)." While the availability of biosimilar insulins can potentially lead to lower insulin costs and increased access for patients with diabetes worldwide, clinicians and regulatory agencies have raised several concerns regarding the safety and efficacy of these new formulations.

The European regulatory agencies have established guidelines for market approval of biosimilar insulins; however the FDA has yet to establish guidelines for numerous issues surround the development and commercialization of biosimilar insulins including effective postmarketing surveillance protocols, determination of product interchangeability, and product identification/labeling (Dolinar 2014).

GA could play a critical role in the post-marketing surveillance likely to be required by FDA as part of the biosimilar insulin approval process. Because biosimilars are basal insulins, episodic monitoring by SMBG is unlikely to be sensitive enough to show a change in average glycemic level. GA would allow any change in control resulting from a switch to a biosimilar to be detected much sooner that it could be detected by HbA1c.

Emergency Room Screening

Knowledge of the glycemic status of patients who are brought into a hospital emergency room for treatment can be critical to providing proper care, especially for those requiring surgery or critical care. Such patients are likely to be unable to inform doctors of preexisting medical conditions such as diabetes, and may not even be aware of their condition. A test such as glycated albumin that can give physicians information on short-term recent glycemic status beyond the single point reading given by a blood glucose test could improve patient care, reduce the incidence of complications and reduce treatment costs.

Advances in Sensor Technology for Glycated Proteins

Keypoint

In the light of the growing recognition of the limitations of HbA1c and the lack of a convenient rapid point-of-care method for glycated albumin estimation, development of a robust, sensitive glycated albumin assay is extremely desirable in order to fill the gap in the present diagnostic landscape. Epinex Diagnostics has already taken a leading role in the development of a handheld device to measure GA and is looking ahead to further advances in sensor technology.

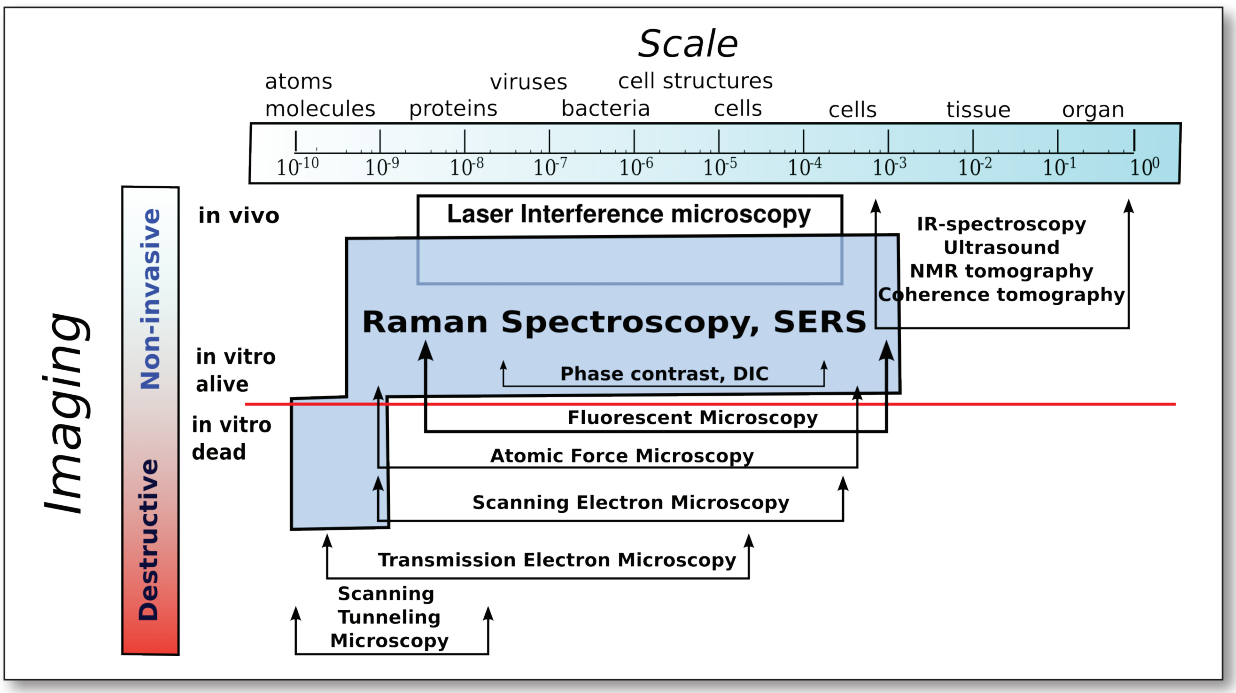
A 2015 review article by scientists from the MIT Biomedical Research Center, Harvard Medical School and the Johns Hopkins Department of Mechanical Engineering evaluated emerging trends in optical sensing of glycemic markers for diabetes monitoring. They note that detection of such glycemic markers is challenging, especially in a point-of-care setting, due to the stringent requirements for sensitivity and robustness.

A recent push to develop and standardize a glycated albumin assay has yielded significant developments. Advances in monoclonal antibody development specific to glycated albumin, while beneficial in principle, requires considerable sample preparation and have not increased the availability of commercial glycated albumin assays to date. However, various optical-based technologies have shown promise.

The review states that by using Raman spectroscopy glycated albumin can be differentiated from its non-glycated counterpart with 100% accuracy.

Other techniques include refractive index-based measurements and an aptamer-based surface-plasmon sensing approach (see below). While none of the these methods has been validated in an adequate number of clinical samples, the emergence of novel spectroscopic and imaging techniques suggests the potential development of a truly label-free, glycated albumin-specific, high-throughput photonic assay. Although only a few of the emerging optical methods have been tested in clinical patient samples, they have demonstrated the feasibility of clinical application by offering high levels of accuracy and precision for physiologically relevant levels of these biomarkers.

A wide array of methods has been designed based on differential markers in native and glycated proteins. Of these methods, vibrational spectroscopy, due to its label-free, real-time detection capabilities, shows the greatest promise, although significant work still needs to be done in order to reduce the cost and the size of instrumentation. Ultimately, according to the reviewers, the goal is to design and develop both a table-top and a hand-held miniaturized spectrometer to be used in conjunction with lab-on-a-chip devices that can together offer multiplexed, high-throughput, clinically viable detection of the glycemic marker panel. (Pandey 2015)



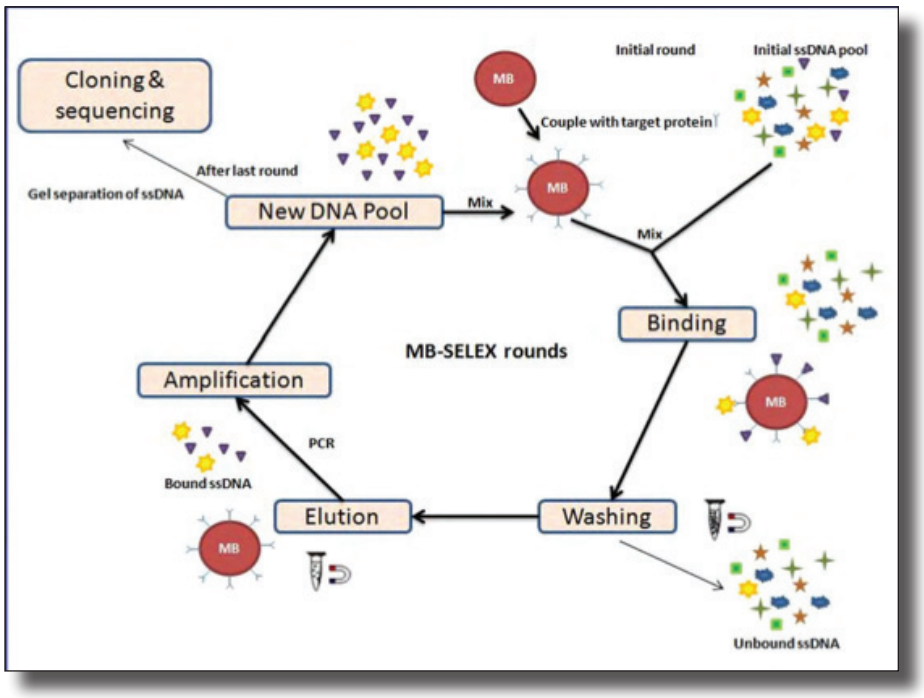
Aptamer Technology for Glycated Albumin

Keypoint

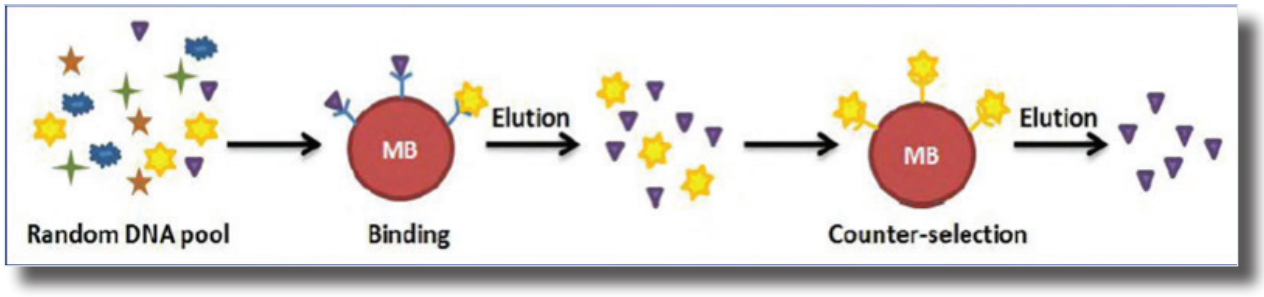
In 2011, scientists in the Departments of Bioengineering and Chemical and Environmental Engineering at the University of Toledo began development of an aptamer-based technology for detection of glycated proteins, with a focus on glycated albumin. Epinex Diagnostics is working closely with the University of Toledo to translate their aptamer technology into a robust rapid test for glycated albumin that will complement the company's existing proprietary research.

Aptamer Selection

Aptamers are oligonucleotide molecules that bind to a specific molecular targets such as small molecules, proteins, nucleic acids, and even cells, tissues and organisms. Aptamers are usually created by selecting them from a large random sequence pool. Aptamers are useful in biotechnological and therapeutic applications as they offer molecular recognition properties that rival that of antibodies. In addition to their discriminate recognition, aptamers offer advantages over antibodies as they can be engineered completely in a test tube, are readily produced by chemical synthesis, possess desirable storage properties, and elicit little or no immunogenicity in therapeutic applications.



The breakthrough in aptamer selection achieved by the University of Toledo team was to modify the selection process so that aptamers could be developed and selected for proteins that were very similar to each other, specifically, the glycated and non-glycated forms of the same protein.



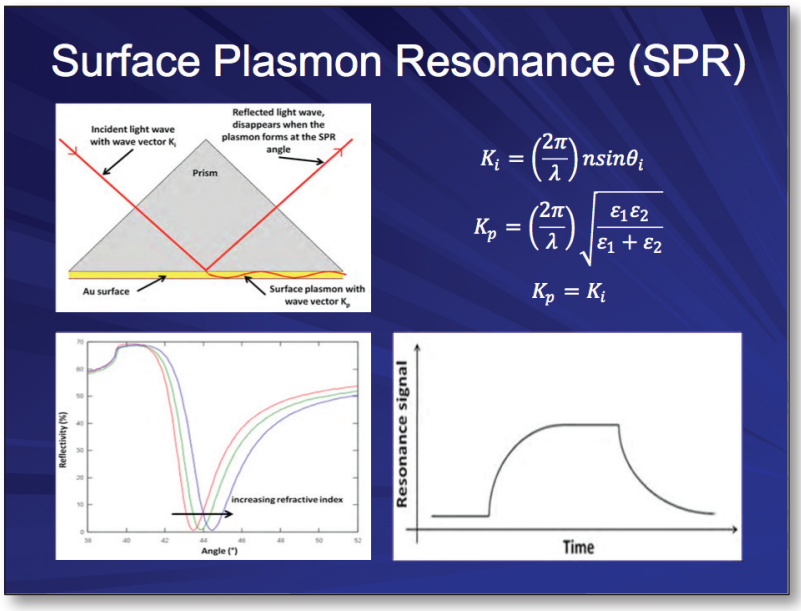
Aptamer Detection and Binding

Keypoint

A successful aptamer will demonstrate highly selective binding to glycated albumin and reject similar molecules.

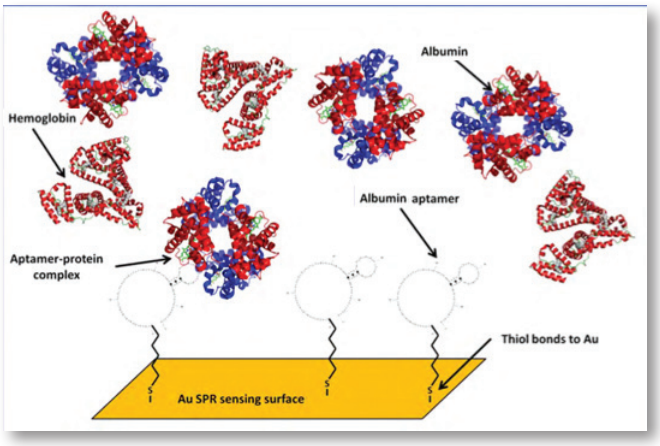
Detection

The University of Toledo team researched the utility of Surface Plasmon Resonance detection, a method for detecting very small amounts of a particular molecule. It is particularly useful for immunoassay tests because it does not require that the target be attached to another molecule, as is required for standard laboratory ELISA testing.



Surface Plasmon Resonance with Aptamers

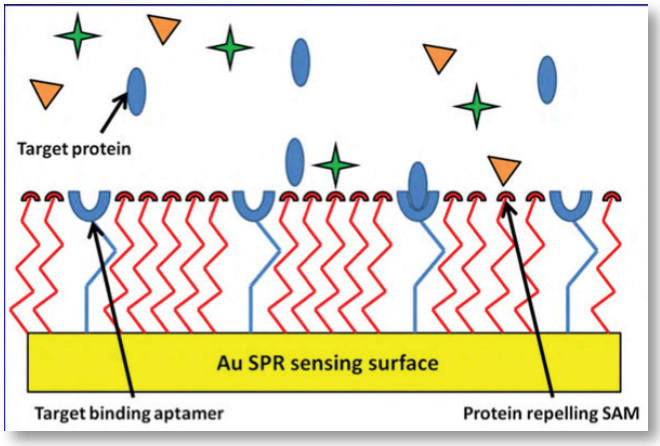
- SPR is very sensitive but not selective
- Aptamers may be used to make SPR selective
- Aptamers may be immobilized to the SPR surface

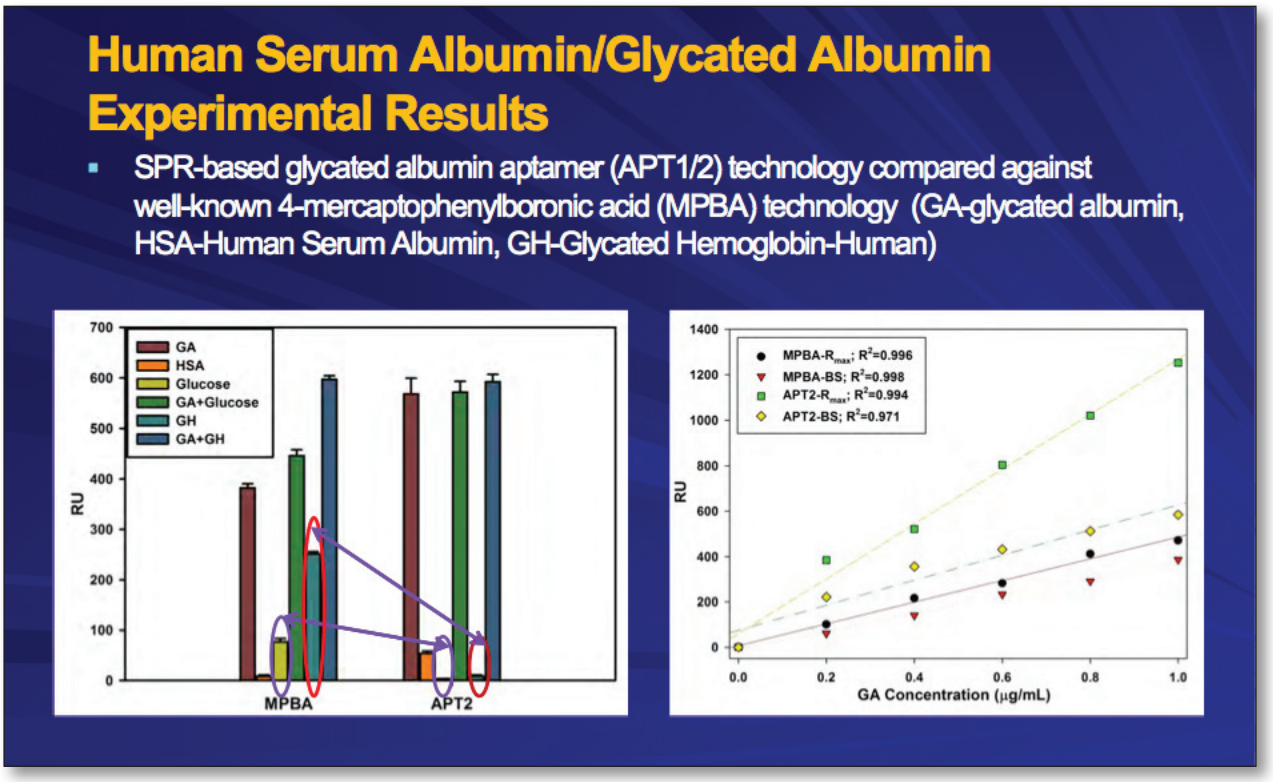


In order to get precise and accurate results from aptamer-based SPR, the aptamer needs to be bound to a gold surface and treated in a way that the mix of similar molecules found in a sample do not interfere with the binding of the target molecule.

Aptamer-based SPR sensor characteristics

- Immobilizes selective aptamer onto the SPR sensing surface
- Surface needs to repel all non-target proteins (i.e. the only binding that occurs is the target protein at the aptamer site)





Experimental results show that the aptamer binds strongly and selectively to glycated albumin but not to human serum albumin, glycated hemoglobin or glucose. The degree of binding reflects the glycated albumin concentration in a highly linear fashion.

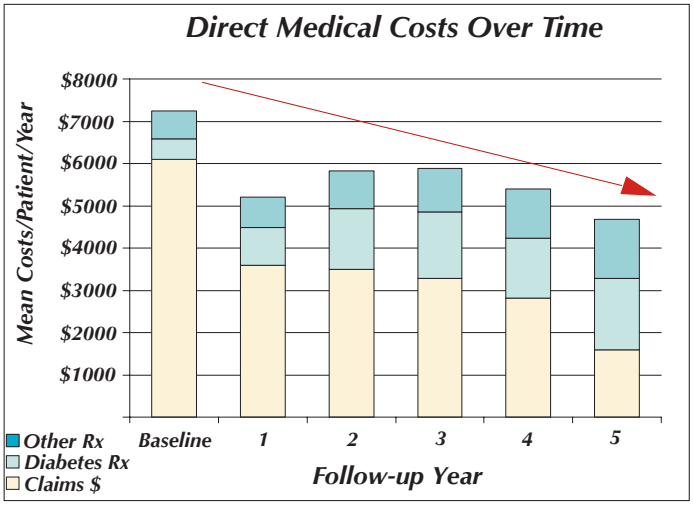
Defining The Solution: A New Diabetes Care Paradigm

Keypoint

The Asheville Project first demonstrated that diabetes could be controlled by through a community program that centered on a monthly consultation between a diabetic and healthcare professional such as a pharmacist, nurse, or counselor. The results of the Asheville Project were reproduced in the Diabetes Ten City Challenge. These programs were the foundation of similar efforts begun nationwide.

Only in the past few years have governments, major health care providers, and pharmaceutical companies begun to respond to the scale of the looming diabetes disaster by focusing on finding effective programs for diabetes prevention and control. The way forward was indicated beginning almost 15 years ago, when the Asheville Project and the Diabetes Ten City Challenge demonstrated that a community–based prevention strategy based on monthly counseling improved patient outcomes, conserved expensive medical resources and more than paid for itself in reduced medical costs and increased worker productivity.

City of Asheville, North Carolina (Downtown)



Asheville Study Results (Cranor et. al.)

Passage of the Patient Protection and Affordable Care Act (PPACA - Obamacare) in 2010 has had the most significant impetus for the exploration and adoption of prevention–based approaches to diabetes. The PPACA called for the creation of the National Prevention Council, which in turn released the National Prevention Strategy in June of 2011 – a document that outlines a detailed policy for the implementation of prevention–based programs that will improve health and significantly reduce healthcare costs.

Diabetes 10 City Challenge Participants
Charleston, SC
Chicago, IL
Pittsburgh, PA
Tampa Bay, FL
Colorado Springs, CO
Northwest, GA
Milwaukee, WI
Los Angeles, CA
Honolulu, HI
Cumberland, MD

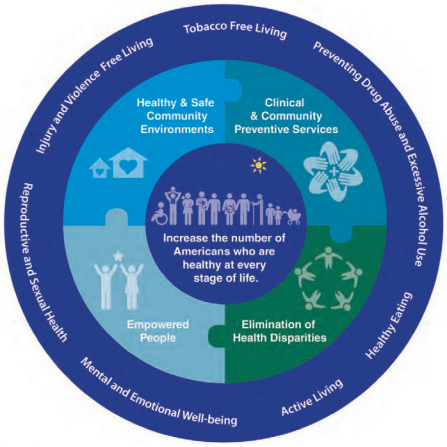
The ACA included federal support for the creation and expansion of prevention initiatives such as

Authority for funding grants to model sites,

The establishment of a training and outreach program for lifestyle intervention instructors, and a

Centers for Disease Control (CDC) programs to determine eligibility for those entities delivering services.

The PPACA has also provided grants to states to prevent chronic diseases, including diabetes, in the Medicaid population. Grant funding supports initiatives that provide incentives to enrollees to participate in programs that improve health and outcomes through the adoption of healthy behaviors.

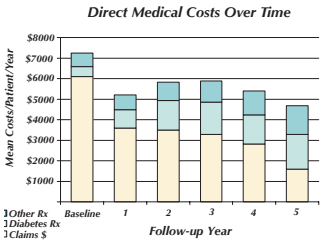


Current Diabetes Prevention & Control Programs

Keypoint

Evidence-based diabetes prevention programs based on monthly counseling are now widespread throughout the U.S., with community organizations such as the YMCA supported by national organizations such as the American Association of Diabetes Educators. The health insurance industry has embraced the logic of diabetes prevention and is in the process of implementing a national strategy for diabetes prevention.

The National Diabetes Prevention Program (DPP) implemented by the Centers for Disease Control (CDC) is an evidence-based lifestyle change program for preventing type 2 diabetes. The year-long program helps participants make real lifestyle changes such as eating healthier, including physical activity into their daily lives, and improving problem-solving and coping skills. Participants meet with a trained lifestyle coach and a small group of people who are making lifestyle changes to prevent diabetes. Over 625 organizations offer the program nationally.



As executed by the YMCA, the Diabetes Prevention Program is a 12-month evidence-based program that includes 16 weekly sessions followed by monthly sessions. To date, more than 25,000 people have attended at least one YMCA's Diabetes Prevention Program session (2014). The YMCA's Diabetes Prevention Program is available at 1,033 locations in 41 states. The program has achieved a 58% reduction in risk overall, with a 71% reduction in risk for adults over. The program has enrolled 19,500 participants in 850 programs, in 41 states.

One-year, group-based program

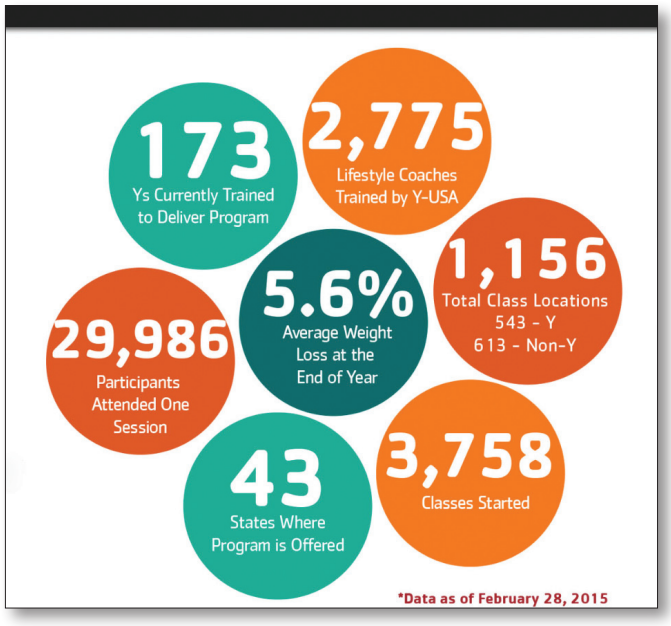
Weekly and monthly sessions led by a trained Lifestyle Coach

Who Qualifies:

- **Overweight**
- **At risk or diagnosed with prediabetes**
- **Previous diagnosis of gestational diabetes**
- **Not already diagnosed with type 1 or type 2 diabetes**

American Association of Diabetes Educators AADE is currently funding a total of 45 nationally certified Diabetes Self-Management Education (DSME) programs in 16 states to implement the National DPP. AADE is also coordinating with CDC and other national organizations, state health departments, employers groups, insurance groups and other third-party payers to support the coverage of DPP as a medical benefit. The AADE is using the success of its DPP model to demonstrate that offering the National DPP as a reimbursable benefit is cost effective and often cost saving for the third party payer.

As part of a cooperative agreement awarded to the trade organization America's Health Insurance Plans (AHIP) by the CDC, health insurers is working in collaboration with participating health insurance plans to launch the National Diabetes Prevention Program (National DPP) in six states across the United States.



Results achieved by YMCA Programs

The Financial Stakes of Diabetes Prevention

Keypoint

The financial incentives that will drive effective programs for diabetes prevention and control are enormous. The UnitedHealth Group report provides a detailed estimate of the potential savings opportunity offered by effective diabetes prevention. The scope of these savings will drive the market to search for and implement the most effective means of realizing these benefits.

Projected Savings with a Monthly Diabetes Management Program

An analysis prepared by the UnitedHealth Group clearly shows how much is at stake, and how large the benefits could be if the diabetes epidemic were to be brought under control.

If all U.S. adults with prediabetes were to enroll in the DPP (Diabetes Prevention Program):

Diabetes prevalence could be reduced by 8 percent by 2020.

The number of individuals expected to convert from prediabetes to diabetes would be reduced by three million individuals over the next decade.

Cumulative health system savings will be about $105 billion (net of the estimated implementation costs).

$61 billion would be realized through savings to the federal government through a reduction in Medicare and Medicaid spending and exchange subsidies.

A more comprehensive program would yield even more substantial benefits and returns. Based on a simulation of a combined medical compliance intervention and lifestyle intervention focused on weight loss for people with diabetes model:

The potential to reduce diabetes–related complications would be about 10 percent by 2020.

Diabetes prevalence would be reduced by 9 percent by 2020.

Prediabetes prevalence would be gradually lowered on net by 7 percent by 2020.

Estimated projections made specifically in conjunction with a program of diabetes management based on monthly consultation with a pharmacist (the Asheville Project model) would produce estimated health care savings of $34 billion over the next ten years. Savings would accrue from a reduction in the number of diabetes-related complications resulting from improved health status among people with diabetes moving from non-compliant to compliant status. Savings to the federal government were estimated to be $21 billion over the period.

If a program based on monthly consultation with a diabetes professional were administered to all diabetes patients, new cases of diabetes complications could come down significantly. Cost savings estimates rise to $88 billion in health care savings over 10 years. Savings to the federal government might be $53 billion over the period.

For a combination of all projected interventions the United Healthcare Study estimates a net reduction of around $250 billion in medical costs ($357 billion gross) and a gain of $239 billion in productivity.

Two Integrated Paths to Diabetes Control

A comprehensive program to stem the onslaught of diabetes will require a coordinated effort along two broad lines of attack: screening and monitoring. Patient empowerment will be at the heart of any successful effort – all of the studies and success stories to date point to the combination of patient involvement and effective counseling as key to improving health and patient outcome. These are also the methods that have proven the most cost effective.

- People in the prediabetes stage need to be informed that they are at risk in order to provide them the time and methods to make the behavioral and lifestyle changes that will prevent or postpone their condition from becoming full–blown diabetes.
- People who have been identified as at risk or who already have diabetes need to have a way that they and their healthcare support system can monitor their condition. Are the actions they are taking effective, leading to improvement, or are different or additional approaches to care required?

Basic research on glycated albumin continues to advance

The recent explosion of research on glycated albumin as a marker for diabetes control has opened a new and deeper level of understanding of how the interactions of this key molecule affect the onset and course of this debilitating disease. Glycated albumin has been increasingly linked to a wide range of diabetes complications, including chronic kidney disease, cardiovascular disease, neuropathy and other conditions traceable to restricted capillary flow. And new research is demonstrating that glycated albumin is seen as a causative agent, not just as a factor that can be monitored.

New technology may offer a point-of-care (POC) rapid test that can screen for diabetes and prediabetes

Even more promising is research that highlights the prospect of using glycated albumin as a full diagnostic tool for diabetes. Large-scale screening in Japan, as well as smaller studies in Korea, have demonstrated that GA screening is an effective way to alert subjects to their condition with regard to developing diabetes.

Development of a simple and cost-effective rapid test for glycated albumin is a key to unlocking the potential application of GA to the range of potential uses highlighted by recent research. A new technology based on aptamers, small pieces of artificial nucleotides that can be constricted to bind to large molecules such as proteins, appears to offer the solution to constructing such a rapid test. Epinex Diagnostics is deeply involved in exploiting this technology and bringing it to completion.

A paradigm shift in diabetes prevention and management is under way

We have known for a long time that the keys to managing diabetes are prevention and, when it becomes necessary, self-management of the diabetic condition. The search for an effective strategy to accomplish these goals has been long, and at times elusive. Now, at last, an effective and reproducible paradigm for diabetes monitoring and control has been demonstrated: based on monthly counseling in conjunction with evidence-based benchmarks that puts simple and easy-to-understand tools in the hands of diabetics, under the guidance of their counseling partner. Empowering diabetics provides incentives for them to meet their treatment goals, while shifting the front line of diabetes control from doctors to pharmacists, nurses, and trained counselors lowers costs significantly. These programs more than pay for all their costs through reduced medical expenses and increased productivity. This strategy has been aimed initially at people who already have diabetes, with the goal of slowing or reversing the degeneration of physiological functions affected by diabetes, and postponing or eliminating the onset of the many serious complications associated with the disease. Glycated albumin has the potential to be tied directly to the monthly treatment paradigm, where it can provide the evidence-based scientific result that will reinforce treatment.

A monthly POC test for GA could have a powerful impact to improve patient outcome and control the costs of diabetes

As this report shows, glycated albumin testing has been increasingly coming to the forefront of diabetes monitoring. It is a measurement of a marker directly linked to several of the most serious complications of diabetes. It has been shown to be a better reflection of the efficacy of treatment than other tests. It has recently been shown to be an effective marker to screen for diabetes at an early stage. If GA testing can be brought into widespread general use, it has the potential to bring about real and significant improvements in diabetes care. Patients will be better able to monitor their condition and take better charge of their life choices. Doctors will be able to better predict the status of many of the most debilitating complications of diabetes. Clinics and public health agencies will have a tool that can screen for undiagnosed diabetes and prediabetes, in time to enact measures for prevention and early intervention for the affected individuals.

Epinex Diagnostics has been at the forefront of developing a new mode of POC diagnostics for diabetes care

All of these outcomes have the potential to save healthcare systems vast amounts of money. Treatment of diabetes complications is by far the largest financial cost of the disease; any program that can reduce the incidence or severity of complications will produce significant savings. Blood glucose testing by non-insulin using type 2 diabetics remains a significant expense for individuals and for government healthcare systems, even though there is widespread evidence that such testing is of limited effectiveness as a means for people to control their diabetes. Several studies suggest that many diabetics will be able to reduce or eliminate frequent blood glucose testing if they have access to a system of short term glycemic monitoring such as GA, which should result in considerable cost savings. The remaining step is to provide this superior test in a rapid, convenient, and cost-effective format; something that is only now becoming available. We believe that the Epinex G1A™ Rapid Diabetes Monitoring Index Test could provide this essential tool that, in conjunction with the new treatment paradigm, can transform diabetes care.

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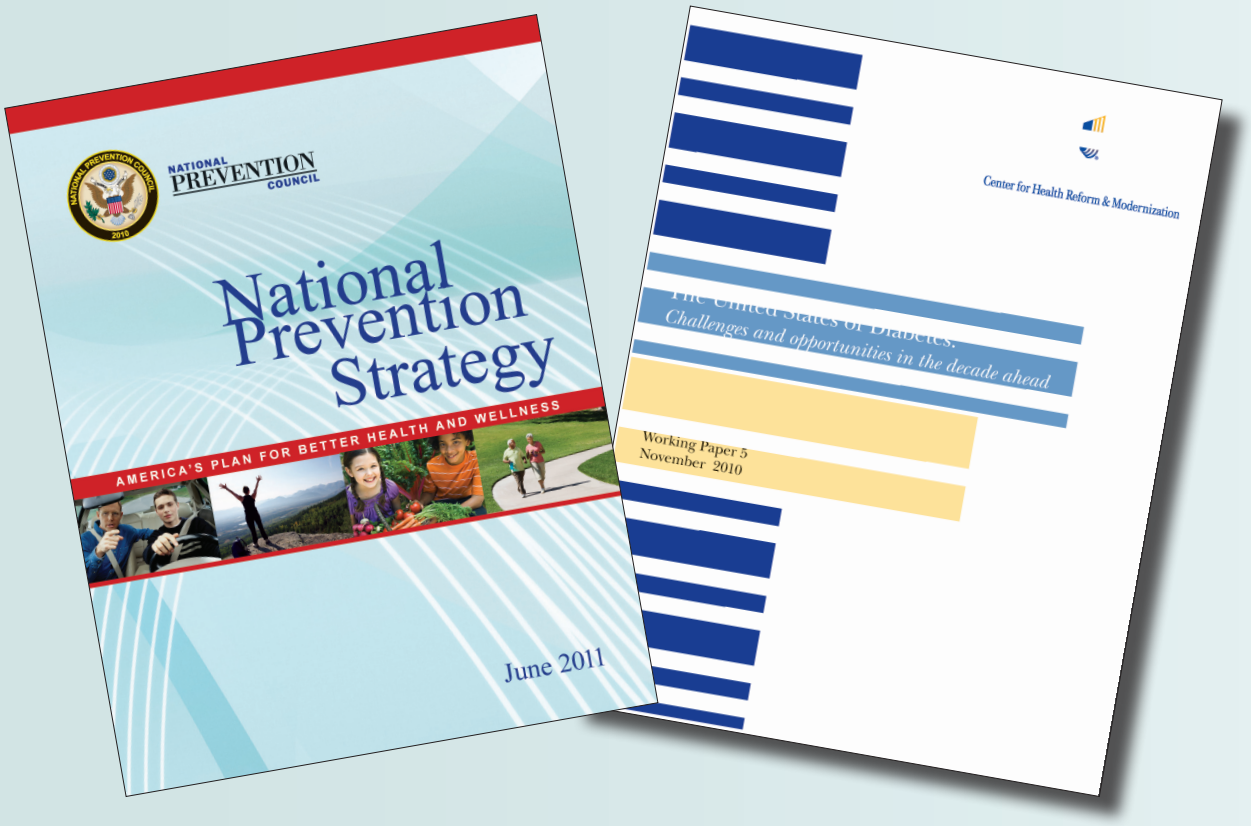

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