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
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 semiquantitative 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 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 traditional 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 monitoring devices approved by the FDA for 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” glu-
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** — 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 (4,5,10,84–92).

**Glycated hemoglobin testing**

Glycated hemoglobin (GHB), also commonly referred to as glycosylated hemoglobin, glycohemoglobin, HbA1c, 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 HbA2 and fetal hemoglobin (HBF), like sickle-cell hemoglobin, are products of alternate globin chain genes, and others such as HbA1c 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 HbA1a, HbA1b, and HbA1c, 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 HbA1c, resulted from a posttranslational modification of HbA by glucose and that there was a relationship between HbA1c 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 NH2-terminal valine of the β-chain (HbA1c) as well as the NH2-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
HbA1c A specific term used to describe all GHb species as measured by affinity chromatographic methods.

HbA1c: 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 physiologic 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—Hemoglobin nomenclature

<table>
<thead>
<tr>
<th>Hemoglobin</th>
<th>Description</th>
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<tr>
<td>HbA</td>
<td>The major form of hemoglobin, a native, unmodified tetramer consisting of two α-chains and two β-chains</td>
</tr>
<tr>
<td>GHb</td>
<td>A general term for glucose bound nonenzymatically to hemoglobin and with a ketoamine structure</td>
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<tr>
<td>HbA1</td>
<td>GHb species that are more negatively charged forms of HbA detected by cation-exchange chromatographic and electrophoretic methods, which include HbA1a, HbA1b, and HbA1c, also called the “fast” hemoglobins</td>
</tr>
<tr>
<td>HbA1c</td>
<td>A specific GHb that is an adduct of glucose attached to the β-chain terminal valine residue</td>
</tr>
<tr>
<td>Total GHb</td>
<td>A term used to describe all GHb species as measured by affinity chromatographic methods</td>
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Glycated hemoglobins (e.g., glucose-lysine adducts) are also formed in excess under hyperglycemic conditions. 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 HbA1c and is an NCCLS-designated comparison method.
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 significant 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% HbA1c), 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 shows the relationship between HbA1c 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 non diabetic 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 indi-
vidual 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 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 ketones, 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 hemoysis) 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 brom cresol 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 immunosassay (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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