When HbA1c and Blood Glucose Do Not Match: How Much Is Determined by Race, by Genetics, by Differences in Mean Red Blood Cell Age?

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Almost 20 years ago, one of us (R.M.C.) became intrigued by patients (both his and those reported by many others) whose hemoglobin A1c (HbA1c) and blood sugar levels did not line up. Relevant questions included the underlying mechanisms and the implications for clinical decision making. The emphasis was not on those subjects with discrepancies due to hemoglobinopathy but rather on those with normal hematology whose HbA1c and glucose levels missed each other by just enough to not make sense. This was before continuous glucose monitoring was available and memory glucose meters had become the norm. When asking fellow clinicians about their experience with this clinical problem, there were generally two types of response:

1. It seems like I see at least one person each week for whom there is an issue, but I don't really have time to think about it.
2. I thought it came from patients not recording their finger-stick glucose levels accurately or from the great variability among the different HbA1c assays I have to use.

But few caregivers (us included) were aware of a study published in 1990 (1) that demonstrated use of laboratory glucose measurements and a single HbA1c assay in people whose HbA1c level was either high or low, respectively, compared with their glucose tolerance and remained persistently high or persistently low with repetition over time. Translation: The discordance between measured HbA1c and blood glucose levels in a substantial fraction of people is a reproducible biologic characteristic of these individuals and is not due to technical problems. If that is the case, we need to examine our assumption that these two measures are equal and consider under what circumstances this may cause clinical errors.

It is helpful to consider the determinants of HbA1c. In physiologic terms, it depends on the level of Hb glycation at a defined "zero time," the integrated average glucose concentration exposure of the Hb molecule, the glycation rate constant, the duration of the exposure of Hb to glucose for this nonenzymatic reaction, and other minor contributions such as the loss of Hb from red blood cells (RBCs) and the potential reversal of the glycation reaction. Historically, we were taught that "the RBC life span" is the issue, but it really is "the mean RBC age" (M_{RBC}) (2) that most directly determines HbA1c level in a routine blood sample containing cell ages ranging from youngest to oldest. The key to scientifically addressing nonglycemic variation in HbA1c levels was having RBC-independent measures of glycemic control to compare with HbA1c values. We and our colleagues addressed this issue using glycated serum proteins as a measure and demonstrated that although there was a strong linear correlation between HbA1c and fructosamine levels,

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Abbreviations: AG, average glucose; CGM, continuous glucose monitoring; HbA1c, hemoglobin A1c; M_{RBC}, mean red blood cell age; RBC, red blood cell.

there was also substantial dispersion around the population regression line (3). Furthermore, when serial paired HbA1c and fructosamine measurements were performed in patients who happened to have wide variations in their control, the within-subject relationship became essentially a perfect correlation. Translation: This provided additional evidence that there was a tight physiologic relationship between the two glycemic measures that was stable over time within individuals, even if it deviated from the average population relationship. That supports the notion that the dispersion or "noise" reducing the correlation in population studies is not exclusively due to technical assay issues but rather to real biologic differences ***between people***.

In the early 2000s, racial differences in HbA1c values were recognized across the spectrum, from prediabetes through advanced, poorly controlled diabetes. Eventually, variables apart from glucose were whittled away by epidemiologic approaches, and a debate arose about the importance of racial differences (4–8). The importance of the debate is whether subgroups of people either are not adequately treated or are actually harmed when criteria for the threshold for diabetes diagnosis or the targets for glycemic control related to prevention of complications, both chronic microvascular conditions and hypoglycemia, could be personalized but are not. Rhee *et al.* (9) demonstrated in a population of U.S. veterans that those whose HbA1c value was highest relative to blood glucose level had a 56% higher frequency of emergency department visits for hypoglycemia than those whose HbA1c value was either proportionate or lowest for blood glucose level. Black subjects were disproportionately represented in the group with highest A1c value for glucose level and higher hypoglycemia frequency, although both blacks and whites were affected (9).

Hivert *et al.* (10) report a study on the association of genetic factors with racial difference in HbA1c. The authors examined genetic markers associated with hemoglobinopathies, HbA1c itself, and African vs European ancestry. The strongest association with racial difference in HbA1c values was observed with ancestry markers, there being weaker associations with sickle cell trait and with genetic variants for HbA1c itself. Sickle cell trait was associated with higher HbA1c value, in contrast to the opposite association recently reported (11). The difference between the two results is attributed to assay differences; if that proves true, the significance of the finding would tend to be diminished. The primary finding of an association with broad, nonspecific measures of ancestry strengthens the evidence that the differences are real. The strengths of the study include the examination of a large cohort at a fairly homogeneous stage of disease and the presence of impaired glucose tolerance, with a remarkable set of genetic markers.

Limitations of the study include the limited glucose and hematologic phenotyping of the subjects. For all that we learn from this study, and it is substantial, it does not address mechanism or physiology in sufficient detail to progress to clinically useful next steps. If the problem of variation in the HbA1c–blood glucose relationship were better understood at a physiologic level and if we could apply a tool to measure the underlying physiologic difference in a clinically practical manner, we could open the door for taking a correction factor into account. Lessons learned from evaluating renal function with simple serum creatinine values to progressively more refined estimates of glomerular filtration rate could be applied in diabetes, with the potential to further reduce hypoglycemia and chronic complications of the disease. We are making clinical decisions relying upon smaller and smaller differences in HbA1c value than those who pioneered the use of HbA1c in diabetes care likely ever envisioned. That demands greater precision in the relationship than was established when HbA1c was first studied and adopted.

Of course, now continuous glucose monitoring (CGM) has become progressively more available and reliable, allowing a more direct integrated measure of glycemic control, albeit with all the technical limitations we have come to associate with this technique. CGM, memory glucose meters, and new standardization of HbA1c assays came together in the A1c-Derived Average Glucose study, which established that a linear approximation of the HbA1c–average glucose (AG) relationship across the range of elevated glucose levels (12) provides a strong and useful correlation (~0.8) for many people with diabetes. Nevertheless, the data from that study confirmed that a dispersion remained in the HbA1c-AG relationship and that this linear approximation would be unsatisfactory for some. As an example, at an AG of 150 mg/dL, the HbA1c value could range from 5.5% to 7.5%; conversely, at HbA1c 6.5%, which was subsequently labeled the threshold for diagnosis of diabetes, AG could range from 115 to 165 mg/dL. It was fair to argue that this relationship was valid for the ***majority of people with diabetes.*** But when we are talking about more than 30 million people in the United States and nearly a half billion people worldwide, accounting for only a majority means a lot of people could still be affected (13). More recently, Bergenstal *et al.* (14) demonstrated a racial difference in the HbA1c-AG relationship. Their data demonstrated not only statistically different regression lines between black persons and white persons but also substantial overlap of the individuals within each group as well as dispersion about both regression lines.

In the intervening years, we have come to know more about the physiology of the relationship between HbA1c and glucose levels. Use of a biotin *ex vivo* labeling technique with reinfusion of labeled RBCs has

demonstrated (i) that variations in M_{RBC}, either with or without diabetes, are wide enough to cause clinically important differences in HbA1c value at a given level of glycemic control and (ii) that at steady-state glycemic control, HbA1c synthesis *in vivo* in these people is linear with time, with a shared near zero *y*-intercept (15). A more recent modernization of a technique for measuring RBC survival/M_{RBC} with an orally administered stable isotope label incorporated during heme synthesis (16) would allow multicenter population studies that are not practical with the biotin technique.

Taken together, these studies allow us to state that a given percentage change in M_{RBC} should result in the same percentage change in HbA1c value. If we consider a factor that changes HbA1c value from 6% to 6.6% as clinically important, that means that a 10% difference in M_{RBC} between two people at the same average blood glucose level is clinically important. Put another way, a difference in M_{RBC} results in an analogous change in the slope of the HbA1c-AG relationship. Data from the A1c-Derived Average Glucose Study and racial differences studies warrant reconsideration in the context of this information on the distribution of M_{RBC} and the HbA1c synthesis pattern in hematologically normal people with and without diabetes. Adjusting the HbA1c-AG linear relationship from each of these studies to reflect the published 95% CIs for M_{RBC} includes 95% and 89%, respectively, of all points in the populations represented in references (12) and (14). In contrast, the black and white population regression lines are quite close together, and there is substantial representation of data points from each racial group on each side of both regression lines in the racial differences study. Translation: (i) There is a very real difference on average between blacks and whites in HbA1c values, which, when accounted for over a large population, could represent real differences in when diabetes is diagnosed and in outcomes, such as hypoglycemia and diabetic vascular complications. (ii) However, there is much greater variation in the HbA1c-AG relationship in the population at large without regard to race than there is on average between the races with the widest variations, which is therefore likely to have an even greater effect. Race would be one surrogate marker; however, if we could measure M_{RBC} routinely and modernize HbA1c by normalizing for M_{RBC} analogous to the calculation of estimated glomerular filtration rate while taking account of other factors, would that in fact refine the personalization of diabetes management in a clinically valuable way?

This progress provided an opportunity to think more precisely about the effect that M_{RBC} variation has on the relationship between HbA1c and AG, but we did not have sufficiently precise means to apply this knowledge to the routine clinical care of people with diabetes. More recently, Higgins and collaborators developed two models of systems biology, one based on detailed information available from certain complete blood count analyzers (which provide the single-cell distribution of cell volume and cell hemoglobin in mature RBCs and in reticulocytes) (17) and the other calculated from the relationship of a period of continuous glucose monitoring and an individual HbA1c determination (18), which offer the prospect of estimating M_{RBC} from tools now widely available in the United States and other developed countries. With CGM-HbA1c modeling for estimating M_{RBC}, the mathematical derivation confirms that changes in M_{RBC} would be expected to alter the slope of the HbA1c-AG relationship. When the assumption is made that M_{RBC} variation accounts for essentially all of the common non–hemoglobinopathy-mediated mismatches in people with normal hematology, one set of HbA1c-AG data can be analyzed to estimate M_{RBC}, which can then be used to test whether it can correct future HbA1c value to improve its fit with the corresponding CGM data. If adjusting HbA1c for M_{RBC} could make a difference in clinical outcomes, such as reducing the frequency of hypoglycemia and chronic diabetic complications and possibly better guiding the management of type 2 diabetes mellitus to reduce treatment-associated weight gain, it is potentially within our grasp upon adequate validation of these tools. The challenge at this time is to validate these models against gold standard methods for M_{RBC} determination, such as the stable isotope or biotin methods, to test whether the assumptions are indeed valid and then conduct the necessary trials to answer whether this approach actually improves clinical outcomes.

What are the take-home messages? (i) Recognizing and addressing racial differences in the HbA1c-AG relationship may have practical importance for minimizing risks of inappropriate overtreatment or undertreatment of diabetes, with implications for preventing hypoglycemia and weight gain on the one hand and vascular complications on the other. (ii) Identifying genes underlying these differences adds credence that the difference is real and that genetic strategies may be useful for modifying clinical decisions in the future to accomplish those goals. A genetic approach could be one way to adjust for mismatches in clinical decision making. Its limitation is that it does not account for one of the commonest situations we confront, changes in the HbA1c-AG relationship associated with RBC changes in moderate and advanced chronic kidney disease. (iii) Variations in the HbA1c-AG relationship are a larger problem within races than between races, representing even larger magnitudes of risks, which we have alluded to in this commentary.

A "next-generation" test to modify interpretation of HbA1c value with normalization for measures of M_{RBC} is

within our grasp, and it is time to devote the resources and ask the necessary questions to see if this will improve the care of diabetes.

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