Hemoglobin A1c in Diabetes: Panacea or Pointless?

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Glycation is the nonenzymatic attachment of a monosaccharide to amino groups of proteins. The reaction has been recognized for many years in the food industry, where it is known as browning (also termed the Maillard reaction) and is responsible for the formation of commonly ingested items, such as toast. In patients with diabetes, glucose accumulation results in enhanced glycation of many proteins, both in tissues (e.g., the lens) and in the blood. Of these, glycated hemoglobin (GHb) is by far the most frequently measured in patient care. Hemoglobin (Hb) in healthy adults consists predominantly of HbA, which has 2α- and 2β-chains. Glucose can attach to several amino acid residues in these chains. HbA1c is formed when glucose attaches specifically to the NH2-terminal valine of the β-chain. Formation of HbA1c is essentially irreversible, and its concentration in the blood depends on both the life span of the red blood cell (RBC), which averages ~120 days, and the blood glucose concentration. Because the rate of formation of HbA1c is directly proportional to the concentration of glucose in the blood, HbA1c represents integrated values for glucose over the preceding 8 to 12 weeks (Table 1).

CLINICAL VALUE OF HbA1c

Initially described 57 years ago (1), GHb was first reported to be increased in patients with diabetes in the late 1960s (2). The clinical value of GHb was soon realized and the American Diabetes Association (ADA) began encouraging the routine measurement of GHb in all patients with diabetes (3).

The fundamental role of GHb in diabetes was accentuated by the publication in 1993 of the Diabetes Control and Complications Trial (DCCT) (4). The study, which compared intensive to conventional insulin therapy in patients with type 1 diabetes, documented a direct relationship between blood glucose concentrations (assessed by GHbA1c) and the risk of microvascular complications. The absolute risks of retinopathy and nephropathy were directly proportional to the mean HbA1c concentration. (To prevent assay variability [see "Development of Accurate HbA1c Measurements" below], all GHb assays in the DCCT were performed in a single laboratory that measured HbA1c).

Analogous correlations between HbA1c and complications were observed in patients with type 2 diabetes in the UK Prospective Diabetes Study (UKPDS) (5). Although mean HbA1c for intensively treated and conventionally treated type 2 diabetic patients differed by an apparently small amount (values were 7.0 and 7.9%, respectively), microvascular complications in the intensively treated group were ~25% lower. Both the DCCT and the UKPDS demonstrated that the HbA1c value predicts the risk of microvascular complications in patients with diabetes. Importantly, these two large, randomized, prospective studies revealed that the reduction of HbA1c was associated with a significantly slower progression of microvascular disease (4,5). Lowering HbA1c in subjects with type 1 or type 2 diabetes also significantly reduced myocardial infarction (6,7), a macrovascular complication that is the most common cause of death in people with diabetes.

HbA1c has many favorable attributes, including no requirement that the patient be fasting, the sample may be collected any time of the day, and the concentration in the blood is independent of acute factors such as stress or exercise (Table 1) (8). Based on these—and other unique qualities—HbA1c is firmly established as an index of long-term blood glucose concentrations. In addition, HbA1c values are used to guide therapy, and target goals have been designated by a number of groups (9). More recently, HbA1c has been accepted by several influential diabetes organizations as a criterion for the diagnosis of diabetes (10,11).

FACTORS OTHER THAN GLYCEMIA MAY ALTER HbA1c VALUES

Notwithstanding the ubiquitous use of HbA1c in diabetes, analogous to any other laboratory test, effective use in patient care requires comprehension of the factors that may influence HbA1c results (Table 1).

Biological variability. Unlike blood glucose, which fluctuates widely, HbA1c varies minimally (~1%) in a healthy individual (12). However, substantial interindividual variation has been observed (13). Several elements may contribute to this. A concept of variable glycation, with high and low glycators, has been proposed to account for differences observed between HbA1c and blood glucose values (14). However, minimal evidence has been published to support the hypothesis, and it remains contentious.

Accumulating data support the concept that race influences HbA1c, with higher HbA1c concentrations in African Americans, Asians, and Hispanics than in whites (13). The etiology is unknown. Some authors posit that the increased HbA1c accurately reflects higher glucose values in these populations (15). Regardless of the cause, the differences are small (~0.4% HbA1c) and the clinical significance, if any, remains to be established.

Interpretation of HbA1c depends on RBCs having a normal life span. Patients with hemolytic disease or other conditions with shortened RBC survival exhibit a substantial reduction in HbA1c (16). This problem could be resolved if one could correct for the age of RBCs, but unfortunately it is extremely difficult to measure RBC life span.
Factors that may interfere with measurement by some methods. The effects of hemoglobin variants (such as HbS, HbE, HbD, and HbC) are contingent upon the specific method of analysis used (17). Depending on the particular hemoglobin variant and assay, results may be spuriously increased or decreased. Most manufacturers of HbA1c hemoglobin variant and assay, results may be spuriously increased or decreased. Most manufacturers of HbA1c have modified their methods to eliminate interference from many of the common hemoglobin variants. Therefore, accurate measurement of HbA1c is possible by selecting an appropriate instrument, provided the RBC life span is not altered (see www.ngsp.org for additional information).

There are isolated reports in the literature of chemical modifications to Hb that affect HbA1c measurements. Many of these articles are old and use methods that are now obsolete, so their relevance to patient care is not clear. A posttranslational modification of Hb that does interfere with some current methods is carbamylation. The non-enzymatic reaction of isocyanic acid with the NH2-terminal valine of Hb (the same residue to which glucose attaches) forms carbamylated Hb. Patients with chronic kidney disease (CKD), a common occurrence in diabetes, have increased carbamylated Hb because of the increased urea, which is in equilibrium with ammonium cyanate. Nevertheless, the majority of the interferents produce relatively small effects, and HbA1c can be measured accurately in most patients with diabetes.

Factors that may affect interpretation. In addition to uremia, patients with CKD have shortened RBC survival and many are on erythropoietin treatment. Together these factors contribute to HbA1c underestimating glycemic control in patients with CKD (18). Iron deficiency anemia, by contrast, is associated with higher HbA1c concentrations (19). While a population-based study of 10,535 adults in the U.S. observed that iron deficiency was associated with small shifts in HbA1c values (20), it seems prudent to correct iron deficiency before measuring HbA1c.

DEVELOPMENT OF ACCURATE HbA1c MEASUREMENTS

Tests to measure GHB were launched in 1978 by several companies and the number of methods grew rapidly, with >120 available at the time of writing this article. This created a practical problem as the various methods measured different forms of GHB, resulting in considerably different values for a single patient sample. The situation was compounded by the complete absence of standardization, producing as much as a twofold difference in GHB values (e.g., 4.0 and 8.1%) in a sample analyzed by two different methods (9). This variability, which was not well recognized by most clinicians, substantially limited the value of GHB measurement in patient care.

The necessity for accurate HbA1c measurement motivated the formation of the NGSP, which standardizes HbA1c results to those of the DCCT. This function is performed by a network of laboratories that are centered around the Primary Reference Laboratory, which uses the HbA1c method used in the DCCT (21). The NGSP laboratory network collaborates with manufacturers of HbA1c assays so that their instruments will report the same HbA1c value as that reported in the DCCT (21). In other words, an HbA1c of 8.0% in a patient sample should be identical to a value of 8.0% in the DCCT. Using mass spectrometry, a working group of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) developed a reference method to accurately quantify HbA1c (22,23). This reference measurement procedure is not intended for routine analysis of patient samples, but has traceability to a standard of higher metrologic order. The complementary efforts of the NGSP and the IFCC have led to significant improvements in the accuracy of HbA1c measurement in routine patient testing (21).

HOW IS HbA1c REPORTED?

HbA1c has traditionally been reported as a percentage of total Hb. IFCC numbers are lower by 1.5–2.0% HbA1c than NGSP/DCCT numbers, most likely because of the increased specificity of the IFCC method. While there is a tight linear correlation between the NGSP and the IFCC methods, the slope and intercept differ significantly from 1 and 0, respectively (23). To conform with Système International (SI) units, IFCC results are now reported as mmol HbA1c per mol Hb (24). Several countries, predominantly in Europe, have elected to report HbA1c in SI units. An HbA1c of 7% (in DCCT/NGSP units) corresponds to 53 mmol/mol. The conversion cannot be performed by simply multiplying or dividing (as can be done for interchanging glucose between mg/dL and mmol/L). Instead a linear equation, derived from multiple comparisons between the NGSP and IFCC networks, is required. The master equation is: NGSP = 0.09148(NGSP) – 2.152 or IFCC = 10.93(NGSP) – 23.50. These equations allow HbA1c results to be converted from DCCT/NGSP units to SI units and from SI units to DCCT/NGSP units. Several journals, including Diabetes, now require authors to report HbA1c in both sets of units. In order to facilitate this conversion, a table and calculator are available on the NGSP website (http://www.ngsp.org/convert1.asp).
PERSPECTIVE
Measurement of HbA1c is integral to the management of patients with diabetes and regular analysis is recommended by many preeminent clinical organizations. A few other glycated proteins have been evaluated in patients with diabetes. The best studied is fructosamine, a measure of all glycated proteins in serum (25). A test for glycated albumin alone has also been developed (26). These extracellular analytes reflect glycemia over ~10–14 days (the half-life of albumin) and are independent of both RBC life span and Hb modifications. However, they are altered by changes in albumin turnover and suffer from a dearth of clinical studies. A PubMed search in humans resulted in 23,210 “hits” for HbA1c, but only 1,526 and 478 for fructosamine and glycated albumin, respectively. More importantly, there are neither outcome data that unequivocally link these analytes to diabetes complications nor agreed target values for optimum glycemic control. While they have a role in situations where HbA1c cannot be used, their clinical value is limited until more data become available.

It is important to emphasize that the measurement of HbA1c provides valuable information for the overwhelming majority of diabetic patients. Knowledge of the conditions that alter HbA1c enables the appropriate use of HbA1c, which will remain, for the foreseeable future, essential for the management of patients with diabetes.

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