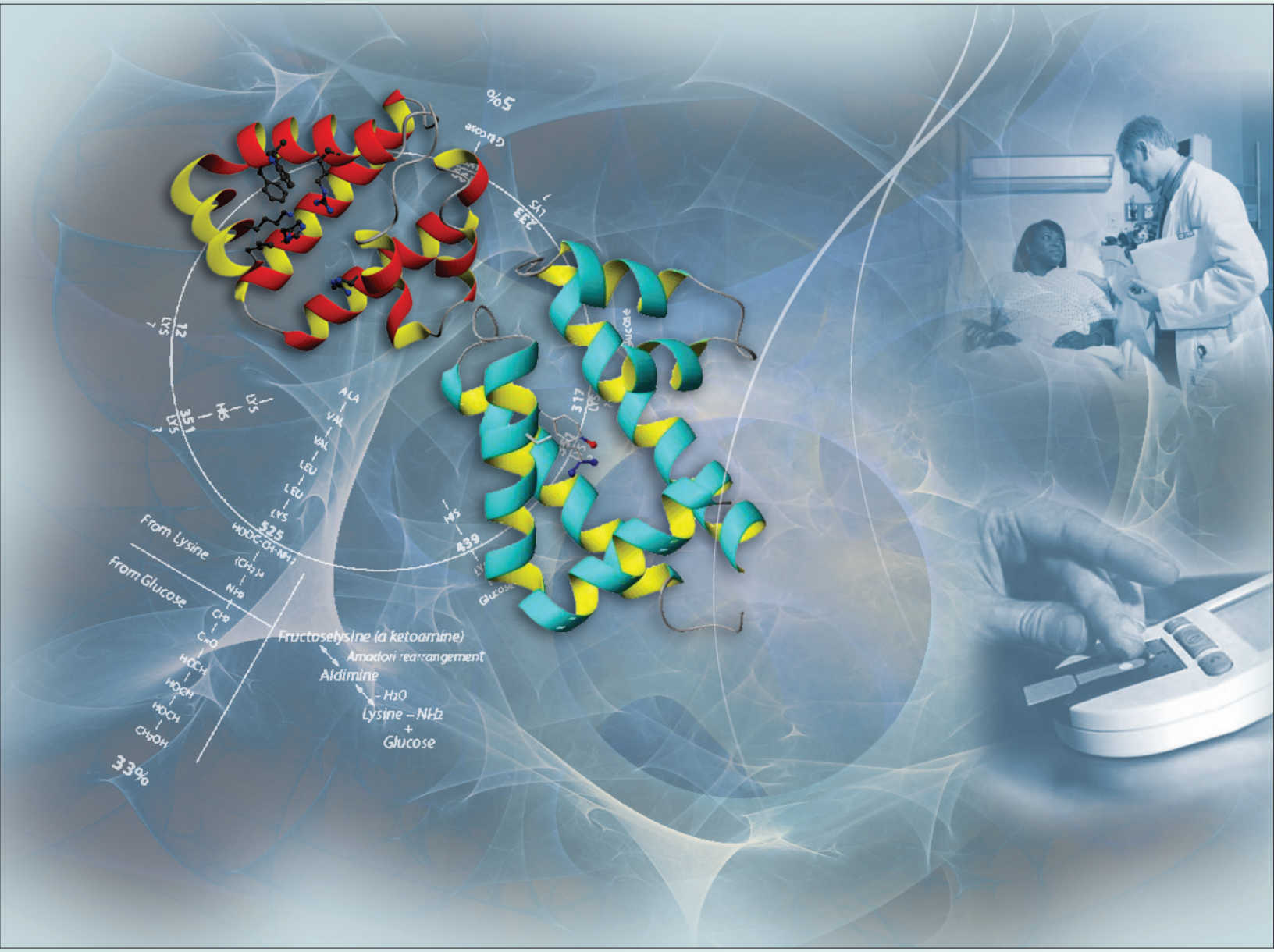


Glycated Albumin and Diabetes Monitoring

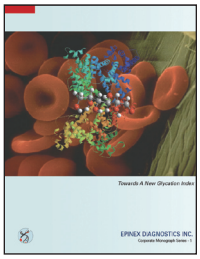
EPINEX DIAGNOSTICS INC.

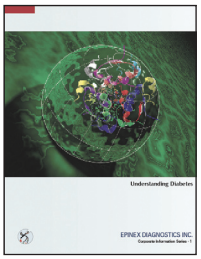
Corporate Information Series - 2

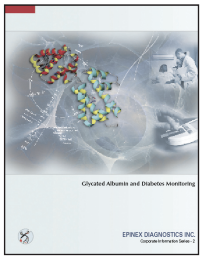
Foreword

This is the second 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, prepared by our Director of Corporate Communications, Dr. David Trasoff, assisted by Serop Gharibian, Executive Assistant, and interns Jaycee Delizo and Bo Du, presents a detailed analytical review of glycated albumin. It describes the glycation process and the resulting structure of the glycated albumin molecule; the mechanisms by which GA causes damage to tissues in the body; and the connection to many of the most common and most devastating complications of diabetes. We then present a detailed description of the existing and potential clinical applications for a rapid test that measures intermediate glycemia using glycated albumin. I hope that this presentation will provide you with useful information and will encourage discussion that may help to improve the available options for patient diagnosis and treatment.

Asad R. Zaidi, President
Epinex Diagnostics Inc.







Glycated Albumin and Diabetes Monitoring

Glycated Albumin: An Intermediate Glycemic Index
An Analytical Review

EPINEX DIAGNOSTICS INC.
Corporate Information Series - 2

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CORPORATE OFFICES
Epinex Diagnostics, Inc.
2815 McGaw Avenue
Irvine, CA 92614
Phone: (949) 660-7770
Fax: (949) 660-7772
email: info@epinex.com
www.epinex.com

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Abstract

The progressive complications of unmanaged diabetes include heart disease, blindness, kidney failure, amputation of extremities due to circulation problems, and nerve disorders, as well as other chronic conditions. Decades of research have established that prolonged exposure to excess glucose is the cause of diabetes complications, and that long-term control of blood glucose levels is required to avoid or lessen the damage caused by excess glucose. The process of protein glycation is now understood to be both a marker for the progress of diabetes complications and an underlying cause of many of the most serious complications.

Monitoring blood sugar levels in individuals with diabetes mellitus is currently managed by a combination of short-term and long-term testing. Epinex Diagnostics has identified glycated albumin (GA) as the ideal marker for an intermediate index to measure glycation. Albumin is the most common protein found in serum, forming approximately an 80% concentration of the circulating blood plasma protein. It is replaced in the body approximately every 20-25 days. As with other proteins in the body, it is subject to nonenzymatic glycation by excess sugar. This leads to structural changes that can affect protein function.

Glycated albumin plays a double role in diabetes complications. In addition to being a marker for glycation, glycated albumin has been directly implicated for a role in several major complications of diabetes, including atherosclerosis, nephropathy, retinopathy and cognitive function.

Several recent studies have confirmed that point measurements of glycated albumin and glycated hemoglobin (HbA1c) are closely correlated, and that values for glycated albumin accurately represent the equivalent values for HbA1c. Levels of glycated albumin change more rapidly over time in response to changes in treatment (as reflected by changes in fasting plasma glucose) than do levels of HbA1c.

Numerous studies conducted over the past 25 years have concluded that glycated albumin is a better indicator of glycemic control than HbA1c. A recent survey of endocrinologists has demonstrated physician support for an intermediate index for glycemic control based on glycated albumin. With such a test available, doctors would recommend a substantial reduction in the level of self-monitoring blood glucose (SMBG) testing for their patients. Studies in the last decade using a fructosamine test showed promise for a positive effect on diabetes control in a workplace population.

Glycated albumin testing has been strongly recommended for control of gestational diabetes, and has been suggested as a means to monitor glycemic control for diabetics undergoing hemodialysis and as a test for coronary artery disease associated with diabetes. Such a test can also serve as a monthly report card for type 1 diabetics.

Current methods of diabetes monitoring have demonstrated inherent shortcomings. SMBG can only provide a snapshot of blood glucose levels and does not monitor glycation. Recent studies have shown no benefit of SMBG testing in improving glycemic control for type 2 diabetics. The HbA1c test cannot effectively measure glycation within a three-month period, during which time diabetes complications can advance unchecked. In addition, biological and clinical variability in HbA1c testing raise potential concerns about its reliability as a monitoring tool.

A regimen of monthly consultations with a pharmacist or other diabetes care counselor has proven to be an effective method for diabetes control. Several municipalities and corporations that have implemented this new paradigm have documented improved health for diabetes patients, lowered healthcare costs and increased productivity by reducing absenteeism. There is a demonstrated need for an intermediate glycation index to monitor diabetes. A test based on glycated albumin could provide a stable monthly index of glycemic control.

The progressive complications of unmanaged diabetes include heart disease, blindness, kidney failure, amputation of extremities due to circulation problems, and nerve disorders, as well as other chronic conditions. These complications are the cause of the immense personal, financial and societal costs of diabetes. Decades of research have established that prolonged exposure to excess glucose is the cause of diabetes complications, and that long-term control of blood glucose levels is required to avoid or lessen the damage caused by excess glucose.

The process of ***protein glycation*** is now understood to be both a marker for the progress of diabetes complications and an underlying cause of many of the most serious complications. Excess glucose binds to proteins throughout the body, changing their shape and properties in ways that have been shown to cause damage to both the structure and function of body organs. It is therefore extremely important that blood glucose be kept at an acceptable level over time through diet, exercise, meal planning and medications (both insulin and other pharmaceutical agents used in diabetes control).

Diabetics must control their levels of blood sugar (blood glucose) in order to manage their diabetes. To achieve this control, diabetics must monitor the way that sugars are being processed in their bodies. Serum proteins – proteins that circulate in the blood such as hemoglobin or albumin – are among the proteins affected by the glycation process. Because glycation of serum proteins can be measured in vitro, diabetes monitoring has depended on the development and refinement of tests that indicate the level and progression of diabetes-related damage by measurement of serum protein glycation in a sample of blood.

Keypoint

Protein glycation is both a marker for diabetes complications and an underlying cause of those complications. The purpose of diabetes monitoring is to help diabetics control glycation. Diabetes is currently monitored by a combination of daily testing (SMBG) and long-term testing (HbA1c). A monthly diabetes monitoring test based on glycated albumin has the potential to provide better information for monitoring glycation.

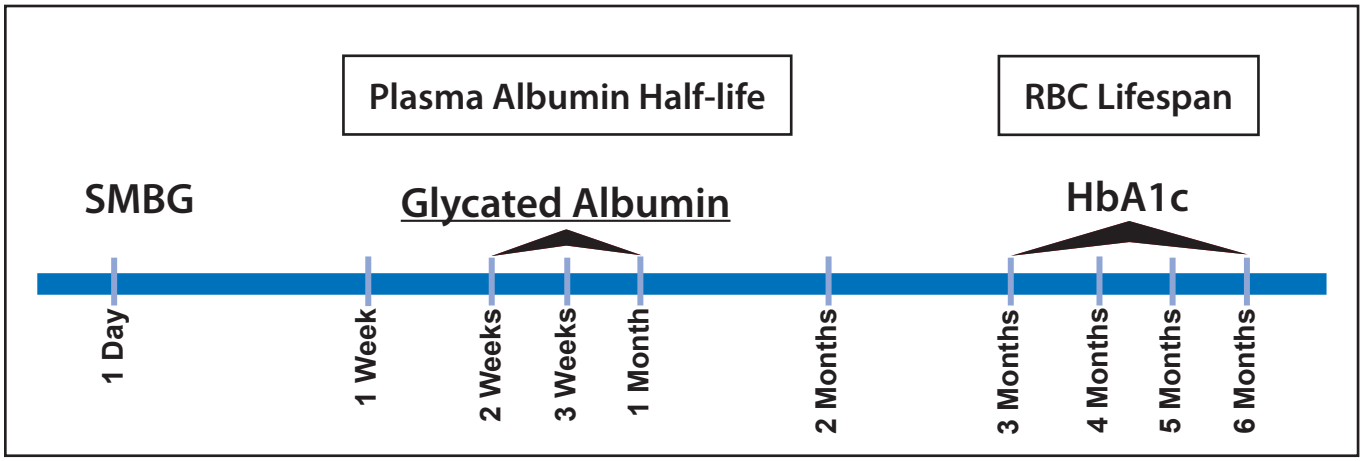
Serum Protein Glycation, Glycated Albumin and Diabetes Monitoring

Monitoring blood sugar levels in individuals with diabetes mellitus is currently managed by a combination of short-term and long-term testing. Diabetics are currently encouraged to self-test their blood sugar levels several times a day in order to get a spot measurement of blood glucose (self-monitored blood glucose or SMBG). SMBG can only provide a snapshot of glucose levels at a point in time, and cannot measure protein glycation. Protein glycation is currently assessed using a test for glycated hemoglobin (hemoglobin A1c or HbA1c), which is typically administered by a physician every 3-6 months with the results processed in a clinical laboratory.

The HbA1c test, based on a variant of hemoglobin, was the first diabetes-monitoring for serum protein glycation to be developed and standardized. This test has become established as a "gold standard" test for glycation, in spite of an inherent shortcoming in the basic chemistry of the test. The human body replaces the red blood cells (RBC) and the hemoglobin they contain every 90-120 days. Therefore, the HbA1c test cannot be performed more frequently than this 90-120 day time frame and yield a meaningful result. While the HbA1c test does provide information on glycation, that information is of limited use in helping diabetics and their healthcare providers in monitoring and modifying treatment, because the interval of the test, which may be as much as four to six months, is too long. Diabetes complications can advance unchecked during this interval. Because the HbA1c test is commonly sent out to a clinical laboratory, and the results are reported to the doctor at a later time, there is little or no opportunity for doctor-patient feedback and immediate treatment modification. In addition, the HbA1c test does not directly measure serum protein glycation, an immediate causal factor for serious diabetes complications such as heart disease, blindness and kidney failure.

Epinex Diagnostics has identified glycated albumin (GA) as the ideal marker to measure glycation. Albumin is the most common protein found in serum, forming approximately an 80% concentration of the circulating blood plasma protein. It is replaced in the body approximately every 20-25 days. As with other proteins in the body, it is subject to glycation by excess sugar. A comparison with total albumin provides a simple, stable index of glycation over a three weeks to one month test period.

A glycated albumin index complements current methods for monitoring diabetes in two ways: the short turnover period for albumin allows it to be used as an intermediate glycation marker for diabetes complications by showing damage to proteins over the previous 2-3 weeks, closing the existing information gap between daily blood glucose testing and glycated hemoglobin testing. In addition, glycated albumin plays a role in tissue damage. Recent research has pinpointed glycated albumin as one of the principal causes of diabetes complications. Byproducts of albumin glycation have been specifically implicated as causal factors in coronary artery disease and kidney failure, two of the most extensive and most serious complications of diabetes.



Time Differential of Diabetes Monitoring
Based on Blood Glucose, Glycated Albumin, and Glycated Hemoglobin

Keypoint

Excess sugar circulating through the body binds to proteins such as albumin in a process called glycation or glycosylation. This leads to structural changes that can affect protein function.

Albumin is the most common protein found in serum, making up about an 80% concentration of the circulating blood protein. It is replaced in the body approximately every 20-25 days. As with other proteins in the body, it is subject to nonenzymatic glycation by excess sugar. The glycation process is a condensation reaction between carbohydrate and free amino acid at the amino terminus of proteins or at the epsilon amino groups of lysine residues of proteins. The reaction is initiated with attachment of the aldehyde function of acyclic glucose to a protein amino group via nucleophilic addition, forming an aldimine, also known as a Schiff base. This intermediate product subsequently undergoes an Amadori rearrangement to form a 1-amino-1-deoxyfructose derivative in a stable ketoamine linkage, which in turn can cyclize to a ring structure (figure 1). This bimolecular condensation of free saccharide with protein constitutes a mechanism by which proteins are subject to post-ribosomal modification without the influence of enzymatic activities (Cohen 1994).

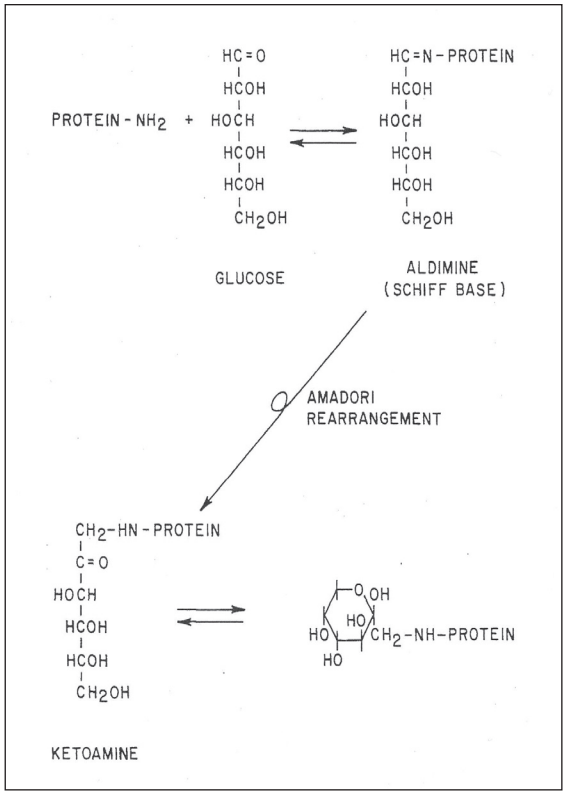


Figure 1: Nonenzymatic glycation of albumin (Dean 1986)

Non-enzymatic glycation of albumin occurs at multiple sites: glucose can attach at Lysine199, Lysine281, Lysine439, and Lysine525, other minor lysine and arginine residues, and also at N-terminal residues of polypeptides. Lysine525 accounts for 33% of all glycation (figure 2). Complex multi-step reactions ensue that cause formation of early and advanced glycation end-products (AGEs). Initial reactions are reversible, but subsequent reactions involving protein unfolding and refolding give rise to irreversible cross-linked rearranged products of glucose with proteins. This results in a stable form of glycated albumin that persists at markedly elevated levels in the plasma of diabetics.

AGEs lead to significant alterations in secondary structure and slight changes in tertiary structure of human serum albumin, resulting in the formation of thermodynamically more stable high molecular weight aggregates that may interfere with normal albumin function (Iberg 1986, Khan 2007).

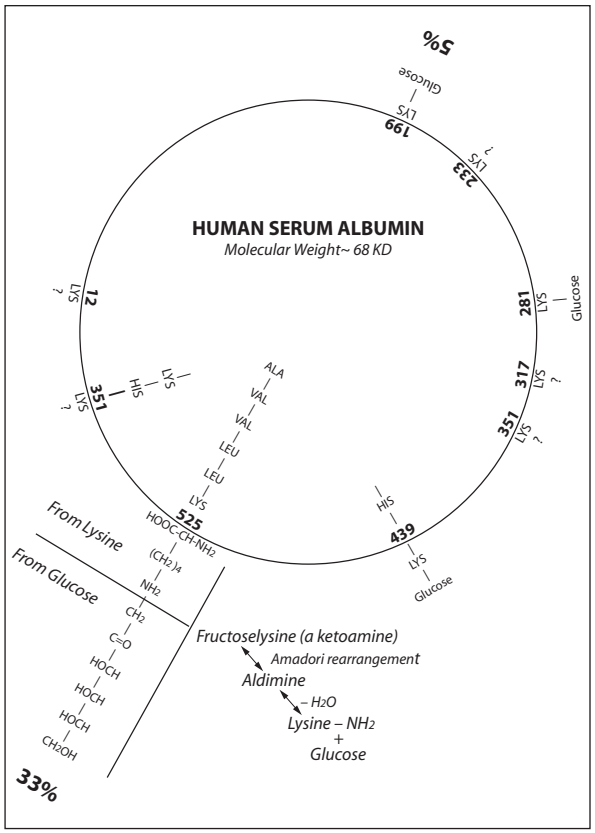


Figure 2: Glycation sites of human serum albumin

Glycation of Albumin Affects Its Conformation and Protein Binding Function

Albumin is a multi-purpose plasma transport protein that binds to ligands such as fatty acids. Glycation of albumin significantly reduces this transport ability. Conformational change associated with glycation cause a decrease in binding affinity, as shown by a study monitoring the fluorescence emission characteristic of the only tryptophan residue on an albumin molecule (Shaklai 1984). After both in-vivo/in-vitro glycation of albumin, the emission of the tryptophan residue is reduced by 30% and maximum wavelength of emission is shifted shorter, indicating a conformational change (figure 1). Compared to the nonglycated form, the affinity of GA is reduced by 50% for bilirubin and 20 fold for the long chain fatty acid *cis*-paranaric acid. This means that the glycation of serum albumin will have a negative impact on the transportation of serum molecules (figure 2).

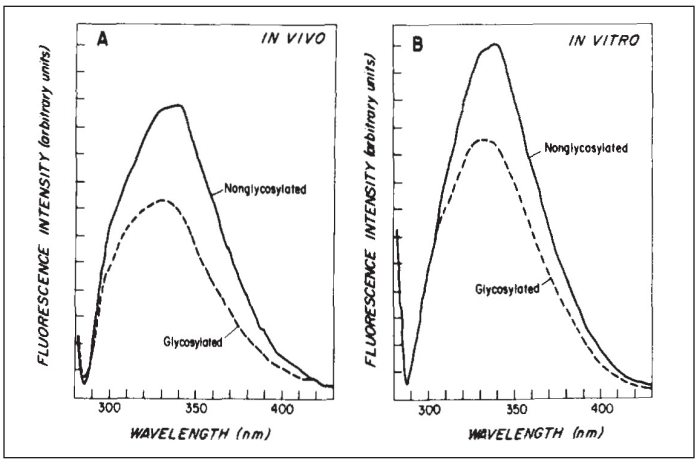


Figure 1: Relative quantum yields and fluorescence of human serum albumin unmodified and glycosylated in vivo (A) and in vitro (B) (Shaklai 1984)

Glycation Reduces Antioxidant Activity of Albumin

There is ample evidence that serum albumin has significant antioxidant activity and may actually represent the major circulating antioxidant in plasma. Glycation of albumin results in impairment of its protein structure and consequently its antioxidant properties (Singh 2007). Amadori products such as glycated albumin generate oxygen free radicals at physiological pH levels and cause lipid peroxidation. It has been shown that oxidative stress has a deleterious impact on adiponectin secretion and lactate production in murine adipocyte cell lines (Unno 2004). In vitro studies also indicate that incubation of adipocytes with glycated albumin in pathological concentrations could be sufficient to induce greater carbonylation of the cell proteins and were associated with decreased adipocyte viability (Chesne 2006). It has been shown that total serum antioxidant defense status in diabetic mothers and their babies was diminished as compared with control subjects (Grissa 2007). These results suggest that human gestational diabetes and macrosomia are associated with down-regulation of antioxidant status.

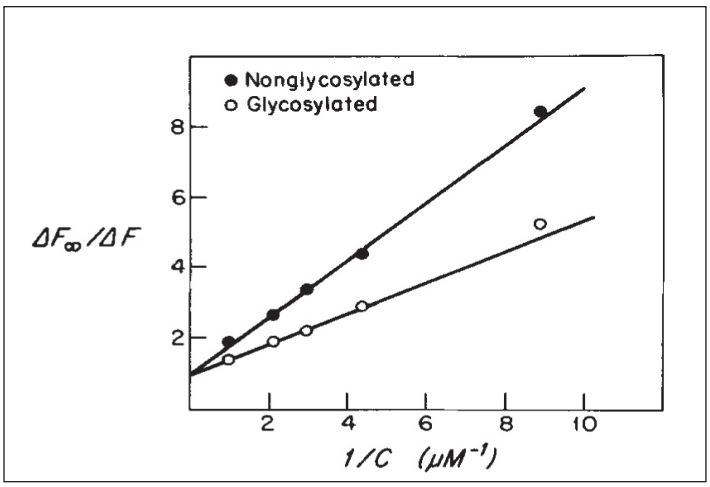


Figure 2: Binding of bilirubin to albumin (Shaklai 1984)

Keypoint

Glycated albumin plays a dual role, as an indicator or marker of intermediate glycation, and as a causative agent for diabetes complications. These complications include: cardiovascular disease, kidney failure, retinopathy, and cognitive degeneration.

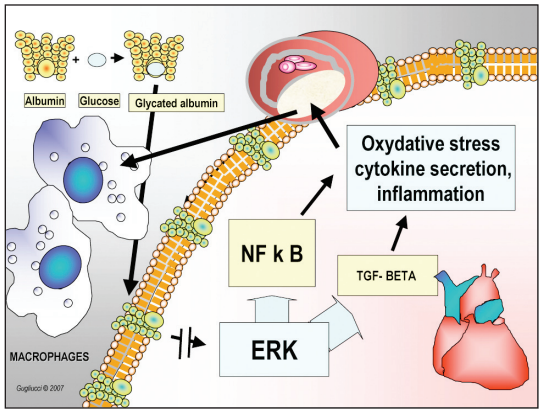
Glycated Albumin Plays A Dual Role In Diabetes Complications

A link between glycated serum albumin and the disease processes of several of the complications associated with diabetes has been the subject of scientific and medical research for more than ten years. As testing for glycated albumin levels has become a more established practice, more and more scientific and clinical research has pointed to the increased levels of glycated albumin associated with diabetes as a direct cause of several significant areas of diabetes complications. It has become clear that glycated albumin plays a dual role: as an indicator or marker of intermediate glycation, and as a causative agent of the damage of diabetes complications. Byproducts of albumin glycation have been specifically implicated as causal factors in atherosclerosis (including coronary artery disease) and kidney failure, two of the most extensive and most serious complications of diabetes in research published by Epinex Advisory Board member Dr. Alejandro Gugliucci (2000). Clinical research in China has further reinforced the linkage between levels of glycated albumin and coronary artery disease, leading these researchers to call for glycated albumin testing as a means of screening for coronary artery disease (CAD). Glycated albumin has also been implicated as a factor in diabetic retinopathy and as a link to the onset of Alzheimer's Disease.

Glycated Albumin and Atherosclerosis

Macrophages in the artery walls can recognize glycated albumin via specific receptors and, in turn, trigger activation of ER kinase, a potent cell-signaling pathway that activates NF kappa B, a key player in inflammatory reactions. This also produces potent cytokines like TGF beta, the corollary being a perpetuation of the inflammatory pathways in the artery wall that characterizes the evolution of the atheroma plaque (Hattori 2002). Recent clinical studies showed a significant correlation between increased serum glycated albumin level and the presence and severity of coronary artery disease in patients with type 2 diabetes mellitus and suggest the use of glycated albumin as a screening test for CAD (Pu 2007).

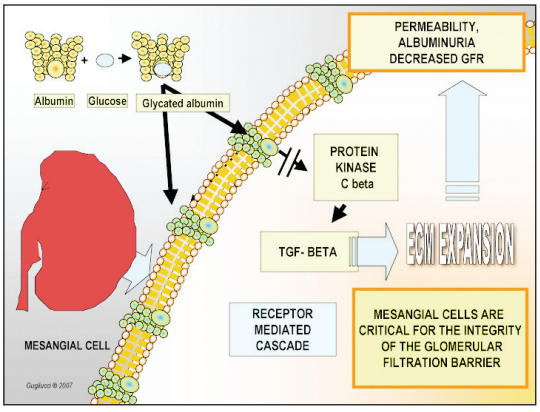
Extravascular protein accumulation, such as the observed thickening of capillary basement membranes observed in diabetic microvascular disease, may be attributed to protein glycation. In vitro studies indicate that glycated albumin in physiologically relevant concentrations possess several pro-atherogenic effects which includes promoting oxidative stress, production of inflammatory mediators, endothelial damage, and vessel wall hypertrophy (Cohen 2006).



Early glycation products such as glycated albumin undergo a variety of degradations, dehydrations, and rearrangements to form non-enzymatic browning pigments that have highly reactive carbonyl groups which are capable of reacting with other proteins to form intermolecular crosslinks which may eventually lead to vessel wall hypertrophy in the capillaries (Brownlee 1983).

Glycated Albumin and Diabetic Nephropathy

The albuminuria and mesangial expansion that are known hallmarks of diabetic nephropathy can be generated by the interaction of glycated albumin with receptors in the mesangial cells, independently of the direct actions of hyperglycemia. Through an amplification cell signaling cascade, involving protein kinase C and secretion of potent cytokines like TGF beta, a series of deleterious effects occur that produce glomerular dysfunction and albuminuria (Cohen 1999, Thomas 2005, Ziyadeh 1998). Researchers were able to retard the progression of diabetic nephropathy in mice by injecting them with monoclonal antibodies that specifically bound glycated albumin, preventing the glycated albumin from causing further harm in the kidney (Cohen 1995).



The functional changes seen in diabetic nephropathy may be the result of an increase in permeability of the glomerular basement membrane to glycated proteins. In the normal kidney, the transglomerular passage of serum proteins and macromolecules is highly selective due to impermeability of glomerular basement membrane and distribution of molecular charge on the capillary

wall, which results in the exclusion of some anionic proteins and proteins larger than 80 kDa in urine (Bohrer 1979). In diabetic nephropathy, there is an increase in the thickness of the glomerular basement membrane and the appearance of proteins larger than 100kDa in urine. It has been proposed that glycosylation of serum albumin results in increased sequestration by endothelial vesicles, which may be a mechanism for transendothelial transport across continuous endothelium in vivo (Williams 1985).

Glycated albumin has also been implicated in reduced nephrin expression, which may be an early event in the progression of diabetic nephropathy. In vitro studies of human kidney visceral epithelial cells show that glycated albumin inhibited nephrin synthesis through the engagement of the receptor for advanced glycation end-products (RAGE) and that nephrin loss and redistribution in glomeruli is present in patients with type 1 and type 2 diabetes (Doublier 2003). The reduction of glycated albumin concentrations and/or blocking of its biologically active epitopes has demonstrated a beneficial influence in the pathogenesis of diabetic nephropathy (Cohen 1994).

Glycated Albumin and Diabetic Retinopathy

A recent article on proliferative diabetic retinopathy (PDR) published in the Japanese Journal of Ophthalmology (Okumura 2007) discusses the involvement of glycated albumin in stimulating angiogenesis in the retina. Involvement of AP-1 (Activator Protein-1) has been implicated in both in vitro and in vivo studies of angiogenesis. The study shows that glycated albumin stimulates the phosphorylation of c-Jun, a component of the transcriptional factor AP-1 in retinal glial cells. AP-1 upregulates the mRNA level of cytokine vascular endothelial growth factor (VEGF), stimulating increased levels of VEGF and proliferation of unregulated capillary growth. When the newly formed capillaries invade the retina, leakage of blood plasma damages the retinal area, inducing macular degeneration. The result is a loss of vision in the central retinal area.

Glycated Albumin and Cognitive Function

Alzheimer's Disease

Accumulation of advanced glycation end products occurs in the brain with ageing and has been proposed to be involved in the pathogenesis of Alzheimer's disease. A study performed in 2001 (Shuvae) shows that patients with Alzheimer's Disease (AD) have higher levels of glycated protein in the cerebrospinal fluid (CSF) compared to healthy individuals (figure 1), and that all proteins in the CSF are highly glycated in AD patients, including albumin, apolipoprotein E and transthyretin (figure 2). The study also shows that glycated beta amyloids rapidly form plaque. In the case of albumin, AD patients showed a 1.5 times greater level of glycation compared to healthy individuals. It was proposed that the increased early glycation of CSF proteins in the Alzheimer's patients may stimulate the formation and the consequent deposition of advanced glycation end products as well as oxidative stress in the brain.

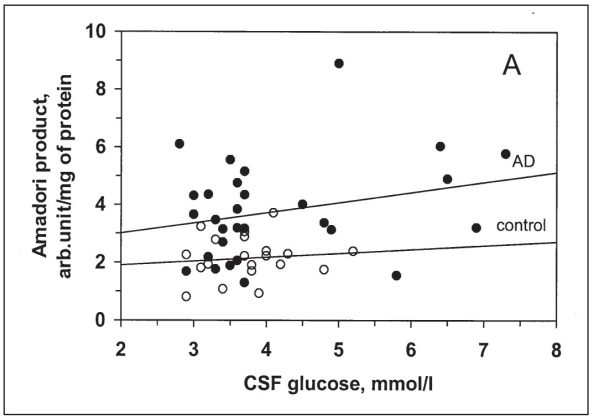


Figure 1: Increased levels of Amadori products in the cerebrospinal fluid of patients with Alzheimer's Disease (Shuvae 2001)

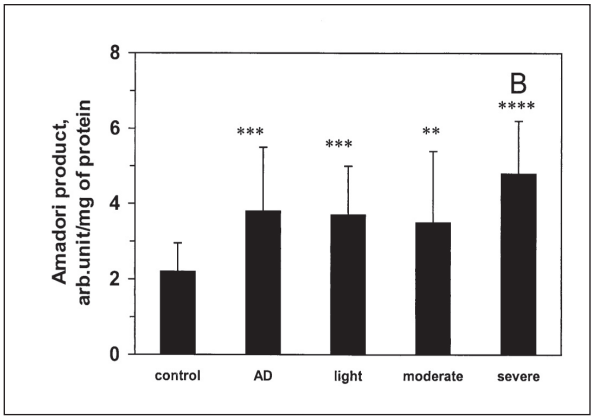


Figure 2: Glycated protein levels in the cerebrospinal fluid of Alzheimer's patients (Shuvae 2001)

Correlation Between Glycated Albumin and Hemoglobin A1c

Keypoint

There is a strong linear relationship between measurements of glycated albumin and hemoglobin A1c. Studies indicate that glycated albumin levels reflect a quicker response to short-term changes in diabetes treatment and glycemic control than levels of hemoglobin A1c.

Several recent studies have confirmed that point measurements of glycated albumin (GA) and glycated hemoglobin (HbA1c) are closely correlated, and that values for glycated albumin accurately represent the equivalent values for HbA1c in diabetic patients not subject to physiological conditions that disturb hemoglobin metabolism. In these cases, glycated albumin has been found to be a better indicator of glycation than HbA1c. As expected, levels of glycated albumin change more rapidly over time in response to changes in treatment (as reflected by changes in fasting plasma glucose) than do levels of HbA1c.

The kinetics of HbA1c, fructosamine (FA), and glycated albumin in response to blood glucose dynamics have been studied by Tahara and Shima (1995) and have been found to reflect the weighted mean of the preceding plasma level for 100, 40, and 30 days, respectively. When compared to hemoglobin A1c or fructosamine concentration, changes in GA have been found to have a closer correlation to changes in mean blood glucose in the first few weeks after intensification of insulin therapy in type 1 diabetics.

A recent clinical study performed a comparison of glycated albumin and glycated hemoglobin in type 2 diabetic patients over 16 weeks (Takahashi 2007). The study demonstrated that GA and HbA1c were significantly correlated in patients with type 2 diabetes who had an HbA1c level below 7.5% with less than 0.5% variation for at least a year. The GA/HbA1c ratio displayed a normal distribution and a mean value of 2.9. The mean value did not differ among the 4 groups studied irrespective of their treatment for diabetes. When combined analysis of the four study groups was performed, GA and HbA1c showed a weak, but significant correlation.

The study also found a rapid decrease of GA (figure 1) reflecting the faster turnover of plasma albumin than that of red-blood cells as displayed by the steeper slope. Figure 1 demonstrates the correlation between GA and HbA1c over the 16-week period.

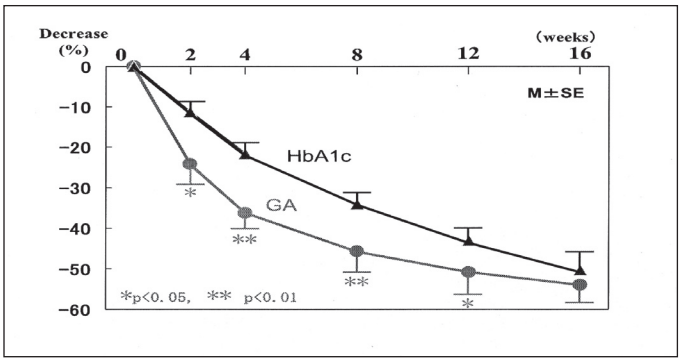


Figure 1: Time course of percent decrease of GA & HbA1c during intensive insulin treatment (Takahashi 2007)

A study that examined physiological and pathological conditions affecting glycated albumin compared GA and HbA1c levels in 209 diabetic patients whose glycemic control had been stable for at least the past three months (Koga 2006). The results showed a strong correlation of HbA1c levels with GA levels in the study populations (figure 2).

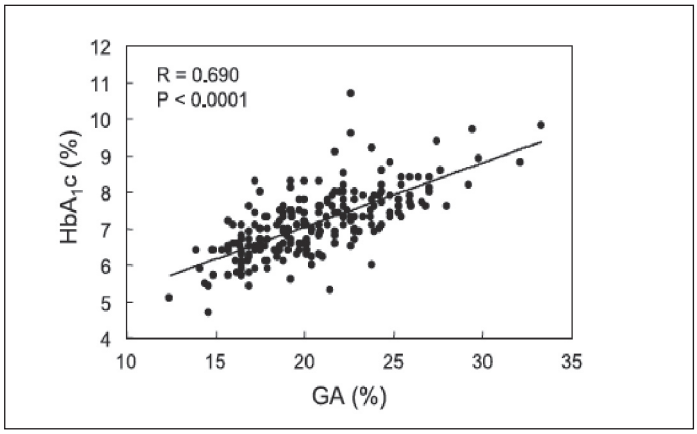


Figure 2: Correlation of HbA1c levels with GA levels in 209 diabetic patients (Koga 2006)

Another study evaluated the clinical utility of employing GA as an indicator of glycemia. Values of GA and HbA1c were compared to daily values of SMBG in 60 type 1 patients and 50 type 2 patients. Results show a very strong correlation of GA with HbA1c in type 1 and type 2 subjects. GA was also significantly correlated with maximum blood glucose in type 1 and type 2. The study concludes that glycated albumin is a reliable indicator of average glucose levels in patients with type 1 and type 2 diabetes. This study also found a strong correlation of GA to pentosidine, an advanced glycation end product known to be a major cause of diabetic vascular complications in type 1 and type 2 diabetics, while HbA1c showed a correlation with type 1 diabetics only (Yoshida 2005).

A study measuring the clinical utility of an enzymatic method for the measurement of glycated albumin in plasma followed a sub-group of type 2 diabetic patients for 18 weeks as they progressed from severe hyperglycemia (HbA1c >or=10.0%) toward better glycemic control (Paroni 2007). The study showed strong correlations between glycated albumin and fasting glucose and between glycated albumin and HbA1c for type 2 diabetics with good control (figure 3) and type 2 diabetics with poor control (figure 4). GA was better correlated with fasting plasma glucose than HbA1c for both groups of type 2 diabetics (with good and poor control), and GA decreased more rapidly than HbA1c during intensive insulin therapy.

The study concludes 1) that plasma glycated albumin is better correlated to fasting plasma glucose than HbA1c, and 2) that glycated albumin is a more sensitive indicator of short-term variations of glycemic control than HbA1c during treatment of diabetic patients.

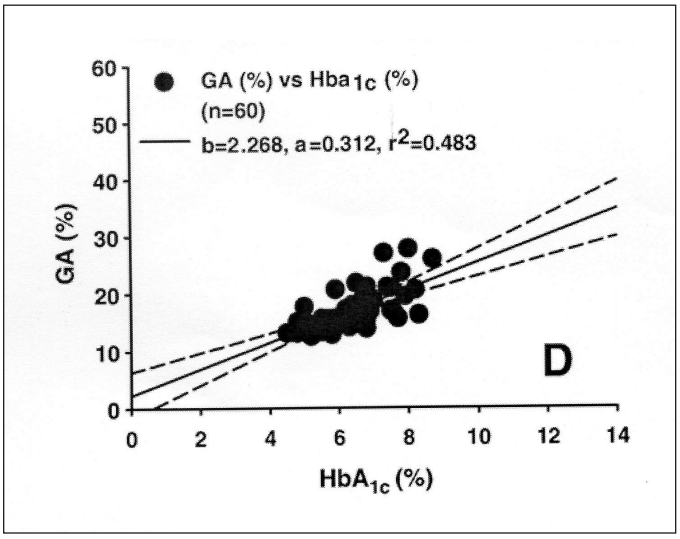


Figure 3: Correlation of GA with HbA1c for type 2 diabetics with good control (Paroni 2007)

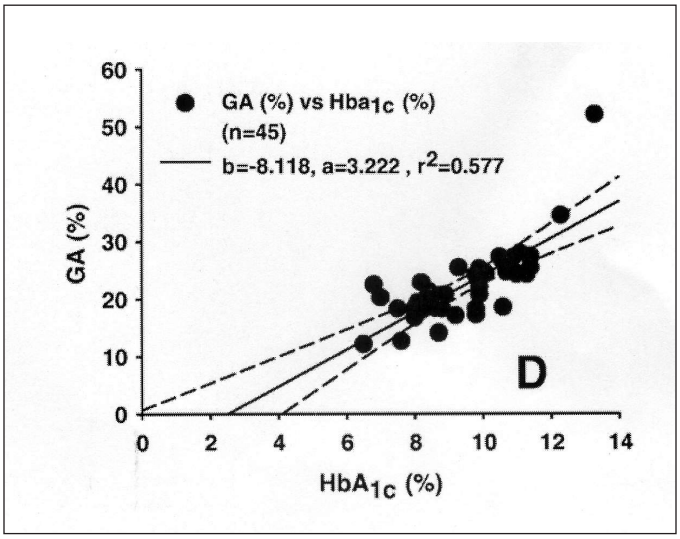


Figure 4: Correlation of GA with HbA1c for type 2 diabetics with poor control (Paroni 2007)

Keypoint

Immediate and potential clinical application for glycated albumin testing include general diabetes monitoring, gestational diabetes, screening for coronary artery disease in diabetics, and monitoring for diabetics on hemodialysis. Physicians have indicated strong support for an intermediate glycation index, and would reduce their recommendations for blood glucose testing in conjunction with such a test.

Existing Studies Have Demonstrated Clinical Utility for GA Monitoring

Articles demonstrating the utility of glycated albumin as an alternative index to glycated hemoglobin for diabetes control have been published for 25 years. As early as 1983, Jones et al took note of the more rapid turnover of albumin as compared to hemoglobin, and tested GA, HbA1c, and fasting blood glucose in newly diagnosed diabetes patients undergoing treatment. They found that GA decreased in step with fasting glucose over the initial 4 weeks of treatment, while HbA1c did not. In 1989, Wincour determined that changes in glycated albumin had a closer correlation to changes in mean blood glucose in the first few weeks after intensification of insulin therapy in type 1 diabetics when compared to hemoglobin A1c or fructosamine concentration. Similarly, Wörner (1993) compared glycated albumin, glycated hemoglobin and fructosamine and concluded that glycated albumin gave the most precise data in medium term diabetic control.

More recently, published and unpublished studies in Japan, using a laboratory-based methodology for measuring glycated albumin, have confirmed the clinical utility of glycated albumin as a methodology for diabetes monitoring. A study of 18 type 2 diabetic patients for 16 weeks as they progressed from untreated severe hyperglycemia (HbA1c>/=9.0%) to good glycemic control (HbA1c</=6.5%) by intensive insulin treatment found that GA decreased more rapidly than HbA1c during intensive insulin therapy, but the percent reduction of HbA1c eventually corresponded with that of GA by 16 weeks after the start of treatment. This result demonstrates that GA provides a more responsive indication of therapeutic treatment than the HbA1c test (Takahashi 2007). A study performed at the Juntendo University School of Medicine, Japan, also demonstrated the effectiveness of using GA as an indicator to monitor diabetes therapy (Kawamori 1996). An unpublished study prepared by the Asahi Kasei Pharma Corp. showed data indicating that short-term therapeutic effectiveness was observed through GA monitoring, with no observable change in HbA1c levels during the observation period.

GA Better Than HbA1c as a Short-term Marker of Glycemic Control

Another Japanese study tested whether GA was a more useful tool to monitor rapidly changing blood glucose than HbA1c. The study was performed on patients hospitalized for diabetes control (51 men & 47 women). Patients were administered oral anti-diabetic drugs and 4-point SMBG tests daily. 7-point SMBG tests were done the third and tenth hospital day. GA & HbA1c were performed the second and thirteenth hospital day. Results from the second day demonstrated a good correlation of blood glucose with HbA1c & GA (p=0.0001). However, on the thirteenth day only GA correlated well with blood glucose (p=0.0001) as opposed to HbA1c (p=0.019). The study concluded that GA measurement is more accurate for determining rapidly changing blood glucose than HbA1c due to the shorter half-life (10-14 days) of GA as compared to the half-life of HbA1c (2-3 months) (Tamemoto 2007).

Physician Support in the U.S. for an Intermediate Glycation Index – Endocrinologist Survey Report

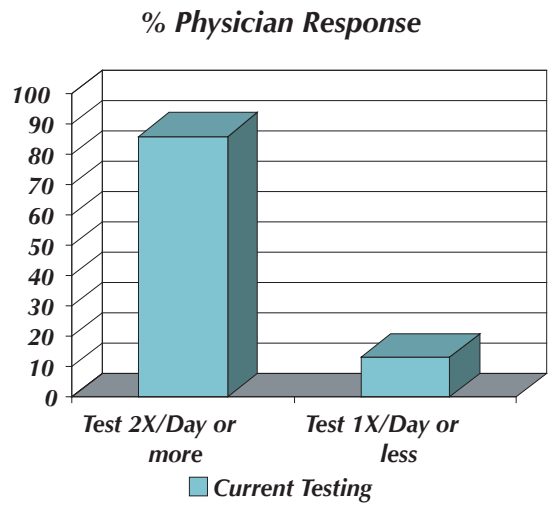
In September 2005, Epinex Diagnostics surveyed clinical and research endocrinologists and diabetes specialists about their current diagnostic practices for type 2 diabetes patients and their opinion of the utility of a monthly test for glycation based on glycated albumin. The Company received a highly positive response to the product concept and technology from survey respondents. A six-page questionnaire was sent to more than 3500 endocrinologists and diabetes specialists, who collectively treat approximately 2.5 million diabetic patients, which constitutes approximately 20% of patients in the U.S. diagnosed with type 2 diabetes. Doctors were surveyed regarding their practice and patient load, their views on different methods of diabetes testing currently available and, following an explanation of the basic science of a rapid test for intermediate glycation based on glycated albumin, were asked to evaluate the potential usefulness of such a test (6% rate of return, margin of error: ±6.5% at a 95% confidence level).

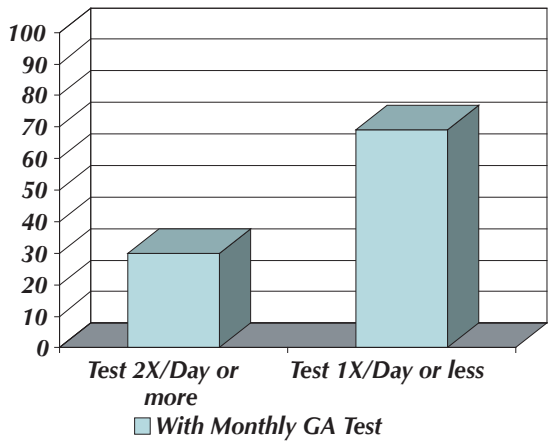
Diabetes Monitoring

Reduction in Daily Blood Glucose Testing

Diabetes specialists surveyed by Epinex indicated that they would recommend a reduction in daily blood glucose testing for stable type 2 patients in conjunction with a monthly glycated albumin test from a weighted average of 2.12 tests per day to 1 test or zero per day. This would translate to a yearly reduction of more than 1 billion daily tests, and their associated expense and inconvenience.

The charts show that at present, 86% of physicians recommend blood glucose testing 2X or more per day for type 2 diabetic patients, while only 13% recommend 1X or less per day. Physicians surveyed responded that they would reduce daily testing in conjunction with a monthly glycated albumin test. 30% would then recommend testing 2X or more per day, and 69% would recommend 1X or less per day. This reduction in the number of daily tests will reduce patient inconvenience, save patients and healthcare providers money, and improve patient acceptance for an intermediate glycemic index test measuring glycated albumin.





Potential Value of a Glycated Albumin Test

Diabetes professionals strongly support the need for a test for intermediate glycemic control, which could be used as a tool for diabetes screening as well as diabetes monitoring.

- 64% of doctors believe there is a need for a test for intermediate glycemic control. Conversely (and more importantly) only 14% of the doctors surveyed do not think there is a need for intermediate testing.
- 69% agreed on the need for a screening test for potential undiagnosed diabetes.
- 61% of endocrinologists surveyed agreed that there is presently no rapid test for diagnosing incipient or existing diabetes.

The Company's survey of endocrinologists showed strong support for a glycated albumin test as an important tool to improve their ability to monitor intermediate glycemic status. Of the endocrinologists responding to the survey:

- 57% believe that a glycated albumin test could be an indicator of short-intermediate glycemic status and control (95% positive or 'not sure'). Only 5% of the doctors believed that a GA test would not be a good test.
- 67% of doctors responding would recommend that their diabetic patients use a glycated albumin test for intermediate glycation.
- 90% of the responding endocrinologists expressed interest in learning more about the test.
- Over 60% wished to participate in evaluating a glycated albumin test or being part of a focus group.

Epinex believes that the survey results show that 1) there is a significant need for a reliable intermediate index for glycation; and 2) a rapid test for glycated albumin could have exceptional acceptance by doctors.

Benefits of Glycated Albumin in Diabetes Monitoring

Keypoint

Glycated albumin has been shown to be a better marker than HbA1c for glycemic control in diabetic hemodialysis patients. Glycated albumin testing has been suggested as a screening tool for coronary artery disease in diabetes patients, and has been recommended for immediate adoption as a test to monitor gestational diabetes. Clinical studies using glycated albumin, as well as earlier studies show patient benefit from an intermediate glycemic index.

Glycated Albumin Testing for Diabetic Hemodialysis Patients

The significance of a comparison of GA with casual plasma glucose (PG) and HbA1c (HPLC) was evaluated as an index of glycemic control in hemodialysis patients with type 2 diabetes. As expected, mean GA, PG, and HbA1c values in 538 diabetic hemodialysis patients were increased above those in 828 hemodialysis patients without diabetes. However, when the three values in all hemodialysis patients (1366) were compared to a third group of 365 type 2 diabetics without renal dysfunction, HbA1c levels were significantly lower than simultaneous PG and GA levels with hemodialysis. A significant negative correlation was found between GA and serum albumin in diabetics on hemodialysis; in the same group HbA1c correlated positively with hemoglobin concentration and negatively with weekly doses of erythropoietin, which had no effect on PG and GA. Multiple regression analysis led to the conclusion that GA testing estimates glycemic control in diabetic hemodialysis patients better than the HbA1c test, which leads to underestimation when erythropoietin is used (90% of dialysis patients) (Inaba 2007).

Glycated Albumin and Coronary Artery Disease

In a clinical study published in 2007, examination of patients with type 2 diabetes revealed significant coronary stenosis in 237 out of 320 subjects. Serum glycated albumin and TNF-alpha levels were significantly higher in patients with coronary artery disease than in controls, although serum HbA1c level did not significantly differ between the groups. Serum glycated albumin levels correlated with the number of diseased arteries. The study concluded that there is "a strong and specific connection or association between elevated glycated albumin levels and coronary disease, with no correlation to HbA1c levels." The article suggested that testing for glycated albumin could provide a useful marker for predicting the onset of coronary artery disease in people with type 2 diabetes (Pu 2007).

Gestational Diabetes

There are approximately 4 million pregnancies to term every year in the U.S.A. Up to 10% will develop gestational diabetes and the numbers are increasing. After pregnancy, 5-10% of women with gestational diabetes are diagnosed with type 2 diabetes, and 20-50% develop type 2 diabetes within 5-10 years (CDC). Gestational diabetes occurs when a woman who does not have diabetes develops a resistance to insulin because of the hormones of pregnancy. Women with gestational diabetes may be non-insulin dependent or insulin dependent. Gestational diabetes can cause complications to the baby, including macrosomia, birth injury, hypoglycemia, and respiratory distress (difficulty breathing). Therefore, it is extremely important for doctors to monitor pregnant women for any signs of glucose intolerance. Medical authorities have declared that ALL pregnant women should be tested.

A study conducted in Glasgow compared the respective value of serial measurements of GA, GPP (Glycated Plasma Proteins), and HbA1c (Glycated Hemoglobin), determined by affinity chromatography, in early pregnancy in 14 insulin-dependent diabetic women (Leiper 1985). As the patients showed rapid improvement in glycemic control with intensive diabetic education and monitoring, the observed rate of decline in the concentration of GA or glycated plasma proteins was approximately twice that of the decline in concentration in HbA1c concentration. The results demonstrate that measurement of GA or GPP gave an earlier indication to the clinician of improved diabetic control. The study also proposed that GA and GPP were less likely than HbA1c assays to be affected by non-diabetic conditions, such as patients who are anemic, received blood transfusions or are treated by hematinics. Hematinics are commonly prescribed in pregnancy and can cause misinterpretation of HbA1c values.

The researchers raised the question of the validity of GA and GPP for patients with conditions characterized by protein loss and depressed protein synthesis (renal and hepatic diseases); however, their initial experience with assays of patients with severe hepatic and renal pathologies suggested that the absolute percentage of circulating plasma proteins which undergo glycation is relatively unaffected by elevation or reduction of total plasma protein concentration. This was confirmed by making serial measurements of GA and GPP in a pregnant diabetic patient who developed nephrotic syndrome and another patient receiving treatment for alcoholic liver disease. No changes in glycosylated protein percentages were observed despite the onset of heavy proteinuria for the kidney patient and the steady rise in plasma protein levels for the liver disease patient.

In its conclusions the Glasgow study stated the importance of a GA test for clinicians monitoring gestational diabetes patients. "The

modern emphasis on adequate control of glycemia in diabetic pregnancy is paralleled by an equal desire to conduct management on an out-patient basis until as late as possible in the pregnancy." Outpatient management can only be justified if the "clinician is satisfied that good diabetic control has indeed been achieved." This can only be achieved by regular tests performed by the patient (SMBG) and less frequent biochemical assays (glycated protein tests). Of these tests, the study concluded that a GA test would be the most suitable since it provides a rapid confirmation of patients' glycemic control.

Paroni (2007) concluded that "GA could be a better marker for glycometabolic control with respect to HbA1c in cases of pre-gestational diabetes" (i.e. in pregnancy of type 1 or type 2 diabetic women) because of larger excursion of glycemic levels in these subjects, with respect to gestational diabetes pregnancies. A symposium held in 1999 on point-of-care testing recommended the immediate adoption of glycated albumin testing for gestational diabetes. This recommendation has not yet been acted upon due to the lack of a convenient and inexpensive test for glycated albumin (Hicks 2001).

GA as a Monthly Report Card for Type 1 Diabetics

The clinical study comparing Glycated Albumin (GA) and Glycated Hemoglobin (HbA1c) in which GA decreased more rapidly than HbA1c for type 2 diabetic patients on intensive insulin treatment suggests that type 1 diabetics would also benefit from a GA test providing earlier glycemic control information enabling earlier therapeutic intervention upon discovery of increases in glycation levels (Takahashi 2007).

Glycated Albumin as a Monthly Indicator for Tight Glycemic Control

Because albumin is replaced by the body every 20-25 days, changes in the levels of albumin glycation can be observed on a monthly basis. The Takahashi study measuring GA and HbA1c over 16 weeks showed that glycated albumin is a sensitive marker for detecting early improvement of glycemic control when starting or modifying the treatment of diabetes, while HbA1c is a marker reflecting the overall glycemic control for several months. That study also observed that GA increases prior to HbA1c in patients with deteriorating glycemic control (Takahashi 2007).

Positive Results From Previous Intermediate Glycation Testing Projects

Clinical Trials Based On Fructosamine Testing

Over the past decade a number of clinical trials were completed based on the use of a fructosamine (FA) test for intermediate glycation. These trials demonstrated the potential for including an intermediary index for glycemia in clinically managing diabetes (Lindsey 2004, Edelman 2000, Carter 2001). Experience with fructosamine testing has pointed out the potential value of an intermediate index to retrospectively evaluate changes in diet and exercise habits, possibly allowing faster evaluation of changes in medication dosages and other control measures, and to serve as an inexpensive rapid screening test for impaired glycemic control (Carter, 2001).

Narayanan (1991) summarized several potential interference factors for early generation FA tests, including lipid interference in a colorimetric assay. Mature versions of the FA test are still faced with the fact that fructosamine represents a collection of proteins that may vary during intercurrent disease states and that a baseline condition is difficult to standardize. As an intermediate index, glycated albumin is a single, highly representative molecule (80-90% of the protein measured by FA is glycated albumin). Glycated albumin can be reliably measured immunochemically and rendered as a ratio to total albumin, thus offsetting most potential interference concerns.

The fructosamine test, which was promoted 10-20 years ago as a short-term measure of average blood glucose level, did not succeed in the market. Major differences exist between an index based on a glycated albumin test and the fructosamine test. The key deficiencies of the fructosamine test are: 1) 'Fructosamine' is not a single product, but a name used for a series of glycation products, 2) results are variable and unstable, 3) there is no established standard reference range, 4) clinicians do not trust the results. Glycated albumin testing does not share these shortcomings. Glycated albumin is a specific marker that can be measured with accuracy and stability. The test is designed as an index or ratio that is compared to total albumin, which sets up a controlled baseline for each patient. Glycated albumin testing is already supported in the scientific literature and accepted by clinicians.

Issues Affecting Current Diabetes Monitoring Methods

Keypoint

SMBG can only provide a snapshot of blood glucose levels and does not monitor glycation. Recent studies have shown no benefit to SMBG testing in improving glycemic control for type 2 diabetics. Studies reveal three areas of potential uncertainty exist in HbA1c testing: biological variability, red blood cell variability, and clinical variability.

Blood Glucose Monitoring

Blood glucose testing measures a point-in-time glucose (sugar) concentration in the blood. Testing for blood glucose does not provide any direct information regarding protein glycation – the underlying cause of diabetes complications. Large numbers of test results must be tracked and analyzed to determine if patient therapy is effective. Because blood glucose levels can vary widely over the course of a day or several days, results can be difficult to interpret and translate into an effective plan for diabetes management.

The Fremantle Study of 1,286 type 2 diabetes patients over 5 years, as well as a study of nearly 3,000 type 2 diabetes patients on OAD (oral medication) or diet alone in Germany and Austria found "no benefit" for daily blood glucose testing "regardless of treatment." (Davis 2007; Schutt 2006)

A recent article published by the scientific journal of the American Diabetes Association declared that "early and intensive treatment can affect patients' psychological outcomes, resulting in higher anxiety and less self-efficacy." (Davis 2006) "For patients who do not receive insulin, self-monitoring [of blood glucose] is associated with poorer metabolic control and greater psychological distress." (Pellegrini 2001)

A definitive article on self-monitoring of blood glucose has been published by BMJ, the journal of the British Medical Association. In this BMJ study, type 2 patients with non-insulin treated diabetes were divided into three groups and followed for 12 months (Farmer 2007). All were given the same education as to how they could maintain or improve their condition: diet, exercise, etc. One group was given education and HbA1c testing every 3 months. The second group was given in addition a blood glucose meter, trained in its use, and told to test themselves 2 days a week and call a doctor if their results were above or below certain values. The third group was further given extensive training in using and interpreting the meter and encouraged to use it for multiple daily tests and to try to coordinate their lifestyle choices with meter results. After 12 months, the study found "no significant improvement in glycemic control," for any group, in spite of setting conditions for the intensive group "into a framework that, based on psychological theory, should have optimized its effect." (Thoolen 2006)

HbA1c Testing

Current literature shows that there are 3 areas of uncertainty in HbA1c testing: biological variability, red blood cell variability, and clinical variability.

Biological Variability – Hemoglobin A1c values have been found to vary significantly within an individual and between individuals. Inter-individual variation is much larger and due primarily to two components: (1) those that are glycemia-related and (2) red blood cell (RBC) variability. About two-thirds of the between-individual variation is due to the latter, which is genetically controlled and possibly mediated by red-cell fragility or enzymatic deglycation (Jeffcoate 2004).

A poster presentation at the 67th ADA meeting in 2007 demonstrated RBC lifespan variations in non-anemic pre-menopausal women due to menstruation factors. Hemoglobin, hematocrit, MCV (mean corpuscular volume), and MCH (mean corpuscular hemoglobin) were negatively associated with HbA1c. By contrast, any RBC and iron metabolism indices were not associated with serum GA levels. These findings imply that HbA1c levels should be interpreted with caution when assessing pre-menopausal diabetic women. Serum GA was suggested as for a better index for chronic glycemic control in pre-menopausal women (Kasayama 2007).

Red Blood Cell Variability – In non-diabetic individuals, it has been shown that there are subjects whose HbA1c levels are high relative to their blood glucose levels and whose HbA1c levels therefore do not accurately reflect their mean blood glucose levels (Gould 1997; Chalew 2005). It has been hypothesized that these discrepancies may be due to variability of erythrocyte permeability to glucose (Rendell 1985; Gould 1997), differences in glycolytic enzyme activity, intracellular pH, 2,3-DPG concentrations (Gould 1997; Hudson 1999; Madsen 1982), deglycation of hemoglobin (Szwergold 2003), and kinetic differences in HbA1c glycation (Gould 1997). See Tables 1 and 2, page 20.

It has been shown that 62% of the population variance in HbA1c levels is genetically determined (Jeffcoate 2004). Tables 3 and 4 (page 21) show that variations in hemoglobin affect the HbA1c levels and result in erroneous measurement of glycemic control (Schnedl 2001).

Time	Low glycators (*n* = 5)			High glycators (*n* = 7)			Plasma insulin	
	Plasma glucose (mmol/l)	Intra-erythrocyte glucose (mmol/l)	Ratio of intra-erythrocyte to plasma	Plasma glucose (mmol/l)	Intra-erythrocyte glucose (mmol/l)	Ratio of intra-erythrocyte to plasma glucose	Low glucators (mU/l)	High Glycators (mU/l)
Fasting	5.65 ± 0.73	4.79 ± 1.20	0.84 ± 0.13	6.04 ± 0.88	5.59 ± 0.54	0.93 ± 0.06	11.0 ± 3.8	12.5 ± 3.3
30min	9.87 ± 2.1l	7.11 ± 1.65	0.72 ± 0.10	10.06 ± 1.86	9.27 ± 1.61	0.93 ± 0.12	64.1 ± 29.6	93.2 ± 43.5
1 h	9.09 ± 1.53	7.34 ± 2.14	0.79 ± 0.13	9.11 ± 2.38	8.11 ± 1.59	0.92 ± 0.11	76.5 ± 55.7	116.1 ± 71.8
2 h	7.59 ± 1.49	5.69 ± 1.81	0.74 ± 0.12	6.49 ± 2.14	6.33 ± 1.86	0.98 ± 0.15	77.7 ± 42.2	65.8 ± 60.9
Area under the GTT curve (min/mmol per l)								
	1018 ± 154	786 ± 202			995 ± 228	917 ± 150		

Values are mean ± S.D.

Table 1: Plasma glucose, intra-erythrocyte glucose and their ratios, and plasma insulin concentrations given by low and high glycators during an oral glucose tolerance test (Gould 1997)

Measurement	Low glycators (n = 5)	High glycators (n = 7)	Correlation with mean GHb
(a) Direct effects			
Intra-erythrocyte 2,3-Diphosphoglycerate (mmol/1)	4.81 -+ 0.24	5.61 + 0.26 a	NS
Plasma inorganic phosphate (mmol/1)	0.97-+0.19	1.06+0.07	NS
Intra-erythrocyte inorganic phosphate (mmol/l)	0.46 + 0.21	0.36 + 0.21	NS
Intra-erythrocyte pH	7.05+0.19	7.17+0.11	0.55 b
(b) Indirect effects			
Plasma vitamin C (µmol/1)	62.6 _+ 36.8	54.0 + 22.4	NS
Intra-erythrocyte vitamin C (µmol/1)	50.1 _+20.1	37.8+29.9	NS
Plasma urea (mmol/l)	4.64 +_ 0.69	4.64 _+ 1.28	NS
Plasma NEFA (mmol/1)	0.49 -+ 0.18	0.61 -+ 0.14	NS
Plasma total amino acids (mmol/1)	1.28+0.16	1.09+0.16	--0.57 b
Intra-erythrocyte total amino acids (mmol /l)	1.12+0.17	0.98_+0.12	NS
Urine amino acids (mmol/24 h per mmol creatinine)	0.29--+0.14	0.23-+ 0.11	NS

Values are mean _%_+ **S.D.**

aP<0.001; bp<0.05 versus low glycators.

Table 2: Comparison of physiological factors other than glucose concentration that may affect glycation in low and high glycators (Gould 1997)

Hb variant [reference]	Mutation	Method of HbA1c determination	HbA1c (reference values)
Hb Raleigh [15,26,27]	$\alpha_2\beta_2$1(NA1)ValpAla	HPLC 2, 5	46% (4–6)
	IAG		Falsely low
Hb Niigata [28]	α2β21(NA1)ValpLeu	HPLC 5	13,6% (NR)
		IAG	Falsely low
Hb South Florida[29]	α2β21(NA1)ValpMet		
	amino terminus extended with a methionyl residue	HPLC 7	14.8% (3.5–6.9)
Hb Graz [12,30]	α2β22(NA2)HispLeu	HPLC 4	48.3% (4.1–6.2)
		HPLC 2	52.5% (3.8–6.1)
		IAG	Falsely low
Hb Long Island [31]	α2β22(NA2)HispPro	HPLC 1	51% (NR)
Hb Okayama [32]	α2β22(NA2)HispGln	HPLC 4	44–47% (NR)
Hb S [14,33]	α2β26(A3)GlupVal	HPLC 2, 4	Falsely low+high
Hb C [14,33]	α2β26(A3)GlupLys	IAG	Falsely low+high
Hb J-Baltimore [15,18,27]	α2β216(A13)GlypAsp	HPLC 2, 5	Falsely low
		IAG	Normal
Hb G-Coushatta [23]	α2β222(B4)GlupAla	HPLC 6)	4.8% (NR
Hb E [15]	α2β226(B8)GlupLys	HPLC 2	Normal
		HPLC 5	Falsely low
		IAG	Normal
Hb Tacoma [27]	α2β230(B12)ArgpSer	IEF	28% (NR)
Hb K-Ibadan [27]	α2β246(CD5)GlypGlu	IEF	Falsely high
Hb North Manchester[34]	α2β251(D2)PropHis	HPLC 6	2.7% (3.8–5.5)
Hb Hamadan [35]	α2β256(D7)GlypArg	HPLC 1)	0.0–1.2% (NR
Hb J-Lome [36]	α2β259(E3)LyspAsn	HPLC 5	Falsely low
Hb Rambam [37]	α2β269(E13)GlypAsp	HPLC 1	Falsely low
		IAG	Falsely low
		BA	Normal
Hb N-Baltimore [38]	α2β295(FG2)LyspGlu	HPLC 8	Normal
Hb Moriguchi [39]	α2β297(FG4)HispTyr	HPLC 6	~4% (NR)
Hb Sherwood Forest [40]	α2β2104(G6)ArgpThr	HPLC 4	51.8% (4.1–6.2)
		HPLC 2	49.2% (3.8–6.1)
		HPLC 3	1.2% (3.6–5.7)
Hb Toranomon [41]	α2β2112(G14)CyspTrp	HPLC 5	4.9% (4–6)
Hb Hafnia [42]	α2β2116(G18)HispGln	IEF	~50% (4–6.5)
Hb Hijiyama [43]	α2β2120(GH3)LyspGlu	HPLC 1	51%
Hb Riyadh [44]	α2β2120(GH3)LyspAsn	HPLC 5	3.0% (NR)
Hb D [45,46]	α2β2121(GH4)GlupGln	HPLC 2–4	Falsely low
		HPLC 9	Falsely high
Hb Camden [15]	α2β2131(H9)GlnpGlu	HPLC 2	Falsely low
		IAG	Normal
Hb Hope [15]	α2β2136(H14)GlypAsp	HPLC 2	Falsely high
		IAG	Normal
Hb Himeji [47]	α2β2140(H18)AlapAsp	HPLC 6	Normal
Hb Old Dominion/	α2β2143(H21)HispTyr	HPLC 1	44.2%
Burton-upon-Trent [48,49]		BA	7.3%
Hb Andrew-Minneapolis [50]	α2β2144(HC1)LysAsp	HPLC1	37.4%

Table 3: Summary of ß-chain hemoglobin (Hb) variants reported to interfere with HbA1c determination methods (Schnedl 2001)

Hb variant [reference]	Mutation	HbA1c determination method	HbA1c value (reference values)
Hb Tatras [16]	β2α27(A5)LyspAsn	HPLC 1	Falsely high
Hb O Padova [17]	β2α230(B11)GlupLys	HPLC 2, 3	Falsely low
Hb Hasharon [18]	β2α247(CE5)AsppHis	HPLC 4	Normal
Hb Ube-2 [19]	β2α268(E17)AsnpAsp	HPLC 5	2.8% (4.3–5.8)
		IAG	Normal
Hb Les Andelys [20]	β2α283(F4)LeupPro	HPLC 2	Normal
Hb Broussais [21]	β2α290(FG2)LyspAsn	HPLC 4	3.3% (4.5–5.8)
Hb Cemenelum [21]	β2α292(FG4)ArgpTrp	HPLC 4	18.1% (4.5–5.8)
Hb Turriff [22]	β2α299(G6)LyspGlu	HPLC 4	21.6% (NR)
Hb Manitoba [23]	β2α2102(G9)SerpArg	HPLC 6	6% (NR)
Hb J-Meerut [24]	β2α2120(H3)AlapGlu	HPLC 5	4.6% (5.2–6.7)
Hb Pavie [25]	β2α2135(H18)ValpGlu	HPLC 1	25%

Table 4: Summary of alpha-chain hemoglobin (Hb) variants reported to interfere with HbA1c determination methods (Schnedl 2001)

Red Blood Cell Variability (continued) – Erythrocyte viability (lifespan) is also a source of HbA1c level variation. The mean lifespan is about 117 days in normal adults and the half-life ranges from 25 to 40 days with a mean of 27 days. It has been proposed that HbA1c is only useful if erythrocyte turnover is not abnormal, on the assumption that if increased erythrocyte turnover results in a lower HbA1c in relation to glycemia, this would be a potential source of error in interpretation of test results (Jeffcoate 2004). A study has shown that this is important for patients undergoing dialysis because erythrocyte lifespan changes due to periodic blood sampling, residual blood in the dialysis circuit, mechanical hemolysis, erythropoietin administration and blood transfusion (figures 1 & 2) (Chujo 2006). This same study was also able to show that the consistent correlation between blood glucose control and glycated albumin levels, was unaffected by erythrocyte life span.

Clinical Variability – Complexity in patient management arises from the broad disease spectrum encompassed by type 1 and 2 diabetics, especially the latter. While a strong correlation has been demonstrated between fasting glucose and HbA1c for type 1 diabetes (DCCT), the correlation is considerably weaker for type 2 (UKPDS). Regardless, neither fasting glucose nor HbA1c are sensitive predictors for cardiovascular disease and death. In type 2 diabetics, rapid fluctuations of glucose, e.g., postprandial, have recently been found to be particularly damaging and not well monitored by HbA1c. In general there is doubt about the value of HbA1c as a predictor of microvascular disease in type 2 diabetes and its role in the general management of that diabetic population (Jeffcoate 2004).

Loyola University Medical Center uses both the DCA 2000 (Bayer Healthcare) and Variant II (Bio-Rad Laboratories) systems for measurement of HbA1c. In October 2005, they began receiving complaints from clinicians that the differences between DCA and VAR test results in patients who were tested by both methods were larger than the 0.2 to 0.4 % HbA1c unit differences (VAR >DCA) that had been typically observed. This discrepancy was confirmed by reviewing results of their intra-laboratory quality assurance program, in which patient samples with HbA1c values in the range of 4.5 to 13 % were tested by both methods. This variability can lead to clinically significant discrepancies in HbA1c results when two methods are used in the same institution, even when both methods are NGSP certified. Such variability can cause confusion among clients, a loss of credibility for the laboratory, and limits the utility of HbA1c as a measure of glycemic control (Holmes 2006).

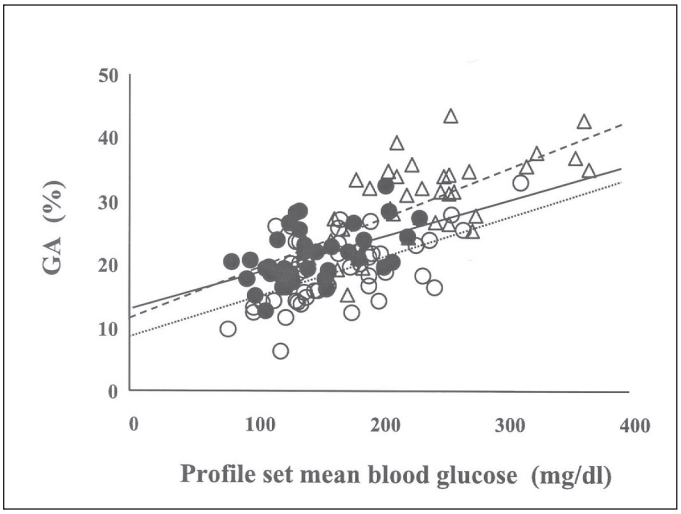


Figure 1: Relationship between blood glucose control and HbA1c in diabetes patients with end-stage renal disease (black circles/solid line), compared to diabetes patients with normal renal function (white triangles/broken line). (Chujo 2006)

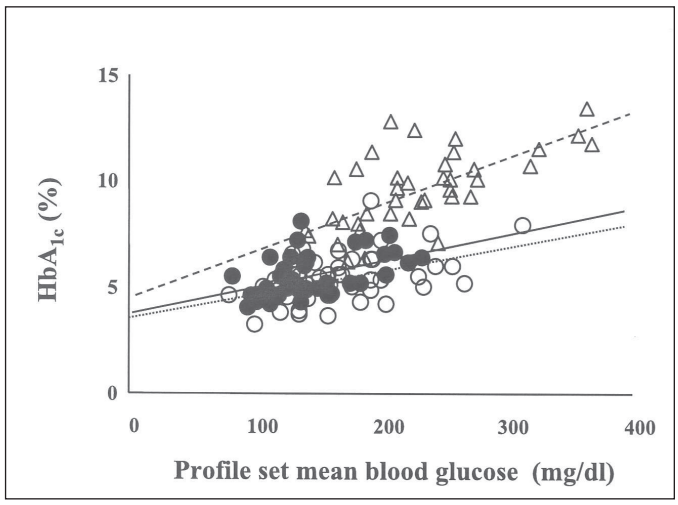


Figure 2: Relationship between blood glucose control and glycated albumin in diabetes patients with end-stage renal disease (black circles/solid line), compared to diabetes patients with normal renal function (white triangles/broken line). (Chujo 2006)

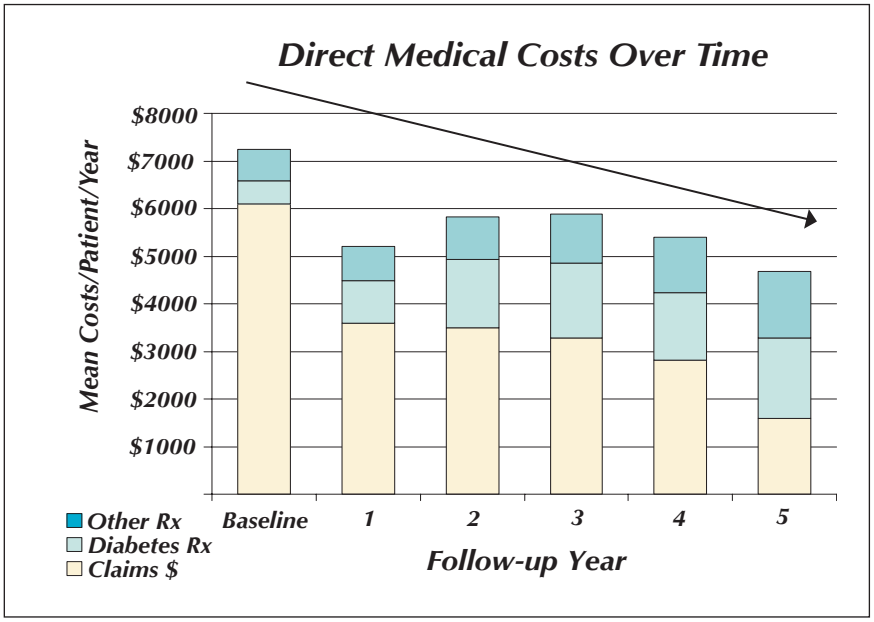
The "Asheville Project" for Diabetes Control

The city of Asheville, North Carolina, teamed with local industries and arranged for city and local company employees who require diabetes monitoring to see a pharmacist for a brief meeting once a month. The purpose of the meetings is to provide feedback for the patients' efforts to control their diabetes through diet, exercise, and, if required, medication. The experiment has generated strongly positive results. Significant improvement in diabetes metrics for enrolled patients has been recorded. Absenteeism related to illness is significantly down in the workplace, and productivity is increased, to the extent that the decrease in healthcare-related expenditure more than pays for the cost of the monitoring (Cranor 2003). This new paradigm for diabetes monitoring based on community care and patient empowerment, is gaining widespread interest and acceptance, and is being reproduced in similar projects throughout the U.S., as illustrated in a recent video feature produced for the Diabetes TV network.

Keypoint

A regimen of monthly consultations with a pharmacist or other diabetes care counselor has proven to be an effective method for diabetes control. Several municipalities and corporations that have implemented this new paradigm have documented improved health for diabetes patients, lowered healthcare costs and increased productivity by reducing absenteeism.

As diabetes consumes an ever-greater share of healthcare resources, the sheer magnitude and urgency of the diabetes epidemic make it evident that the present diabetes care paradigm, in which the patient sees a physician at most twice a year, does not work now and will not work as a means to address the situation in the future. Spiraling, out-of-control healthcare costs also indicate that the present system may not be sustainable. There is general agreement among diabetes care professionals that self-management of diabetes is the single most significant component of the solution to the diabetes epidemic, and that patient empowerment is the key to successful diabetes control. In the past several years, a new paradigm for diabetes monitoring has been pioneered, based on monthly consultation with a pharmacist or other non-physician diabetes caregiver. Community-based diabetes care programs based on monthly consultation with a diabetes educator or pharmacist, supported by municipal and corporate employers, are showing success, as the program pays for itself through lower healthcare costs and higher productivity.



Asheville Project – Health Care Costs

Conclusion

Keypoint

There is a demonstrated need for an intermediate glycation index to monitor diabetes. A test based on glycated albumin can provide a stable monthly index of glycemic control.

Projected Role For A Monthly Glycation Index

The current diabetes monitoring paradigm consisting of SMBG and HbA1c testing shows inherent shortcomings. SMBG testing does not directly measure glycation. HbA1c as a tool for monitoring diabetes has inherent deficiencies, although it will continue to be a gold standard. However, there is a demonstrated need for an intermediate glycation index. Measurement of glycated albumin monitors diabetes complications by showing damage to proteins over the previous 2-3 weeks. A comparison with total albumin provides a simple, stable index of glycation over the test period, and closes the information gap that now exists between daily blood glucose testing and glycated hemoglobin testing.

Recent clinical studies have shown a significant correlation between GA and HbA1c. Studies have demonstrated the utility of a monthly index for glycation based on glycated albumin (Takahashi 2007; Kawamori 1996; Pu 2007; Inaba 2007). These studies have shown that diabetes monitoring based on glycated albumin can reflect changes in treatment more quickly than other methods, and that glycated albumin may be useful as a marker for cardiac disease and kidney problems in diabetics.

An intermediate index for protein glycation based on glycated albumin could be used as a monthly indicator for tight glycemic control and has many immediate applications including testing for gestational diabetes. Such a test would also be a valuable addition to the evolving community-based paradigm for diabetes care based on monthly consultation.

In a period when rapidly escalating costs and demands for universal coverage threaten to overwhelm the healthcare system, the glycated albumin test stands out as a new approach. It has the potential to lower costs, increase patient compliance, and serve a preventative function in response to one of the greatest challenges facing the world today – the diabetes epidemic.

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Notes

CORPORATE OFFICES
Epinex Diagnostics, Inc.
2815 McGaw Avenue
Irvine, CA 92614
Phone: (949) 660-7770
Fax: (949) 660-7772
email: info@epinex.com
www.epinex.com

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