Hemoglobin A_{1c} 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. HbA_{1c} is formed when glucose attaches specifically to the NH_2-terminal valine of the β-chain. Formation of HbA_{1c} 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 HbA_{1c} is directly proportional to the concentration of glucose in the blood, HbA_{1c} represents integrated values for glucose over the preceding 8 to 12 weeks (Table 1).

CLINICAL VALUE OF HbA_{1c}

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 HbA_{1c}) and the risk of microvascular complications. The absolute risks of retinopathy and nephropathy were directly proportional to the mean HbA_{1c} concentration. (To prevent assay variability [see "Development of Accurate HbA_{1c} Measurements" below], all GHb assays in the DCCT were performed in a single laboratory that measured HbA_{1c}).

Analogous correlations between HbA_{1c} and complications were observed in patients with type 2 diabetes in the UK Prospective Diabetes Study (UKPDS) (5). Although mean HbA_{1c} 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 HbA_{1c} value predicts the risk of microvascular complications in patients with diabetes. Importantly, these two large, randomized, prospective studies revealed that the reduction of HbA_{1c} was associated with a significantly slower progression of microvascular disease (4,5). Lowering HbA_{1c} 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.

HbA_{1c} 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—HbA_{1c} is firmly established as an index of long-term blood glucose concentrations. In addition, HbA_{1c} values are used to guide therapy, and target goals have been designated by a number of groups (9). More recently, HbA_{1c} has been accepted by several influential diabetes organizations as a criterion for the diagnosis of diabetes (10,11).

FACTORS OTHER THAN GLYCEMIA MAY ALTER HbA_{1c} VALUES

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

Biological variability. Unlike blood glucose, which fluctuates widely, HbA_{1c} 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 HbA_{1c} 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 HbA_{1c}, with higher HbA_{1c} concentrations in African Americans, Asians, and Hispanics than in whites (13). The etiology is unknown. Some authors posit that the increased HbA_{1c} accurately reflects higher glucose values in these populations (15). Regardless of the cause, the differences are small (≤0.4% HbA_{1c}) and the clinical significance, if any, remains to be established.

Interpretation of HbA_{1c} depends on RBCs having a normal life span. Patients with hemolytic disease or other conditions with shortened RBC survival exhibit a substantial reduction in HbA_{1c} (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.

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TABLE 1
Selected attributes of HbA_{1c}

Positive	Negative
Biology	
Reflects chronic glycemia	Variable glycation
Value independent of acute factors (e.g., stress, exercise)	Age
Very low intraindividual variability	Race
	Erythrocyte life span
Analysis	
Subject need not be fasting	Hemoglobin variants may interfere
Blood may be collected any time of the day	Chemical modification of hemoglobin may interfere
Sample is stable	
Assay is standardized	
Accuracy is monitored	
Clinical	
Monitor long-term glucose control	May be altered by factors other than glucose (e.g., hemolysis, CKD, iron deficiency anemia)
Used to guide therapy	
Concentration predicts the risk of microvascular complications of diabetes	
Used to diagnose diabetes	

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 HbA_{1c} assays have modified their methods to eliminate interference from many of the common hemoglobin variants. Therefore, accurate measurement of HbA_{1c} 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 HbA_{1c} 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 nonenzymatic reaction of isocyanic acid with the NH_2-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 HbA_{1c} 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 HbA_{1c} underestimating glycemic control in patients with CKD (18). Iron deficiency anemia, by contrast, is associated with higher HbA_{1c} 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 HbA_{1c} values (20), it seems prudent to correct iron deficiency before measuring HbA_{1c}.

DEVELOPMENT OF ACCURATE HbA_{1c} 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 HbA_{1c} measurement motivated the formation of the NGSP, which standardizes HbA_{1c} 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 HbA_{1c} method used in the DCCT (21). The NGSP laboratory network collaborates with manufacturers of HbA_{1c} assays so that their instruments will report the same HbA_{1c} value as that reported in the DCCT (21). In other words, an HbA_{1c} 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 HbA_{1c} (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 HbA_{1c} measurement in routine patient testing (21).

HOW IS HbA_{1c} REPORTED?

HbA_{1c} has traditionally been reported as a percentage of total Hb. IFCC numbers are lower by 1.5–2.0% HbA_{1c} 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 HbA_{1c} per mol Hb (24). Several countries, predominantly in Europe, have elected to report HbA_{1c} in SI units. An HbA_{1c} 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(IFCC) + 2.152 or IFCC = 10.93(NGSP) − 23.50. These equations allow HbA_{1c} 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 HbA_{1c} 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 HbA_{1c} 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 HbA_{1c}, 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 HbA_{1c} cannot be used, their clinical value is limited until more data become available.

It is important to emphasize that the measurement of HbA_{1c} provides valuable information for the overwhelming majority of diabetic patients. Knowledge of the conditions that alter HbA_{1c} enables the appropriate use of HbA_{1c}, which will remain, for the foreseeable future, essential for the management of patients with diabetes.

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