Role of Glycated Proteins in the Diagnosis and Management of Diabetes: Research Gaps and Future Directions

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Blood oligosaccharides are attached to many proteins after translation, forming glycoproteins. Glycosylation refers to an enzyme-mediated modification that alters protein function, for example, their life span or their interactions with other proteins (1). By contrast, glycation refers to a monosaccharide (usually glucose) attaching nonenzymatically to the amino group of a protein. Glycated hemoglobin is formed by the condensation of glucose with select amino acid residues, commonly lysine, in hemoglobin to form an unstable Schiff base (aldimine, pre-HbA1c) (Fig. 1). The Schiff base may dissociate or may undergo an Amadori rearrangement to form a stable ketoamine.

Glycated hemoglobin, particularly HbA1c, has for decades been widely incorporated into the management (and, more recently, the diagnosis) of patients with diabetes. An important attribute is that glycation occurs continuously over the lifetime of the protein, so the concentration of the glycated protein reflects the average blood glucose value over a period of time. This contrasts with the measurement of blood glucose, which reveals the glucose concentration at the instant blood is sampled and which is acutely altered by multiple factors such as hormones, illness, food ingestion, and exercise (2). While HbA1c is by far the most extensively used—and studied—glycated protein (2–4), other glycated proteins that have been evaluated in clinical studies include fructosamine, glycated albumin, and advanced glycation end products (AGEs).

HEMOGLOBIN A1c

HbA1c is glycated hemoglobin in which glucose is attached to the N-terminal valine residue of each β-chain of hemoglobin A (HbA). Glucose can also be attached at other amino acids, predominantly lysine, in either the α- or β-chain of hemoglobin (5). However, modern methods that measure HbA1c do not report these other glycated hemoglobin species. The extent of hemoglobin glycation is influenced by the concentration of glucose in the blood. Since the life span of erythrocytes is ~120 days, HbA1c reflects the average glucose concentration over the preceding 8–12 weeks (3).

HbA1c has been recommended by the American Diabetes Association since 1988 for routine monitoring of patients with diabetes (6). Although the association of chronic hyperglycemia with the risk of chronic complications of diabetes was suspected for many years, landmark trials such as the Diabetes Control and Complications Trial (DCCT) in type 1 diabetes (7) and the UK Prospective Diabetes Study (UKPDS) in type 2 diabetes (8) and their follow-up studies (9,10) confirmed that lowering mean glucose, as measured by HbA1c, significantly reduced the onset and progression of complications. This led to the development of treatment goals for HbA1c and the use of HbA1c as a performance measure. The increasing use of HbA1c in patient management is evident from the increase in the number of clinical laboratories that are enrolled in proficiency testing surveys conducted by the College of American Pathologists (Fig. 2). Note the large (more than threefold) increase in participants during the 4 years after the publication of the DCCT results in 1993.

HbA1c was recently included as a diagnostic criterion for diabetes by the American Diabetes Association (11), European Association for the Study of Diabetes,
International Diabetes Federation, and World Health Organization (12). This recommendation was motivated by improvements in the measurement of HbA1c and by the certain advantages of its measurement over that of glucose, such as the convenience of not requiring the patient to fast and the reduced intraindividual variability compared with fasting or glucose measurements after loading (11).

HbA1c can be measured by immunoassays, high-performance liquid chromatography (HPLC) (the two most commonly used methods in the U.S. and many other developed countries), affinity chromatography, capillary electrophoresis, and enzymatic assays (13). Standardization of methods by the NGSP (formerly called the National Glycohemoglobin Standardization Program) (14,15) and the International Federation of Clinical Chemistry and Laboratory Medicine (16) has yielded highly consistent HbA1c results for a blood sample, regardless of the method used (provided the method is certified by NGSP).

Interference
There are numerous published reports of conditions that change HbA1c independent of glucose (reviewed in refs. 17 and 18). Based on the nature of the interference, these can be conveniently divided into two groups: conditions that influence interpretation (i.e., change HbA1c concentration in ways unrelated to changes in glucose) and conditions that interfere with HbA1c measurement (i.e., analytic interferences) (Table 1).

Factors That Influence HbA1c Interpretation

Physiological Factors. HbA1c concentrations increase by ~0.1% per decade after 30 years of age (19). It is not known whether this gradual increase reflects an effect of age on the relationship of mean glycemia to HbA1c or merely the higher prevalence of prediabetes and diabetes with aging (a true increase in mean glycemia). There is contention surrounding the influence of race on HbA1c concentrations. Herman (20) posits that African Americans have higher HbA1c for any given level of mean glycemia,
whereas Selvin (21) argues that the increased mean HbA1c is a reflection of truly higher mean glycemia in African Americans.

**Chronic Renal Failure.** Chronic renal failure (CRF) is a common complication of diabetes, and diabetes is the leading cause of end-stage renal disease (22). Red blood cell survival is reduced in CRF, decreasing HbA1c. In addition, many patients with CRF are treated with erythropoietin to stimulate erythropoiesis. The subsequent increase in the number of young erythrocytes further reduces the HbA1c. Therefore the HbA1c concentration in patients with diabetes and with CRF may not accurately indicate glycemic control.

**Iron-Deficiency Anemia.** Iron deficiency and iron-deficiency anemia occur frequently. Some studies, generally with small sample sizes, have reported increased HbA1c in individuals with iron deficiency. Two recent systematic reviews reached opposite conclusions regarding the effects of iron deficiency on HbA1c. The first, a meta-analysis and systematic review, concluded that there was no statistically significant difference in HbA1c measured by HPLC in the presence of iron deficiency or iron-deficiency anemia (23). By contrast, another assessment determined that iron deficiency, with or without anemia, increased HbA1c (24). This discrepancy is likely due to the differences in the studies selected and the method of analysis. Several studies included in both meta-analyses of HbA1c in iron deficiency were limited by their small sample sizes and the heterogeneity of the methods. Two large investigations of the National Health and Nutrition Examination Survey (NHANES) data have been conducted. Kim et al. (25) evaluated 6,666 female NHANES participants without diabetes from 1999 to 2006 and concluded that iron deficiency was associated with an increase in HbA1c from <5.5% to 5.5–6.0%; however, this association was not apparent at higher HbA1c concentrations. A second investigation of NHANES data from 1999 to 2002 included 8,296 patients with and without diabetes and found an adjusted increase in HbA1c from 5.46 to 5.56% in the presence of iron deficiency (26). Thus, while HbA1c seems to increase slightly with iron deficiency, the clinical significance of this finding remains to be determined. We agree with Ford et al. (26) that caution should be exercised in diagnosing prediabetes and diabetes when HbA1c is near the decision threshold in patients with iron deficiency.

**Erythrocyte Life Span.** A change in erythrocyte survival alters HbA1c. For example, assume HbA1c is 7.0% (53 mmol/mol), with a normal erythrocyte life span of 120 days. If the red blood cell life span is 10 days shorter or longer, the corresponding HbA1c values would be 6.4% (46 mmol/mol) and 7.6% (60 mmol/mol), respectively. HbA1c does not accurately reflect average blood glucose concentration if erythrocyte survival is significantly altered, as in, for example, hemolytic anemia or severe β-thalassemia. Since measurement of red blood cell life span is extremely difficult, one cannot easily solve this problem by, for example, applying a correction factor for erythrocyte age.

**Variable Glycation.** Intraindividual variability of HbA1c is very low. Nevertheless, interindividual variation occurs and has been ascribed by some to differences in glycation rates (27,28). This postulate is contentious (29,30) because the data validating significantly different rates of glycation are minimal and no mechanism for differences in this nonenzymatic process has been documented. Moreover, a recent analysis, although indirect, reveals that even the rate of glycation of hemoglobin variants S, C, D, E, J, and G is not significantly different from that of HbA (31), undermining the premise of variable rates of glycation of HbA. There has been speculation that the rate of deglycation (i.e., the removal of glucose from HbA1c) might vary among individuals, resulting in different HbA1c concentrations despite similar average glycemia. Although at least three groups of deglycating enzymes have been identified, only one, fructosamine 3-kinase, is found in humans. Importantly, fructosamine 3-kinase has no effect on valine-1 of the β-chain of hemoglobin (32), the residue where glucose is attached in HbA1c, and it cannot deglycate HbA1c. Thus the concept of variable glycation remains to be validated.

**Factors That Interfere With Measurement**

Numerous publications have described interferences in HbA1c measurement, but many reports had small numbers of subjects and described changes that were small and unlikely to have clinical significance (33–35). Furthermore, improvements in analytic methods have eliminated interferences from some factors (e.g., aspirin, bilirubin, and triglycerides) that affected older methods. While the possible interference of all substances in each modern method has not been rigorously investigated, it is likely that few drugs or other factors interfere significantly in current HbA1c assays.

**Uremia.** Isocyanic acid, derived from urea, is covalently attached to proteins. The nonenzymatic process, termed carbamylation, increases when blood urea concentrations are high, yielding increased carbamylation of circulating proteins, including on lysine or arginine residues of the N-terminus of globin. Carbamylated globin altered HbA1c values in some early methods (36), but uremia has no significant effect on HbA1c analysis with most contemporary methods (23,37,38).

**Hemoglobin Variants.** Over 1,200 hemoglobin variants have been identified; the β gene is involved in ~70% of these (39). While the vast majority are uncommon or rare, certain hemoglobin variants, particularly HbA2, HbAC, HbAD, and HbAE, occur at relatively high frequencies in some populations. One cannot measure HbA1c in individuals who are homozygous for these common variants or who have HbSC disease (36) because they have no HbA. While total glycated hemoglobin can be determined using borate affinity methods in patients with these homozygous hemoglobin variants, there is no convincing clinical evidence that these

| Table 1—Nonglycemic factors that may influence HbA1c |
|------------------------------------------------------|
| Factors that may influence interpretation of HbA1c |
| 1. Physiological (e.g., age, race) |
| 2. Chronic renal failure |
| 3. Iron-deficiency anemia |
| 4. Erythrocyte life span |
| 5. Glycation “phenotypes” |
| 6. Drugs (e.g., dapsone, antiretroviral) |
| 7. Other (e.g., vitamin C, vitamin E) |

Factors that may interfere with HbA1c measurement

1. Uremia
2. Hemoglobin variants
3. Drugs (e.g., opiates)
4. Other (e.g., bilirubin, triglyceride, alcohol)

**Factors that may influence interpretation of HbA1c**

- Physiological (e.g., age, race)
- Chronic renal failure
- Iron-deficiency anemia
- Erythrocyte life span
- Glycation “phenotypes”
- Drugs (e.g., dapsone, antiretroviral)
- Other (e.g., vitamin C, vitamin E)
values can reliably be used to monitor
glycemia and predict complications, par-
ticularly since some patients may have
reduced erythrocyte life span because of
hemolytic anemia. Most interferences
are method-specific (36). Manufacturers
of HbA1c methods have considerably re-
duced analytic interference from variant
hemoglobin. Therefore HbA1c can be
measured accurately in the presence of
the overwhelming majority of variant
hemoglobins, provided a suitable assay
is used (40). Since common heterozy-
gous variants rarely alter erythrocyte
life span, accurate and reliable HbA1c
values can be obtained in heterozygous
individuals.

GLYCATED SERUM PROTEINS
Glucose attaches nonenzymatically to
amino groups of proteins other than he-
moglobin to form ketoamines (Fig. 1).
Measures of several glycated serum pro-
teins, including fructosamine and gly-
cated albumin, have been proposed as
markers of glycemia that might comple-
ment or replace HbA1c in select patient
populations. Serum proteins turn over
more rapidly than erythrocytes; for ex-
ample, albumin (the protein found in
the highest concentration in serum)
has a circulating half-life of about 14–
20 days. Therefore the concentration of
fructosamine or glycated serum albu-
min reflects mean glucose over a period
of 2–3 weeks. Additionally, glycated se-
rum proteins are not influenced by
changes in erythrocyte life span or he-
moglobin variants such as homozygous
HbS. Glycated serum proteins have
therefore been proposed as measures
of more rapid changes in glycemia and
to monitor glycemic control in patients
with conditions that alter the normal
relationship of HbA1c to mean glucose
(e.g., hemolysis, blood transfusion).

FRUCTOSAMINE
Fructosamine is the common name for
1-amino-1-deoxy fructose and the generic
name for plasma protein ketoamines
(41,42). All glycated serum proteins are
fructosamines, and since albumin is the
most abundant serum protein, measure-
ment of fructosamine is thought to largely
reflect the concentration of glycated albu-
min, though this has been questioned (43).
The fructosamine assay is readily auto-
ated and is less expensive than measure-
ment of HbA1c. There is disagreement as
to whether fructosamine results are in-
dependent of serum protein concentra-
tions (absent significant alterations in
the latter) or whether fructosamine values
need to be corrected for the concentra-
tion of serum proteins (44). Most agree,
evertheless, that fructosamine is not valid when
serum albumin is <30 g/L.

The first commercial method to mea-
sure fructosamine suffered from several
problems, particularly a lack of specifi-
city and interference by other reducing
substances in the serum, such as urates
(43,45). Thus many early studies of fruc-
tosamine generated confusion regarding
its clinical value, with reviews (covering
many of the same studies) leading to
conflicting conclusions as to whether
fructosamine is a reliable test for rou-
tine clinical use (41,46). The assay was
extensively modified in 1991, which
markedly improved the specificity of
fructosamine (47). Strong correlations
with HbA1c, prognostic value for the de-
velopment of diabetes and microvascular
complications, and good precision have
been demonstrated for fructosamine
using modern assays on automated
platforms (48,49).

There is interest in the role of
fructosamine in special populations for
whom HbA1c may not provide an accu-
rate assessment of glycemic status. One
such potential use of fructosamine is the
diagnosis of gestational diabetes mellitus
(GDM). Hyperglycemia develops relatively
quickly with the onset of GDM, and red
cell turnover may be altered in pregnancy,
precluding the use of HbA1c to diagnose
this form of diabetes. Studies evaluating
this use of fructosamine (50) were gener-
ally small and used various fructosamine
thresholds and diagnostic criteria for
GDM. Measurement of fructosamine is
not currently recommended to screen
for GDM (50).

Other conditions for which fructosamine
has shown a potential role in monitor-
ing glycemic status include end-stage
renal disease, certain types of anemia,
and transfusion (49). Combining HbA1c
with fructosamine has been used as a
screening strategy to identify patients
with prediabetes; however, the combi-
nation was not statistically significantly
better than the use of HbA1c alone (51).
A major limitation of the fructosamine
assay is the lack of an evidence base
linking the test to long-term complica-
tions of diabetes. Hence, unlike HbA1c,
there are no generally accepted treat-
ment targets for fructosamine.

GLYCATED ALBUMIN
Albumin comprises almost two-thirds
of total serum protein and accounts for over
80% of total glycated serum proteins (52).
HPLC tandem mass spectrometry of
human plasma using [13C6]glucose la-
beling has identified 35 glycation sites
on albumin (53). Analogous to HbA1c,
which is most commonly reported as a
percentage of total hemoglobin, gly-
cated albumin is usually expressed as
a percentage of total albumin in the
blood. A number of glycated albumin
assays are commercially available, but
these lack standardization and values
vary widely among methods (54). Spe-
cifically, the reference intervals have
considerable variation depending on
the method and range from 0.8–1.4% to
18–22% (52,54). A U.S. Food and
Drug Administration–approved method
for glycated albumin measurement
manufactured by Diazyme Laboratories
(Poway, CA) is commercially available
(55). A glycated albumin assay developed
by Asahi Kasei in Japan (56) is the method
most widely used globally and most ex-
tensively evaluated in clinical studies.

Values of glycated albumin in blacks
are significantly higher than in whites,
for reasons that are unclear (54). Factors
that influence albumin metabolism may
alter glycated albumin independent of
glycemia. These factors include the
nephrotic syndrome, cirrhosis, thyroid dis-
eease, hyperuricemia, hypertriglyceride-
emia, and smoking (57). As with fructosamine,
glycated albumin concentrations can be
affected by altered protein levels that
occur with liver, thyroid, and renal dis-
eease (58). The clinical use of glycated
albumin is limited by the same caveats
that apply to fructosamine—namely, a
paucity of evidence relating it to clinical
outcomes, specifically the chronic com-
plications of diabetes. As is the case with
fructosamine, further studies are required
to determine its clinical utility in the man-
agement of diabetes (48,59).

A recent investigation by Sumner et al.
(51) identified a potential role for gly-
cated albumin in the diagnosis of pre-
diabetes in African immigrants to the
U.S. Using the oral glucose tolerance
test as the gold standard, the combina-
tion of HbA1c with glycated albumin de-
tected 78% of African immigrants with
pre-diabetes, compared with only 50% detected with HbA1c alone and 72% with HbA1c paired with fructosamine (51). An investigation of 302 adults in Japan found that HbA1c or glycated albumin could diagnose patients at risk for developing diabetes; fructosamine was considered unsuitable as a screening test (60). Glycated albumin is used extensively as a screening test for diabetes among blood donors in Japan, identifying patients who are at risk for the disease (56).

Intriguing data are emerging suggesting that glycated albumin may be a better test than HbA1c for diabetes screening in nonobese patients. Koga et al. (61) found a negative correlation between glycated albumin and BMI in Japanese individuals; this finding has been replicated in other Asian populations (62,63). Similarly, in the study by Sumner et al. (51), the African immigrants whose pre-diabetes was identified by glycated albumin, but not HbA1c, were more likely to have a lower BMI. The converse implication of these data is the potential for glycated albumin to underestimate glycemic status in the obese.

AGEs

Glycation of tissue proteins may contribute to the link between hyperglycemia and the chronic complications of diabetes. Nonenzymatic attachment of glucose to proteins, lipids, or nucleic acids produces stable Amadori products, which can undergo further modifications to form AGEs (64,65). Irreversible rearrangements of Amadori products occur via both oxidative and nonoxidative pathways, or via condensation of the side chains of lysine, arginine, or cysteine, forming reactive dicarbonyl compounds such as glyoxal, methylglyoxal, and deoxyglucosones that ultimately form irreversible AGEs by forming cross-links between many proteins, altering their structure and function (65). For example, glyoxal can form N-(carboxymethyl)lysine (CML), glyoxal-derived lysyl dimer, or N-(carboxymethyl)arginine, whereas methylglyoxal may induce the formation of methylglyoxal-derived lysyl dimer, argpyrimidine, N-(carboxyethyl)lysine (CEL), and others (65). The most common cross-linked AGE is glycosylated, formed by a mechanism of action that has not yet been fully elucidated (65). More than 20 AGEs have been identified (66,67). These products do not return to normal, even when hyperglycemia is corrected, so they accumulate continuously over the life span of the protein; AGEs also accumulate as an individual ages. Hyperglycemia accelerates the formation of protein-bound AGEs, and patients with diabetes have more AGEs than age-matched subjects without diabetes. There is evidence that AGEs in the diet contribute to AGE accumulation in tissues (68).

Through their heterogeneous effects on the functions of proteins and extracellular matrix, AGEs may contribute to the chronic microvascular and cardiovascular complications of diabetes (69,70). Plasma concentrations of CEL, CML, and pentosidine were correlated with incident, but not prior, cardiovascular outcomes in patients with type 2 diabetes (71,72). AGEs have also been linked to other diabetic complications including nephropathy, retinopathy, and neuropathy (73–78). There is significant heterogeneity among these studies in the specific AGEs evaluated and the method of AGE measurement. Of potential interest, certain publications reported no correlation between serum AGE concentrations and HbA1c (71–73). Levels of AGEs in the skin biopsies of patients from the DCCT were found to be a better predictor of retinopathy and nephropathy progression than HbA1c (74,77). Collectively, these results raise the possibility that AGEs may provide additional independent information to predict microvascular diabetic complications. Thus AGE burden may explain why only a subset of patients with poor glycemic control develop complications and why some patients with good glycemic control also develop certain diabetic complications.

Several methods have been proposed to measure AGEs. Some AGE products fluoresce, which has led to the development of noninvasive measurement of skin autofluorescence to estimate the burden of AGEs in tissues. A meta-analysis of seven studies showed that skin autofluorescence was positively associated with mortality, neuropathy, nephropathy, and cardiovascular events (79). Certain studies found that skin autofluorescence predicted microvascular and macrovascular complications of diabetes independent of HbA1c (80,81), whereas others found that adjustment for HbA1c rendered these associations nonsignificant (82). These discrepant findings are possibly accounted for by differences in the patient population and statistical methods. The utility of skin autofluorescence measurements is limited by several factors. First, most AGEs are not fluorescent, specifically CML and CEL, which have been shown to be important in predicting cardiovascular outcomes (67). Second, skin fluorescence is not specific; numerous skin proteins fluoresce with spectra that overlap the spectra of AGEs (83). Furthermore, skin autofluorescence does not correlate directly with AGE burden.

There is considerable interest in the measurement of AGEs in the circulation as a biomarker to monitor the risk of diabetes complications, given the numerous studies correlating AGEs with various diabetic complications (66). Assays to determine total AGE fluorescence have been used in selected studies, but these methods have limitations similar to those of skin autofluorescence, namely, the most important AGEs are not fluorescent and many other serum proteins interfere. Methods for measuring specific AGEs have been developed, many of which use immunoads. However, heterogeneity of the structures (ranging from single molecules to complex cross-linked compounds) and composition of AGEs have resulted in assay variability. Questions have been raised regarding antibody specificity (AGEs such as CML and CEL share certain common epitopes), the use of excess blocking proteins that have oxidized and glycated fragments, and the high temperature and pH of the assay (67). Furthermore, the lack of immunoassay standardization has yielded variable results (84).

Isotope dilution analysis liquid chromatography–tandem mass spectrometry (LC-MSMS), with careful preanalytic sample preparation, is a promising technique for circumventing the problems of immunoassays and fluorescence-based methods (67). Analytes are first separated by HPLC from related compounds that have not been oxidized or glycated; then they are detected based on a specific chromatographic retention time, molecular ion mass-to-charge ratio, and fragment ion mass-to-charge ratio, rendering this technique highly specific for the desired analyte. LC-MSMS quantitatively analyzes the modification of proteins by glycation, nitration, and oxidation,
as well as free adducts, using a small sample volume. This technique has been applied to a number of clinically important AGEs including pentosidine, CML, CEL, 3-deoxymannose, and methylglyoxal hydroimidazolones, and it has aided in the discovery of new candidate AGE products (67,85,86). A limitation of LC-MSMS is the need for specialized (and expensive) equipment and highly trained personnel. Furthermore, isotope-labeled standards are not commercially available for the full range of analytes (67,86), preventing assay standardization.

Certain AGEs activate the receptor for AGE (RAGE), inducing intracellular signaling that results in the production of proinflammatory cytokines and increased oxidative stress (66,69). RAGE is expressed on the surface of several cells, including endothelial and renal cells, raising intriguing hypotheses about the role of RAGE in the pathophysiology of specific diabetes complications. Nevertheless, some studies cast doubt on whether AGE-modified proteins activate RAGE (87). Proteolysis of RAGE leads to a truncated soluble form of RAGE (sRAGE) (66), which is found in serum and can be measured by a commercially available ELISA. There is evidence of clinical value of sRAGE. In a case-cohort study of 3,763 patients with type 2 diabetes, both AGE and sRAGE plasma values predicted decreasing renal function and all-cause mortality, but hazard ratios were only 1.1 to 1.2 (88). There is, however, controversy over the associations between sRAGE concentrations and diabetes complications; some studies show a positive association (89) and others an inverse one (90). The associations between sRAGE and health outcomes remain unresolved. Differences in studied populations and genetic mechanisms have been suggested as a cause of the discrepancies (91,92).

Inhibitors of AGE formation, such as aminoguanidine, prevented signs of microvascular complications of diabetes in animal models, although initial clinical trials in humans failed to show a significant benefit (66). Nonetheless, anti-AGE therapy remains an area of active research. Of interest, patients with type 2 diabetes taking metformin had lower AGE levels than those not receiving metformin (93). Promising studies of the use of recombinant sRAGE in animals suggest the potential of future therapies in humans to reduce the risk of diabetes complications. The recent total synthesis of the lysyl-arginine cross-link glucosamine (94), the main in vivo cross-link in AGEs (95), is likely to permit the generation of relevant reagents (e.g., specific antibodies) to enhance our comprehension of the role of AGEs in disease.

**FUTURE DIRECTIONS**

The development and standardization of HbA1c measurement have revolutionized research and clinical care in the field of diabetes (2–4). The role of HbA1c in diabetes has been extensively studied in large, prospective trials with long-term follow-up (9,10), which has extensively validated the value of HbA1c in predicting many diabetic complications. Additionally, diagnostic thresholds for using HbA1c to diagnose prediabetes and diabetes have been established (11,96). Yet despite the documented utility of HbA1c in diabetes research and care, controversies remain. As argued from opposing perspectives by Herman (20) and Selvin (21) in this issue, whether there are clinically significant differences in the relationship between HbA1c and average glucose in different racial groups remains contested, and similar questions exist about age groups. If there are differences in what HbA1c “means” in different groups, what are the implications for the diagnosis and management of diabetes? Considerable progress has been made in reducing interference from hemoglobin variants and other factors in HbA1c assays and in achieving high levels of standardization of the assay in developed countries. We need to continue to overcome the barriers to worldwide standardization of HbA1c assays, particularly in developing countries.

Since the discovery of HbA1c, other potentially useful additional or adjunct measures of protein glycation, glycated serum proteins, and AGEs have emerged. It is unlikely, however, that the newer measures of glycated proteins will be studied as markers of diabetic complications in the same thorough manner as HbA1c because of limited funding for long-term clinical trials with large numbers of patients. We need to develop innovative strategies to establish the evidence base for the link between other glycated proteins and clinical outcomes, so that treatment targets or diagnostic thresholds can be developed. Furthermore, glycosylated albumin and AGE assays need to undergo standardization, as has been done for HbA1c, to enable comparison among studies and decrease imprecision (15).

AGEs have the potential to identify—indepedent of HbA1c—a subset of patients who develop cardiovascular and microvascular complications of diabetes. It is important to determine whether AGEs are a cause or consequence of the pathophysiology of diabetes. Because the term comprises a large group of divergent compounds, future studies of AGEs will require detailed knowledge of the specific compound(s) being studied. Although AGEs may go beyond simple biomarkers into pathophysiology, substantial research needs to be done to use AGE-related measures to improve the prediction of risk for diabetes complications or to ultimately develop risk-reduction therapies based on these pathways.

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