Tests of Glycemia in Diabetes

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Monitoring of glycemic status, as performed by patients and health care providers, is considered a cornerstone of diabetes care. Results of monitoring are used to assess the efficacy of therapy and to make adjustments in diet, exercise, and medications in order to achieve the best possible blood glucose control.

The purpose of this review is to summarize current knowledge about the tests used most widely in monitoring the glycemic status of people with diabetes. The review addresses both patient- and physician/laboratory-based testing, and it includes tests of urine glucose and ketones and tests of blood glucose and glycated proteins (hemoglobin and serum proteins). The major emphasis is on the advantages and limitations of each test for routine clinical practice. Use of these tests for diabetes screening and diagnosis will not be addressed in this review. Since this review was first published in 1995, there have been many advances in the field, most notably standardization of glycated hemoglobin testing and new approaches to self-monitoring of blood glucose (SMBG), including minimally invasive continuous glucose monitoring over hours to days at a time. These and other advances are presented in detail in a recent report that was prepared by the National Academy of Clinical Biochemistry (NACB) and published as an American Diabetes Association (ADA) position statement (1). This review will attempt to complement, rather than duplicate, the material in the NACB report.

Conceptual framework

If there was an ideal method of monitoring glycemic status, it might be a small noninvasive device, perhaps similar to a wristwatch that people with diabetes could wear to continuously monitor their blood glucose level. The device would warn of impending hypoglycemia. It also would store blood glucose data and perform a variety of calculations such as hourly, daily, weekly, or monthly blood glucose averages. Unfortunately, such a monitoring device is not available. Recent advances in the field, however, have provided a number of new testing tools and have increased our understanding of how to use both new and traditional tests together to improve glycemia.

Terminology

Many terms have been used to describe the degree to which the diabetic state has altered the normal metabolic milieu. Examples include "diabetic control," "metabolic control," "glucose control," and "glycemic control." The interchangeability and lack of precise definitions illustrate both the limitations of current testing methods and uncertainties regarding recommendations for specific glycemic goals. For convenience, in this report the various terms used to describe metabolic or glycemic status are considered to be equivalent. Diabetes is characterized not only by hyperglycemia, but also by other metabolic derangements involving carbohydrates, lipids, and proteins (2). The relative contribution of the individual metabolic abnormalities to the pathogenesis of chronic diabetic complications is unknown.

Because hyperglycemia is the defining hallmark of the diabetic state and because glucose is relatively easy to quantify, most monitoring methods have focused on glucose determinations (ketone testing, which relates to lipid metabolism, is one major exception). Further research is needed to determine whether metabolic perturbations other than hyperglycemia predict risk for chronic complications. It also will be important to develop more precise definitions of altered metabolic status in diabetes and, in particular, to avoid using ambiguous terminology such as "tight control," "good control," "poor control," and so forth.

Historical perspective

Before 1975, routine patient monitoring consisted of urine sugar/glucose and ketone determinations (3–5). Typically, physicians monitored occasional laboratory blood glucose determinations and reviewed patient home urine testing records. Most often, the primary purpose of monitoring was to provide information to the patient's health care provider to guide changes in therapy to relieve symptoms due to hyperglycemia—polyuria, polydipsia, and nocturia—rather than to achieve specific glycemic goals.

Since 1975, dramatic changes have taken place in both the methods and goals of monitoring. The changes were driven by both technical advances in testing and steadily increasing evidence that the chronic complications of diabetes were

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Abbreviations: AACC, American Association for Clinical Chemistry; ADA, American Diabetes Association; AGE, advanced glycation end product; CAP, College of American Pathologists; DCCT, Diabetes Control and Complications Trial; FDA, Food and Drug Administration; GSA, glycated serum albumin; GSP, glycated serum protein; HPLC, high-performance liquid chromatography; IFCC, International Federation of Clinical Chemistry; NACB, National Academy of Clinical Biochemistry; NGSP, National Glycohemoglobin Standardization Program; SMBG, self-monitoring of blood glucose; UKPDS, U.K. Prospective Diabetes Study.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

the result of chronic hyperglycemia. By the mid-1980s, patient monitoring of capillary blood glucose had replaced urine glucose testing as the recommended method of day-to-day testing (6). At the same time, determinations of glycated proteins were found to be clinically useful measures of average glycemia over weeks and months and gradually have become part of routine monitoring by health care providers.

At present, it is recommended that all patients with diabetes, especially those who use insulin, should monitor their blood, not urine, glucose levels (6–9). Urine glucose testing should be considered only if patients are unable or unwilling to perform SMBG.

URINE TESTING

Urine glucose testing

Urine glucose testing by patients in the home setting typically consists of semi-quantitative measurements based on single voidings or, less often, by more quantitative "blocks" collected over 4–24 h. The rationale is that urinary glucose values reflect mean blood glucose during the period of urine collection and that single-voided specimens reflect blood glucose at the time of voiding, with second-voided specimens being most representative (3–5). Reasons why the use of urine glucose testing to estimate blood glucose concentrations in diabetes management is undesirable include the following (1,9):

1. Although the renal threshold for glucose in healthy adults corresponds to a plasma glucose concentration of ~180 mg/dl (10 mmol/l), there is wide individual variation. Of particular importance are findings that adults, especially those with long-standing diabetes, may have substantial increases in this threshold, resulting in underestimation of the blood glucose level. Conversely, children and, particularly, pregnant women may have very low or variable renal thresholds, resulting in overestimation of the blood glucose level.
2. Fluid intake and urine concentration affect urine test results.
3. The urine glucose value reflects an average level of blood glucose during the interval since the last voiding and not the level at the time of the test.
4. A negative urine glucose test does not distinguish between hypoglycemia, euglycemia, and mild or moderate hyperglycemia. Thus, urine glucose testing is of limited value in preventing hypoglycemia and hyperglycemia.
5. Urine glucose testing, which uses a color chart with which the test strip color is compared, is less accurate than capillary blood glucose monitoring, which typically uses a digital readout from a reflectance meter.
6. Some drugs interfere with urine glucose determinations.
7. Evaluation of urine dipsticks reveals high imprecision at low glucose concentrations. Manufacturers claim that the test strips are positive if urinary glucose concentrations are 100 mg/dl or greater, but the data indicate this does not always occur.

The above recommendations are supported by both clinical experience and extensive research investigations (3,10–17). Unfortunately, despite the well-documented advantages of SMBG over urine glucose testing, most patients with diabetes still do not perform SMBG on a regular basis (18–20).

If patients choose to perform urine glucose testing, they should fully understand the test limitations. Specifically, patients should be taught that although urine glucose measurements correlate with blood glucose measurements, urine glucose testing provides only a rough estimate of prevailing blood glucose levels (3,11–14). Most important, patients should be taught that urine glucose testing provides no information about blood glucose levels below the renal threshold, which for many patients today is the target range for blood glucose (8). Second-voided specimens do not appear to offer any significant advantage over first-voided specimens (3,21,22).

Urine ketone testing

Urine ketone testing is an important part of monitoring, particularly in patients with type 1 diabetes (8,9). The presence of urine ketones may indicate impending or even established ketoacidosis, a condition that requires immediate medical attention. It is recommended that all people with diabetes test their urine for ketones during acute illness or stress, when blood glucose levels are consistently >300 mg/dl (16.7 mmol/l), during pregnancy, or when any symptoms of ketoacidosis, such as nausea, vomiting, or abdominal pain, are present.

The ketone bodies, which are breakdown products of fatty acids, include β-hydroxybutyric acid, acetoacetic acid, and acetone. The relative proportions in which the three ketone bodies are present in blood vary according to the redox state of the cell. In healthy people, β-hydroxybutyrate and acetoacetate, which are present in approximately equimolar concentrations (i.e., 0.5–1.0 mmol/l each), constitute virtually all of the serum ketones. In diabetic ketoacidosis, the ratio of β-hydroxybutyrate to acetoacetate, may increase up to 6:1 or greater (23). Urine ketone levels are proportional to blood levels but like urine glucose are affected by urine volume and concentration. Ketones are normally present in urine but concentrations are usually below the limit of detectability with routine testing methods. Positive ketone readings are found in normal individuals during fasting and in up to 30% of first morning urine specimens from pregnant women (24).

All of the commercially available testing methods rely on the nitroprusside reaction to produce a purple color in the presence of ketone bodies; acetone is detected only if the reagent contains glycine in addition to sodium nitroprusside, and none of the tests detect β-hydroxybutyric acid. Acetest tablets (Bayer Health Care, Tarrytown, NY) contain glycine and sodium nitroprusside. Ketostix (Bayer Health Care) is a test strip (also available as Keto-Diastix, a test strip for urine glucose and ketones) that is a modification of the nitroprusside test. The strip does not contain glycine and therefore does not detect acetone.

Chemstrip uK (Roche Diagnostics, Indianapolis, IN) is a reagent strip (also available as Chemstrip uGK, a test strip for glucose and ketones) that contains sodium nitroferricyanide (sodium nitroprusside) and glycine. The strip will, therefore, detect both acetoacetic acid and acetone. Multitest urine strips that measure multiple analytes, including ketones, are available from manufacturers for use principally in the hospital or office setting. We are not aware of any data showing clinically important advantages of any one ketone-testing product over the others.

Urine ketone tests using nitroprusside-containing reagents are reported to give false-positive results in the presence of several sulfhydryl drugs, including the antihypertensive drug captopril (25–27). False-negative readings have been reported when test strips have been exposed to air for an extended period of time (28) (reagent bottles should be kept tightly capped and should be discarded after the expiration date on the manufacturer's label) or when urine specimens have been highly acidic, such as after large intake of ascorbic acid.

With recent emphasis on SMBG rather than on urine glucose testing for routine monitoring, all patients, particularly those with type 1 diabetes, should be reminded frequently of the indications for urine ketone testing.

Urine ketone testing materials should be available in the office/clinic setting (8). Health care professionals should be aware, however, that urine ketone tests are not reliable for diagnosing and/or monitoring treatment of ketoacidosis (29,30). Although acetoacetic and β-hydroxybutyric acids are reabsorbed by the renal tubules, their final concentrations in urine usually greatly exceed those in blood. Therefore, the presence of ketone bodies in urine cannot be used to diagnose ketoacidosis. Conversely, during recovery from ketoacidosis, ketone bodies may be detected in urine long after blood concentrations have fallen below levels found in ketoacidosis.

Blood versus urine ketones

Enzymatic methods for quantification of β-hydroxybutyric acid in blood have been described (31,32), and blood ketone testing based on these methods is now available. Studies should be performed to determine whether blood ketone testing by patients at home is as good as or better than urine ketone testing in terms of patient acceptability (i.e., frequency of ketone testing when appropriate) and timeliness of detection of established or impending ketoacidosis.

In addition, studies should be conducted to determine whether blood ketone testing by health providers would be useful as a measure of glycemic control in patients with type 1 diabetes. MacGillivray et al. (33) reported that plasma β-hydroxybutyric acid levels were increased in 73% of presumably healthy patients with type 1 diabetes, of whom only 43% showed ketonuria using a nitroprusside-containing tablet method; all patients whose urine specimens tested positive by the nitroprusside reaction showed elevated levels of plasma β-hydroxybutyric acid.

BLOOD GLUCOSE TESTING

Blood glucose testing by patients

The development of SMBG has, within only a few years, revolutionized the management of diabetes (6–8,10,34–40). By using SMBG, patients with diabetes can work to achieve and maintain specific glycemic goals. Now, particularly given the results of the Diabetes Control and Complications Trial (DCCT), there is broad consensus on the health benefits of normal or near-normal blood glucose levels and on the importance of SMBG in treatment efforts designed to achieve such glycemic goals. The DCCT showed that patients with type 1 diabetes who maintained near-normal blood glucose levels for up to 9 years had dramatic reductions in the risk of developing microvascular and neuropathic complications (41).

Virtually identical risk reductions with improved glycemic control were more recently demonstrated in patients with type 2 diabetes who participated in the U.K. Prospective Diabetes Study (UKPDS) (42). Based principally on the DCCT and UKPDS results, it is recommended that treatment of individuals with diabetes should be aimed at lowering blood glucose to normal or near-normal levels (43). To achieve normal or near-normal blood glucose levels, it is further recommended that most patients with diabetes who take insulin injections follow intensive treatment programs that include frequent SMBG (at least three or four times daily). Note that treatment goals should be individualized; more and less stringent targets may be appropriate for selected patients (43). The role of SMBG in patients with type 2 diabetes, particularly those with stable diet-treated diabetes, is not known (44–46).

The subject of SMBG has been addressed extensively at two ADA Consensus Conferences, one in 1986 and one in 1993. The results of those conferences have been published as ADA Consensus Statements (6,7) and, together with the more recent NACB report (1), provide a comprehensive review of the subject, which will not be discussed further in this report except for a few specific issues.

1. Although it is desirable that patients with all types of diabetes perform SMBG as part of routine care, data indicate that only a minority of patients do so. National survey data from 1989 showed that overall only 33% of patients with diabetes (40% of patients with type 1 diabetes and 26% of patients with type 2 diabetes) performed SMBG at least once a day (18). Other more recent studies show similar data (19,20). Clearly, major efforts should be undertaken to substantially increase the use of SMBG by individuals with all types of diabetes. Barriers to increasing use of SMBG appreciably are formidable and include high costs of SMBG, inadequate education of both health care providers and patients about the health benefits and proper use of SMBG testing results, patient psychological and physical discomfort associated with finger-prick blood sampling, and patient-perceived inconvenience of testing in terms of time requirements and complexity of the technique (5,39). Success at increasing both the frequency with which patients perform SMBG and the optimal use of the data to improve glycemia will depend on the degree to which health care providers working with patients can overcome these barriers. At the same time, development of simpler, less costly testing methods, including noninvasive ones, should be a major scientific priority.
2. Accurate results with SMBG are very technique dependent (5,47–52), although newer meters have somewhat reduced the contribution of technique to imprecision. Analytical goals for meters and for patient-performed testing have been recently reported (53–56). If SMBG is prescribed by the health care provider, an effort should be made to assure that the patient's measurement technique is acceptable, both initially and at regular intervals thereafter. Both the ADA and the American Association for Clinical Chemistry (AACC) have recommended that patients who perform SMBG use calibrators and controls on a regular basis to assure accuracy of results (57,58). The technique of meter calibration is meter specific; some devices have automatic calibration, whereas others use lot-specific

code chips or strips. Since 1976, the Food and Drug Administration (FDA) has required that each new blood glucose meter has a package insert that includes information on use of control materials, how often to use them, and what to do if control results fall outside the specified range (59,60). The FDA also requires that manufacturers recommend to patients that they use control materials regularly, but there is of course no means of monitoring patient compliance, except by health care providers.

3. A number of the glucose meters store test results and with a computer interface can provide sophisticated printouts of blood glucose data. Studies are needed to determine the usefulness of these data management systems.
4. In response to the need for less painful glucose self-testing, several manufacturers now provide products that are specifically designed to be used at body sites other than the fingertip, usually the forearm. However, patients and health care providers should recognize that "alternate-site" testing results may be different from fingertip results when glucose levels are changing rapidly (e.g., postprandially or immediately after exercise) (61–64).
5. Based on the DCCT and UKPDS results (41,42), the ADA (43) recommends that most adults with either type 1 or type 2 diabetes aim for preprandial plasma glucose levels (most glucose meters are calibrated to read as plasma glucose) of 90–130 mg/dl (5.0–7.2 mmol/l) and peak postprandial plasma glucose levels of <180 mg/dl (<10.0 mmol/l). It is further recommended that plasma glucose targets be adjusted on an individual basis for the elderly, children, and patients with recurrent severe hypoglycemia.
6. Further studies are needed to determine whether the blood glucose goals currently recommended are appropriate for most patients with diabetes given the limitations of current therapies. It is particularly important to determine whether risks of severe hypoglycemia in type 1 diabetic patients treated with intensified regimens are lower, the same, or greater than they were in similarly treated DCCT patients.

BLOOD GLUCOSE TESTING BY HEALTH CARE PROVIDERS FOR ROUTINE OUTPATIENT MANAGEMENT OF DIABETES

— It is recommended that blood glucose testing be available to health care providers for immediate use as needed (8,65). Although it is almost tradition that fasting blood glucose determinations be performed at routine interval care visits, their value as a means of assessing glycemic control has been questioned, particularly in patients with type 1 diabetes, in whom blood glucose levels fluctuate widely from day to day (10,33,66,67). In patients with type 2 diabetes, several studies indicate that fasting blood glucose determinations in the clinic setting at intervals of weeks to months provide a better measure of long-term glycemia than they do in patients with type 1 diabetes (10,67–69).

Regardless of the type of diabetes, with the development of SMBG and glycated protein testing (see "Glycated protein testing" below), the continued need for routine blood glucose testing by health care providers as a means of assessing glycemic control must be questioned. However, as a method of testing the accuracy of each patient's home blood glucose testing, comparisons between results of patient self-testing of blood glucose in the clinic and simultaneous laboratory testing can be valuable (47,49,50). Health care providers should be aware that the majority of blood glucose strips and meters approved by the FDA for home use quantify plasma glucose (this information is available in each manufacturer's test strip package insert).

Most laboratory methods quantify plasma or serum glucose, but a few quantify whole-blood glucose, which reads 10–15% lower (70). Therefore, it is necessary for health care providers to determine their laboratories' blood glucose measurement procedures. In addition, it is important for health care providers to know that while glucose concentrations in capillary and venous blood are similar when patients are fasting, postprandial samples can have 20–25% higher values in capillary blood (1). While use of venous blood in a meter will eliminate this error, it will not simulate the patient's testing technique.

Many health care providers measure blood glucose levels with glucose meters approved for home use rather than using the more time-consuming and expensive hospital or clinical laboratory (71).

Several studies have shown that performance of glucose meters varies widely and that use of these devices as "gold standards" with which patient monitoring systems are compared should be subject to rigorous quality control procedures (8,47,48,50,57,72–74). Yet, based on current U.S. government guidelines, blood glucose monitoring devices cleared by the FDA specifically for home use are classified as "waived tests," not requiring any proficiency testing (60). The Clinical Laboratory Improvement Amendments of 1988 (CLIA '88) (59,60), in providing a waiver for blood glucose monitoring devices approved by the FDA for home use, also waives these devices even if they are used in an office or hospital setting. (Note: professional-use versions of home-use tests are not automatically waived. However, such professional versions do qualify for expedited waiver review because only differences between the home-use and professional-use versions need to be examined to determine whether the professional version qualities for waiver.) The Joint Commission on Accreditation of Healthcare Organizations does regulate ancillary testing performed in hospitals, including blood glucose determinations using glucose monitoring devices approved by the FDA for home use (75,76). A survey raised serious questions, however, about the effectiveness of these regulations (77).

The College of American Pathologists (CAP) offers a voluntary proficiency testing program conducted three times per year for home-use testing devices (78,79). This survey's results as well as other studies (80) have documented considerable imprecision, both between different meters from the same manufacturer and between different types of meters. Data such as these emphasize the accuracy and precision limitations of home-approved glucose meters.

OTHER APPROACHES TO BLOOD GLUCOSE TESTING

— Despite vigorous and ongoing research efforts, a practical, accurate, real-time noninvasive blood glucose monitoring device is not yet available. There have been, however, a number of important advances in the field. A number of so-called "minimally invasive" glucose monitoring devices have been developed, and at present two types of devices have been approved by the FDA for use by patients and health care providers (1). The first type, the GlucoWatch Biographer (Cygnus, Redwood City, CA), is worn by the subject and consists of two parts: the "Biographer," worn on the forearm, which calculates, displays, and stores data points, and the "AutoSensor," a single-use device that snaps into the back of the Biographer and draws glucose through the skin by a process called reverse iontophoresis.

This technique, similar to that used to perform sweat chloride testing in patients suspected of having cystic fibrosis, uses a low electric current applied to the skin to promote ion movement and, thus, movement of glucose through the skin, where it is collected on gel discs and analyzed by a glucose oxidase method. This device is reported to provide a maximum of 36 readings per 12-h monitoring period. Use of the device requires warm-up for several hours and calibration with one or more fingerstick glucose determinations by the patient using a traditional glucose meter. The GlucoWatch can be set to alarm if the measured glucose value is below a specified level or rises above a specified rate. The device does not provide a "real-time" glucose reading; the interstitial glucose readings correlate with plasma glucose readings 10–20 min earlier. The device is reported to cause considerable local skin irritation, including redness, itching, and blistering. The device is reported to report false low glucose readings with sweating. Some studies have shown that the device is useful to detect unsuspected nocturnal hypoglycemia (81).

Recently, the FDA has approved a continuous subcutaneous insulin infusion device coupled with the glucose-reading device. It should be noted that this is not a "closed loop" system in which the glucose reading would automatically determine the insulin infusion requirement. Numerous reports have documented the usefulness of continuous glucose monitoring systems for detecting nocturnal hypoglycemia or unusual glycemic patterns in selected patients with type 1 diabetes.

At present, there is no convincing evidence that the current FDA-approved minimally invasive glucose monitoring devices should replace routine SMBG by patients with diabetes or glucose measurements by an accredited laboratory. The available data do suggest, however, that these devices can be useful in selected patients to improve their glycemic control and, in particular, to decrease risks for hypoglycemic episodes (82–84).

GLYCATED PROTEIN TESTING

GLYCATED PROTEIN TESTING — Blood and urine glucose and urine ketone testing provide useful information for day-to-day management of diabetes. However, these tests cannot provide the patient and health care team with an objective measure of glycemia over an extended period of time. Measurements of glycated proteins, primarily hemoglobin and serum proteins, have added a new dimension to the assessment of glycemia. With a single measurement, each of these tests can quantify average glycemia over weeks and months, thereby complementing day-to-day testing (4,5,10,40,84–92).

Glycated hemoglobin testing

Glycated hemoglobin (GHb), also commonly referred to as glycosylated hemoglobin, glycohemoglobin, HbA_{1c}, HbA1, or A1C, is a term used to describe a series of stable minor hemoglobin components formed slowly and nonenzymatically from hemoglobin and glucose.

The rate of formation of GHb is directly proportional to the ambient glucose concentration. Because erythrocytes are freely permeable to glucose, the level of GHb in a blood sample provides a glycemic history of the previous 120 days, the average erythrocyte lifespan. GHb testing first became available to the routine clinical laboratory in the late 1970s. Since then, use of the test for both research and patient care has increased steadily. Routine use of GHb testing in all patients with diabetes is recommended, first to document the degree of glycemic control at initial assessment, then as part of continuing care (8). GHb is used both as an index of mean glycemia and as a measure of risk for the development of diabetes complications. The test is also being used increasingly by quality assurance programs to assess the quality of diabetes care (93,94).

History. In normal human erythrocytes, HbA comprises ~90% of the total hemoglobin. Besides HbA, human erythrocytes contain other hemoglobin components that are of considerable interest. Some of these, such as HbA_2 and fetal hemoglobin (HbF), like sickle-cell hemoglobin, are products of alternate globin chain genes, and others such as HbA_{1c} are posttranslational modifications of HbA.

As early as 1955, investigators noted that adult human hemoglobin was heterogeneous (95,96). In 1958, Allen et al. (97) reported that with cation-exchange chromatography human hemoglobin could be separated into at least three minor components that had more negative charges than HbA. These minor components were named HbA_{1a}, HbA_{1b}, and HbA_{1c}, in order of their elution from the column. The significance of this finding in relation to diabetes was not appreciated until Rahbar et al. (98,99), using gel electrophoresis, reported an elevation of these minor hemoglobin fractions in patients with diabetes.

By the mid-1970s it became clear that HbA_{1c} resulted from a posttranslational modification of HbA by glucose and that there was a relationship between HbA_{1c} and fasting plasma glucose, glucose peak during the glucose tolerance test, area under the curve of the glucose tolerance test, and mean glucose levels over the preceding weeks (100–105). By the early 1980s, GHb testing became widely available.

Chemistry and terminology. The ability of reducing sugars to react with the amino groups of proteins is now widely recognized, as is the natural occurrence of many non–enzymatically glycated proteins (106). The initial step in the reaction is the condensation of a free primary amine on hemoglobin with the carbonyl of the glucose, resulting in the formation of a Schiff base (early Maillard reaction). This Schiff base is not stable and may either dissociate or undergo an Amadori rearrangement to form a stable ketoamine. There is now considerable evidence for an Amadori-type rearrangement for the adduct of glucose with the NH_2-terminal valine of the β-chain (HbA_{1c}) as well as the NH_2-terminal valine of the α-chain and for ε-amino groups of certain lysine residues on α- and β-chains. The rate of formation of GHb is directly proportional to the ambient glucose concentration.

Because hemoglobin circulates in each erythrocyte for ~120 days, there is some opportunity in this cell for late Maillard reactions or nonenzymatic browning to occur (the products of these reactions are referred to as advanced glycation end products [AGEs]), and the extent of these changes appears to correlate with GHb

Table 1—*Hemoglobin nomenclature*

HbA	The major form of hemoglobin, a native, unmodified tetramer consisting of two α-chains and two β-chains
GHb	A general term for glucose bound nonenzymatically to hemoglobin and with a ketoamine structure.
HbA_1	GHb species that are more negatively charged forms of HbA detected by cation-exchange chromatographic and electrophoretic methods, which include HbA_{1a}, HbA_{1b}, and HbA_{1c}; also called the "fast" hemoglobins
HbA_{1c}	A specific GHb that is an adduct of glucose attached to the β-chain terminal valine residue
Total GHb	A term used to describe all GHb species as measured by affinity chromatographic methods.

values (107). In the formation of AGEs, the Amadori product is degraded into deoxyglucosones, which react again with free amino groups to form other products (108,109). In tissues that are longer lived (connective tissue, vascular endothelium, etc.), AGEs may be important mediators of diabetes pathology as well as the normal aging process. Although the structures of many AGEs have been elucidated, few have been obtained under physiological conditions, thus making detection in vivo difficult and their pathological role uncertain (110,111). Studies are needed to determine whether measurement of AGEs, presumably reflecting very long–term glycemia (perhaps many months or years), has useful clinical application.

Table 1 summarizes recommended terminology for hemoglobin that has reacted with sugars (86,91). GHb is a general term used to describe hemoglobin that has been modified by addition of glucose through a nonenzymatic process. HbA_{1c} is one of several GHbs that reflect glycemic status; it is the form of GHb that has been studied most extensively, probably because it was the first hemoglobin species observed to be increased in individuals with diabetes. Because circulating erythrocytes are incapable of initiating protein synthesis after the reticulocyte stage (3 days in the circulation), HbA_{1c} and the other forms of GHb are produced only as posttranslational modifications of hemoglobin.

The postsynthetic modifications of hemoglobin to form GHbs are essentially irreversible, and rates of synthesis reflect the minute-to-minute glucose environment in which the erythrocyte circulates. The term HbA_1 is used to describe all the fast-eluting hemoglobins as quantified with cation-exchange chromatographic methods (or fast-migrating ones, as quantified with electrophoretic methods) and include HbA_{1a}, HbA_{1b}, and HbA_{1c}. The term total GHb is a relatively recent addition to hemoglobin nomenclature and describes all GHb species as quantified by boronate affinity chromatographic methods; this type of assay detects ketoamine structures on α- and β-chain NH_2-terminal valine residues as well as on lysine residues.

Assay methods. The different GHb assay methods available to the routine clinical laboratory can be divided into two major categories: those based on charge differences between GHb and non-GHb (these include cation-exchange chromatography, electrophoresis, and isoelectric focusing) and those based on structural characteristics of glycogroups on hemoglobin (these include affinity chromatography and immunoassay) (85,86, 91,112). Most methods quantify HbA_{1c}, defined as HbA with glucose attached to the NH_2-terminal valine of one or both β-chains. Other methods (boronate affinity) quantify "total glycated hemoglobin," which includes both HbA_{1c} and other GHb adducts (e.g., glucose-lysine adducts and glucose α-chain NH_2-terminal valine adducts). Results of methods using different assay principles show excellent correlation, and there are no convincing data to show that any one method or analyte is clinically superior to any other.

A review of preanalytical and analytical variables affecting GHb assays has been published (1). In general, any situation that shortens erythrocyte survival or decreases mean erythrocyte age falsely lowers GHb test results regardless of the assay method (113). Vitamins C and E are reported to falsely lower test results, possibly by inhibiting glycation of hemoglobin (114,115). Iron-deficiency anemia is reported to increase test results. Hypertriglyceridemia, hyperbilirubinemia, uremia, chronic alcoholism, chronic ingestion of salicylates, opiate addiction, hemoglobinopathies, and chemically modified derivatives of hemoglobin may interfere with some assay methods (116–119). Interferences from hemoglobin variants and adducts are summarized by Bry et al. (120) and on the National Glycohemoglobin Standardization Program (NGSP) Web site at www.ngsp.org (120–123). Laboratories should use GHb assay methods with an interassay coefficient of variation (CV) of <4% (ideally <3%). Each laboratory should also determine its own reference interval following NCCLS guidelines (Document C28A).

Each method has certain advantages and disadvantages for the clinical laboratory, and choosing a method can be difficult; none should be considered the "best" method. Laboratory directors have a responsibility to provide clinicians with some basic information about the assay method used. Such information should include the following: type of assay method, nondiabetic reference interval, potential assay interferences, and assay performance (e.g., some measure of assay imprecision, such as CV).

Standardization of GHb. The DCCT set the stage for establishing specific diabetes treatment goals using GHb as an index of mean blood glucose. The correlation between GHb levels and outcome risks demonstrated in the DCCT and later in the UKPDS underscores the need to measure GHb with high precision and in such a manner that results can be directly related to these studies and therefore to outcome risks. By the early 1990s, a number of investigators had already shown that standardization was technically feasible, despite the wide variety of assay methods in use (124–129). In 1996, the NGSP was initiated to standardize GHb test results among laboratories to DCCT-equivalent values (130–133). The NGSP was developed under the auspices of the AACC and is endorsed by the ADA, which recommends that laboratories use only GHb methods that have passed certification testing by the NGSP.

The NGSP Laboratory Network includes a variety of assay methods, each calibrated to the DCCT reference. The DCCT reference is a high-performance liquid chromatography (HPLC) cation-exchange method that quantifies HbA_{1c} and is an NCCLS-designated comparison method (134,135). The assay method has been used since 1978 and has demonstrated good long-term imprecision (between-run CVs consistently <3%) (136). The laboratories in the network interact with manufacturers of GHb methods to assist them first in calibrating their methods and then in providing comparison data for certification of traceability to the DCCT. Certification is valid for 1 year and is available to manufacturers and laboratories. The certification process follows a specific protocol involving evaluation of both precision and accuracy using specific criteria. A method must have a total imprecision (CV) of ≤4%, and the 95% CI of the differences with a network laboratory must fall within the clinically significant limits of ±1% HbA_{1c}. Current lists of NGSP-certified methods and laboratories can be found on the NGSP Web site (www.ngsp.org).

An important adjunct to the NGSP is the GHb proficiency-testing survey administered by the CAP. Since 1996, the survey has used fresh whole-blood samples with NGSP-assigned target values. The CAP data have been used to evaluate the progress of the NGSP. Since 1996, the survey has documented a steady improvement in comparability of GHb values among laboratories, both within and between methods. Based on data from the 2003 GH2 CAP survey, 99% of laboratories (n = 2,042) reported results as HbA_{1c} or HbA_{1c} equivalents. Ninety-eight percent of laboratories used NGSP-certified methods (137). The NGSP Web site contains summaries of recent CAP data.

At an international level, the International Federation of Clinical Chemistry (IFCC) established in 1995 a working group to develop both a reference method and pure standards. An IFCC Laboratory Network that uses mixtures of purified HbA_{1c} and HbA0 to calibrate two different reference methods has been established (138,139). The relationship between the IFCC and the NGSP networks has also been established; a master equation, based on several sample comparisons, has now been accepted (140). The IFCC HbA_{1c} results are significantly lower (~1.3 to 1.9% across the relevant HbA_{1c} range) than NGSP results. There has been much debate about which numbers—the accuracy-based IFCC numbers or the outcomes-based NGSP/DCCT/UKPDS numbers—should be reported worldwide.

Beyond its use as a measure of long-term glycemia and of risk for chronic complications in diabetes, routine GHb testing has been shown to improve glycemia per se. Larsen et al. (141) randomly assigned 240 patients with type 1 diabetes to either a treatment or a control group. GHb testing was performed quarterly in both groups for 12 months; test results were made available, however, only to patients and health care providers in the treatment group. There were no other specific differences in management between the two study groups. After 1 year, GHb values were substantially lower in the treatment group than in the control group. The higher the GHb level at baseline, the greater its decrease after 1 year. Thus, knowledge of GHb seems to alter behavior of health care providers and/or patients, which in turn improves glycemia and lowers GHb results, thereby lowering the patient's risk of developing chronic complications of diabetes.

Optimal frequency of GHb testing has not been well established. The ADA recommends that GHb testing be performed at initial patient assessment and at least two times a year in patients who are meeting goals, and performed quarterly in patients whose therapy has changed or who are not meeting glycemic goals (8). In the DCCT (41), GHb determinations were performed monthly in the intensive treatment group and quarterly in the standard treatment group. Although mean GHb levels were ~2% lower in the intensive treatment group throughout the 9-year study (~7 vs. 9% HbA_{1c}), GHb values were not made available to standard treatment group patients or to their health care providers, and other aspects of patient management in the two treatment groups differed considerably.

Further studies are needed to determine whether current recommendations for GHb testing frequency are appropriate or if more (or less) frequent testing (e.g., monthly determinations) is clinically useful.

Proper interpretation of GHb test results is not easy and requires that health care providers understand the relationship between the test results and average blood glucose, kinetics of GHb, specific assay limitations, and patient factors (other than blood glucose levels) that can affect the results. Most often, and appropriately, GHb is used in the routine care of patients as a surrogate mean blood glucose determination—a measure of glycemic status during the previous weeks and months. Several studies, most notably the DCCT, have defined quantitatively the relationship between GHb and average glycemia (41,142).

Table 2—*Correlation between HbA_{1c} level and mean plasma glucose levels*

	Mean plasma glucose	
HbA_{1c} (%)	mg/dl	mmol/l
6	135	7.5
7	170	9.5
8	205	11.5
9	240	13.5
10	275	15.5
11	310	17.5
12	345	19.5

Table 2 shows the relationship between HbA_{1c} and mean plasma glucose levels based on data from the DCCT. In general, each 1% increase in GHb corresponds to a 35-mg/dl (1.95-mmol/l) increase in mean plasma glucose.

Although the DCCT data show a strong relationship between glycemia and GHb, results also raise important questions about differences among individual patients. Is the relationship between GHb and average blood glucose the same for all patients or are there clinically important differences? Some investigators have suggested that there may be significant interindividual differences in the relationship between GHb and average blood glucose, possibly due to differences in glycation rates (i.e., high and low "glycators") (143,144). However, between-subject variation in GHb has been shown to be minimal in nondiabetic subjects (145,146), and to the extent that differences exist, they may represent differences in mean glycemia rather than differences in glycation rates (146,147).

Another factor to consider is individual differences in renal threshold for glucose. The average kidney threshold for glucose is ~180 mg/dl (10 mmol/l). Above this level, virtually all the additional glucose presented to the kidney is excreted. The higher the threshold, the higher the steady-state blood glucose (and GHb) level that can be attained. Thus, differences in kidney threshold would not affect the relationship between GHb and average blood glucose but would affect the ease with which an individual could achieve a certain level of GHb. All of the above factors may affect interpretation of GHb test results. Further studies are needed to determine the relative importance of each of these variables in GHb results.

Proper interpretation of GHb test results also requires an understanding of GHb kinetics (that is, the rate of change in GHb with a change in glycemia). There is a common misconception among both health care providers and patients that since the GHb test reflects mean glycemia during the preceding weeks and months, large changes in glycemia cannot be detected except after many weeks. Mathematical modeling predicts and in vivo studies confirm that although a change in mean blood glucose on day 1 is not fully reflected in the GHb level until 120 days later (the mean erythrocyte lifespan), a large change in mean blood glucose (up or down) is accompanied by a rapid and large change in GHb. Regardless of the starting GHb level, the time required to reach a midpoint between the starting level and the new steady-state level is relatively constant at 30–35 days. Thus, a large change in mean blood glucose is accompanied by a large change in GHb within a matter of 1–2 weeks, not 3–4 months (85,148,149). In effect, GHb is a "weighted" measure of mean blood glucose during the preceding 120 days; more recent past events contribute relatively more to the final result than earlier events. The mean level of blood glucose in the 30 days immediately preceding the blood sampling (days 0–30) contributes ~50% to the final result, whereas days 90–120 contribute only ~10%.

Glycated serum proteins

Because the turnover of human serum albumin is much shorter (half-life ~14 days) than that of hemoglobin (erythrocyte lifespan 120 days), the degree of glycation of serum proteins (mostly albumin) should provide an index of glycemia over a shorter period of time than glycation of hemoglobin. Measurements of total glycated serum proteins (GSPs) and glycated serum albumin (GSA) correlate well with GHb and have been suggested as alternative methods for routine monitoring of glycemic control in patients with diabetes (112,150–156). The subject has been reviewed fairly recently (85,112,154,155).

Methods. Several methods have been described that measure either GSP or GSA. The major methods can be divided into two categories: those that separate glycated from nonglycated species based on differences in chemical reactivity (e.g., fructosamine assay) and those that separate based on differences in structural characteristics (e.g., affinity chromatography) (85,112). Methods based on charge differences have not found wide application in the measurement of GSP and GSA, as they have with GHb, because glycated and non-GSP components show very little difference in charge.

In 1982, Johnson et al. (150) described a method based on the ability of ketoamines, or fructosamines, to act as reducing agents in alkaline solution. The term fructosamine was originally introduced into the clinical chemistry literature as a general term for glycated protein. However, it has come to be associated with the specific analyte measured by the nitroblue tetrazolium (NBT) assay, and this assay method has come to be known as the fructosamine assay. The assay became commercially available for use on several chemistry analyzers a few years after the initial report.

Since the initial description of this method, there have been numerous reports of interferences (e.g., uremia, lipemia, bilirubin, ascorbate, and hemolysis) and other assay problems, including standardization and matrix effects, dependence on buffer pH and assay timing, and effects of protein concentration (112,154–167). Subsequent modifications to the assay appear to have improved results appreciably (168–171). Still unresolved, however, is the question of whether fructosamine measurements should be corrected for either total protein or albumin concentrations.

Early studies showed that fructosamine measurement was independent of protein or albumin concentration as long as the concentrations of albumin and protein were within the reference range (151,152,158). However, other studies have found statistically significant relationships between fructosamine and either total protein or albumin concentrations and have recommended that fructosamine values be corrected for protein concentrations (153,163,164).

An argument for not routinely correcting fructosamine values for protein concentrations was proposed by Staley (162) and contends that the molar concentration of serum protein and of reactive lysine groups will always be in excess. Therefore, the rate-limiting step will be the glucose concentration and not the serum protein concentration. Schleicher et al. (161) concluded that albumin concentration should not be used to correct fructosamine values because albumin concentration influences its own turnover, which in turn influences the amount of glycation. Conversely, Lamb et al. (160) concluded that correcting fructosamine values for serum albumin or total protein concentration is justifiable because the amount of fructosamine produced is in first-order relation to albumin/protein concentration. Henrichs et al. (168) warned that if fructosamine values are corrected for protein concentration, overall precision may be reduced by the imprecision of the total protein determinations. Furthermore, even when the total protein concentration is normal, dysproteinemias (i.e., qualitative changes in serum protein composition) may affect fructosamine values; this cannot be corrected by simple adjustment by protein concentration (172). Finally, Hill et al. (159) concluded that while in a given population a relationship between serum fructosamine and protein may be apparent, the clinical utility of routine fructosamine correction has not been clearly established.

In summary, it is clear that further studies are needed to resolve the vigorous debate about the need to correct fructosamine values for protein/albumin concentration. In the interim, perhaps fructosamine can be reported as both corrected and uncorrected values.

Affinity chromatography has been used for separation and measurement of both GSP and GSA. Protein determination after separation on affinity columns is accomplished by ultraviolet absorbance at 280 nm or Coomassie Blue reagent at 595 nm. Albumin in glycated and nonglycated fractions can be determined with bromcresol green or enzyme-linked immunosorbent assay (173).

Alternatively, albumin can be separated first by Affi-Gel Blue affinity chromatography or anion-exchange HPLC (174), and boronate affinity chromatography can then be used to determine the GSA fraction. Boronate affinity chromatographic methods for measurement of GSP or GSA are commercially available in the form of minicolumns and automated HPLC. GSA and GSP can also be measured by immunoassay (175).

Clinical utility. Although there are still important unanswered questions regarding assay details (e.g., whether values should be corrected for protein concentration), the most critical question is whether measurement of GSP is a simple and inexpensive alternative to measurement of GHb for routine monitoring of glycemic status in patients with diabetes. Several studies recommend cautious interpretation of GSP measurements unless they are performed frequently; patients can improve their GSP values appreciably by increasing compliance during the week or two before their clinic visit (176,177). Such maneuvers would have much less effect on GHb results. A second important question is whether short-term measures of glycemia are clinically useful (e.g., in diabetes complicated by pregnancy). Most but not all studies show that the level of GSP responds more quickly than that of GHb to changes in the level of blood glucose, although the reported differences are generally small and are dependent on the assay method (177–181). Some studies have shown that GHb actually correlates better than does GSP with average blood glucose as recent as 2 weeks previously (182,183). The clinical usefulness of a home fructosamine test has been evaluated, with conflicting results (184,185).

In summary, the clinical utility of glycated protein determinations other than GHb has not been clearly established, and there is no conclusive evidence that relates their concentration to the chronic complications of diabetes (45). Further studies are needed to determine whether these assays provide clinical information equivalent to GHb for routine management of patients with diabetes and, if so, whether they offer any significant advantages over GHb. The recent availability of a number of improved assays for GSPs may help answer some of these questions.

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