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Do Red Blood Cell Indices Explain Racial Differences in the Relationship between Hemoglobin A1c and Blood Glucose?

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For a clinical test that has been around as long as hemoglobin A1c (HbA1c) and that started with a seemingly straightforward and simple story, there sure have been a series of controversies, many of which are still being debated. To most health care providers practicing today, however, it is essentially noncontroversial dogma that Hb is glycated on specific residues over the "120-day" lifespan of the red blood cell (RBC) and that HbA1c reflects average glycemic control equally well in everyone. Accordingly, you read the result, make a clinical decision, and get on with it, right?

Of course, we have all been taught that there are some conditions in which this is not true, namely, hemoglobinopathies that interfere in certain kinds of assays for HbA1c, and hemolytic diseases, such as sickle cell anemia and hereditary spherocytosis, in which the lifespan of the RBC is shortened. So, what is the big deal with the paper by Hamdan et al[1] in this volume of *The Journal*? Why should the practitioner care? "The big deal" is the increasing recognition of situations in which the ostensibly simple story of HbA1c is not as straightforward as we once thought and we really do not know how often this leads to flawed clinical decisions. We will focus on mechanisms first, because they lead to understanding

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how a seemingly small alteration can significantly interfere with the assumptions underlying HbA1c interpretation.

We often are asked, “How much change in RBC lifespan is required to cause a difference in HbA1c that would alter a clinical decision?” In 1976, one healthy person was described in whom the fraction of glycated Hb increased continuously in vivo throughout the RBC lifespan.[2] More recently, we used the biotin RBC label to demonstrate more precisely that the in vivo formation of HbA1c in people is linear with time at stable glycemic control,[3] which leads to the conclusion that a change in RBC lifespan leads to a proportional change in HbA1c at constant blood glucose. For example, an 8% reduction in average RBC age causes an 8% decrease in HbA1c. In this case, a diabetes-diagnosing HbA1c level of 6.5% would instead be 6.0% (despite abnormal glucose tolerance), causing a missed diagnosis of diabetes. Moreover, our research group has demonstrated by 2 independent methods for measuring RBC lifespan in humans, ex vivo biotin labeling and in vivo stable isotope labeling, that there is a much wider normal range for RBC lifespan than previously appreciated: approximately ± 20 days (2 SD) around a mean of about 120 days.[3,4] This finding indicates that “one size does not fit all,” even for the normal population.

The magnitude of difference in mean RBC age (M_{RBC}) is sufficient to explain much of the variability observed in the HbA1c-mean blood glucose (MBG) relationship in hematologically normal people.[3–5] This could potentially lead to errors in diabetes diagnosis and decision-making. We refer here to “mean RBC age (M_{RBC})” because that is the time characteristic that most directly determines the measured level of Hb glycation, as opposed to the more commonly cited RBC lifespan. Consider 3 healthy people each with identical glucose tolerance, each with M_{RBC} in the normal range, but one 2 SDs below the mean, one 2 SDs above the mean, and one just at the mean. The range of their HbA1c values at the 2 extremes could be ~15% different from the prototypical, middle-of-normal person. Accordingly, if the middle one is at 6.5%, the 2 others could be at 5.5% and 7.5% at equal glucose tolerance.

What are some of the specific implications of these findings that are relevant to practitioners? We know from multiple studies that a HbA1c level of ≥6.5%, the threshold that has been adopted for the diagnosis of diabetes in adults, has a specificity very near 99%, whereas the sensitivity is only in the 40%–70% range.[6,7] This means that among people with glucose tolerance test results that meet criteria for diabetes, 30%–60% will have an HbA1c less <6.5%. Consequently, diabetes would be missed if HbA1c is used alone for diagnosis without some probabilistic model for the likelihood of diabetes.

What could be the reason(s) that these individuals with abnormal glucose tolerance test results have an HbA1c <6.5%? In addition to a shorter RBC lifespan within the much broader range of normal than was once thought (the focus of our work) there could also be (1) variation in the rate of glycation for a given mean blood glucose; (2) mismatches between glucose tolerance and MBG (eg, due to differences in the variation of blood glucose levels throughout the day); or (3) measurement error (assay bias or variability). If there is a difference in M_{RBC} between groups, then the resulting difference in HbA1c would reflect a mismatch between the measurement of HbA1c and blood glucose. If so, one would predict

that the likelihood of diabetes-related complications would correlate more strongly with measurements of blood glucose than HbA1c.

If, on the other hand, there is a difference in the rate of glycation between groups, it is not so clear whether complications would correlate more strongly with blood glucose or HbA1c. One possibility is that HbA1c could be an index of glycation of proteins in the body overall, and these glycated proteins at other sites may be directly involved in the pathogenesis of diabetic complications. If so, the risk of complications risk might correlate more strongly with HbA1c than with a direct measurement of MBG.

In the context of the effort to establish HbA1c as a diagnostic criterion for diabetes in the past decade, the racial mismatches between MBG and HbA1c have received much attention. Within the limits of the ability to define a person's race, one of the largest racial differences in HbA1c occurs between black and white people at equal glucose tolerance. The results reported by Hamdan et al[1] in this volume of *The Journal* confirm their previous results and those of others[8–10] that HbA1c is greater in black individuals by 8%–10% on average than in white individuals, even with equivalent glycemic control. This difference in HbA1c between races matters because it could lead to misdiagnosis and over-or undertreatment of diabetes and prediabetes.

Are there differences in complication rates between the races at equal glucose tolerance? Some say if anything the threshold for retinopathy is lower in blacks than whites, arguing against a greater threshold for diabetes diagnosis in blacks.[11] Others have challenged whether those studies had sufficient power to exclude an effect of the proposed magnitude. If the relationships between target HbA1c on the one hand and the balance between chronic complications vs hypoglycemia on the other differs by race, then prevention of either could be affected if the disparity is not taken into account. Regardless of mechanism, the racial difference in HbA1c-MBG relationship adds to the complexity in the discussion of public health policy related to racial disparities in diabetes, obesity, and cardiovascular health outcomes.[12,13] Is the greater HbA1c observed between black and white individuals due to disparities in access to care, the biologic differences we have discussed, or some combination of the two?

Where do RBC indices fit into all of this, and why did Hamdan et al[1] choose to study their role in the racial disparity in HbA1c in children with type 1 diabetes? Empiric correlations between RBC indices and mortality, cardiovascular disease, and HbA1c, among a variety of seemingly unrelated diseases and measures, have been published in the last few years.[14–16] These have largely been empiric correlations with post hoc speculation about mechanisms by which the disease of interest could affect RBC development and produce disease-associated changes in RBC indices. The most direct reason to invoke a role for RBC indices with the specific question of HbA1c differences between black and white subjects is that there are reductions in cell size and in cell hemoglobin content during normal RBC aging with a strong relationship within individuals between HbA1c and cell volume.[17] As previously mentioned, the data of Hamdan et al[1] demonstrate about a 10% difference in HbA1c for equivalent MBG. This certainly provides a basis for hypothesizing that the finding could be due to a 10% greater RBC mean age in the black population. Their data

showing differences in RBC indices between the black and white groups, in the context of the framework presented here, is another piece of evidence predicting a corresponding difference in RBC lifespan between the groups. That hypothesis, however, remains to be tested with modern sensitive techniques.

We are also challenged, surprisingly, by a continued deficit in our knowledge about the regulation of the senescence of RBCs.[18,19] The fact that there is a fairly consistent 10% difference over the entire MBG range rather than a fixed difference, however, has mechanistic implications. This argues against a mechanism that is dependent on blood glucose level which would be the contention of those who argue for a mismatch between glucose tolerance and MBG as the basis for racial differences in the HbA1c-glucose tolerance relationship. The black group had a lower MCV and greater red cell distribution width coefficient of variation (RDWCV). This could be due to a greater incidence of thalassemia trait, iron deficiency, or both in this group. These conditions, however, are in fact associated with lower rather than higher RBC lifespan within the limits of techniques of the time.[19–22] The group of Higgins et al[23] has developed mathematical models taking advantage of the capability of newer blood cell analyzers to capture the distribution of sizes and hemoglobin contents of individual RBCs and reticulocytes to generate predictions of RBC age distribution. The Higgins model is built on the speculative assumption that there is a homeostatic mechanism related to RBC senescence that would predict lengthened M_{RBC} with the lower MCV and increased RDWCV observed in the black population. It still remains, however, for the Higgins model to be validated against direct measurement of M_{RBC} or RBC lifespan.

It may be plausible that RBC indices will provide a means to explain racial differences in HbA1c. But the way to approach this is to “bite the bullet” and ask the right scientific questions: are there differences in M_{RBC} between black and white populations, and, if so, what could be the underlying mechanisms? Once that is done, the modeling can be done to ascertain whether RBC indices provide a practical means to correct HbA1c for differences in the MBG-HbA1c relationship. The study by Hamdan et al[1] is a good attempt to describe the racial differences in RBC indices between these populations. In the end, they say RDWCV is statistically different in a model predicting HbA1c in conjunction with race, MBG and chronologic age. It remains to be seen whether the inability to detect further relationships between red cell indices and racial differences in HbA1c, however, result from a true lack of a relationship, a lack of sufficient power, or use of the incorrect mathematical model for testing the question.

In summary, HbA1c depends on both MBG and on M_{RBC}. Importantly, the distribution of M_{RBC} in the general population is wider than has been recognized generally, and we don’t understand this distribution in sufficient detail to know how often it contributes to errors in the interpretation of HbA1c for diabetes diagnosis, diabetes management, and cardiovascular risk assessment. Therefore, is this not such a simple story after all, or are there just a few wrinkles that need to be ironed out? Either way, this is an important question with huge implications for biology, clinical care, regulation of drugs, evaluation of health care providers’ performance, and public policy. We need to measure RBC age directly to begin to address these issues.

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Glossary

HbA1c	Hemoglobin A1c
MBG	Mean blood glucose
M_{RBC}	Mean RBC age
RBC	Red blood cell
RDWCV	Red cell distribution width coefficient of variation

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