

Do Race and Ethnicity Impact Hemoglobin A1c Independent of Glycemia?

William H. Herman, M.D., M.P.H.

Abstract

Hemoglobin A1c (HbA1c) is widely used as an index of mean glycemia, a measure of risk for the development of diabetes complications, and a measure of the quality of diabetes care. Emerging literature suggests that, although HbA1c levels change little over time within persons without diabetes, they vary considerably among individuals, suggesting that factors other than glycemia may impact HbA1c. Racial and ethnic differences in HbA1c have been described that do not appear to be explained by differences in glycemia. It is imperative that the nonglycemic factors that affect HbA1c be more clearly defined. Even more important, it must be determined whether differences among individuals or groups correlate with susceptibility to complications or merely reflect variation in hemoglobin glycation.

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Introduction

Hemoglobin is found in erythrocytes and is critical for normal oxygen delivery to tissues. It is a tetramer composed of two α (141 amino acid) and two β (146 amino acid) globin chains that bind four heme groups, each containing one iron atom. Glycated hemoglobin or hemoglobin A1 represents a post-translational modification of hemoglobin A formed by the covalent attachment of glucose or other sugars to hemoglobin. Hemoglobin A1c (HbA1c) is formed by glucose attachment to the N-terminal valine of the β globin chain.

A number of clinically important hemoglobin abnormalities have been described, including the structural hemoglobinopathies, in which mutations alter the amino acid sequence of the globin chain and alter its physiologic properties; the thalassemia syndromes, in which mutations impair globin biosynthesis and result in hypochromia and microcytosis; and the acquired

hemoglobinopathies, in which hemoglobin is chemically altered. Examples of acquired hemoglobinopathies include carboxyhemoglobin, associated with cigarette smoking; carbamylhemoglobin, associated with uremia; acetylhemoglobin, associated with the consumption of large amounts of aspirin; and methemoglobin, associated with carbon monoxide poisoning.

In 1958, Allen and colleagues first described hemoglobin A1 as one of the forms of hemoglobin that could be separated by cation-exchange chromatography.¹ In 1968, Rahbar recognized that HbA1c represented a glycated form of hemoglobin that was increased in diabetes,² and in 1976, Koenig and colleagues suggested that, because HbA1c is formed slowly and nonenzymatically at a rate directly proportional to the ambient glucose concentration, it might be a useful indicator of glucose tolerance or glucose regulation in diabetes.^{3,4} Currently, HbA1c is widely

Author Affiliation: University of Michigan, Ann Arbor, Michigan

Abbreviations: (ADAG) A1c-derived average glucose, (BMI) body mass index, (DPP) Diabetes Prevention Program, (HbA1c) hemoglobin A1c

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Corresponding Author: William H. Herman, M.D., M.P.H., University of Michigan, 3920 Taubman Center, SPC 5354, Ann Arbor, MI 48109-5354; email address wherman@umich.edu

accepted as a index of mean glycemia, a measure of risk for the development of diabetes complications, and a measure of the quality of diabetes care.

A number of different approaches have been used to measure HbA1c in clinical practice. These include ion-exchange high-performance liquid chromatography, immunoassay, boronate affinity, and enzymatic methods. In an effort to standardize these methods and to reduce the variation among them, the American Association for Clinical Chemistry established the National Glycohemoglobin Standardization Program in 1996.⁵ The purpose was to standardize glycohemoglobin test results to Diabetes Control and Complications Trial equivalent HbA1c values. Each year, the College of American Pathologists assesses the comparability of values within and between methods at HbA1c values.⁵ This initiative has resulted in substantial improvement in the comparability of assay methods and has enhanced reliability and precision. Nevertheless, it is clear that a number of factors, including structural hemoglobinopathies, thalassemia syndromes, and chemical alterations of hemoglobin, can either falsely lower HbA1c test results or raise HbA1c test results independent of glycemia.

Any condition that decreases mean erythrocyte age will falsely lower HbA1c test results regardless of the assay method used.⁶ Examples of such conditions include hemolytic anemia and recovery from acute blood loss. The impact of structural hemoglobinopathies and thalassemia syndromes on HbA1c test results depend on the pathologic processes involved and the assay method employed. Hemoglobin S trait, which effects approximately 8% of African Americans, hemoglobin C trait, which effects approximately 3% of African Americans, and hemoglobin E trait, which effects 10% to more than 50% of Southeast Asians in California, are all reported to effect some HbA1c assay methods.⁷ Elevated hemoglobin F, which is associated with thalassemia syndromes, also effects some assay methods.⁷ Uremia, hyperbilirubinemia, hypertriglyceridemia, chronic alcoholism, chronic ingestion of salicylates, vitamin C ingestion, and opiate addiction have all been reported to interfere with some assay methods, falsely increasing results.⁷ In some assays, vitamin C and vitamin E ingestion have also been reported to falsely lower HbA1c test results.⁷ Iron deficiency, which effects up to 20% of menstruating women⁸ and many pregnant women, has been reported to increase HbA1c test results by altering the structure of the hemoglobin molecule and making it easier to glycate.⁹ When interferences are recognized, alternative forms of testing, such as glycated serum

protein testing (fructosamine or glycated albumin), may be employed to assess glycemia. Unfortunately, factors affecting the accuracy of HbA1c measurement may not be recognized clinically.

The American Diabetes Association, the European Association for the Study of Diabetes, and the International Diabetes Federation sponsored a study called the A1c-Derived Average Glucose (ADAG) Study to define the relationship between HbA1c and mean blood glucose.¹⁰ An international group of investigators from ten clinical centers recruited 268 type 1 and 159 type 2 diabetes patients and 80 nondiabetes control patients. Volunteers with conditions that might interfere with measurement of HbA1c, including anemia, reticulocytosis, blood loss and/or transfusions, erythropoietin treatment, hemoglobinopathies, chronic liver or renal disease, and high-dose vitamin C treatment, were excluded. Hemoglobin A1c was measured centrally at baseline and every month for 3 months. In addition, continuous glucose monitoring and 7-point self-monitoring profiles were performed for 2 days every month and 7-point daily self-monitoring of blood glucose was performed 3 days per week. At the end of the study, the mathematical relationship between HbA1c and average glucose was explored. Linear regression analysis between the HbA1c and average glucose values provided good correlation ($R^2 = 0.84$), allowing calculation of an estimated average glucose for HbA1c values. **Table 1** illustrates this relationship. Although the overall relationship was strong, there were mismatches between peripheral blood measurements and HbA1c on an individual subject basis that could lead to erroneous conclusions. (See the study by Chalew and associates in this issue of *Journal of Diabetes Science and Technology*.)

Table 1.
Estimated Average Glucose

HbA1c (%)	mg/dl ^a
5	97 (76–120)
6	126 (100–152)
7	154 (123–185)
8	183 (147–217)
9	212 (170–249)
10	240 (193–282)
11	269 (217–314)
12	298 (240–347)

^a Linear regression estimated average glucose (mg/dl) = $28.7 \times$ HbA1c – 46.7. Data in parentheses are 95% confidence intervals.

Despite recognition of known assay interferences, initiatives to enhance assay standardization, and studies to define the relationship between mean blood glucose and HbA1c, emerging literature suggests that factors other than glycemia may contribute to the variance in HbA1c across individuals. The seminal observation was that, although HbA1c levels change little over time within persons without diabetes, they vary considerably among individuals.^{11–13} Among individuals without diabetes, only approximately one-third of the variance in HbA1c levels is explained on the basis of measures of glycemia.¹¹ This observation suggests that other factors must operate to produce variation in HbA1c levels. Some factors proposed to be associated with variation in HbA1c independent of glycemia include female sex,¹⁴ sex hormones,¹⁵ visceral fat distribution,¹⁶ and genetics.^{17,18}

Racial and ethnic differences in HbA1c have been described in nondiabetes populations that do not appear to be explained by differences in glycemia. Saaddine and coworkers described HbA1c levels among young participants without diabetes in the National Health and Nutrition Examination Survey-3 (NHANES-3) before and after adjusting for age, sex, education, and weight.¹⁹ They found different HbA1c distributions among non-Hispanic white, Mexican American, and non-Hispanic black populations with shifts of the distributions for both Mexican Americans and non-Hispanic blacks to higher levels than those for whites (Figure 1). Eberhardt and colleagues analyzed data from a community-based sample of 3175 adults without diabetes in the South Carolina Cardiovascular Disease Prevention Project.²⁰ After adjusting for age and body mass index (BMI), HbA1c remained 0.3% and 0.4% higher in black men and women with no reported diabetes compared to white men and women with no reported diabetes ($p < .05$). Additional studies comparing HbA1c levels across racial and ethnic groups with type 2 diabetes have generally demonstrated higher HbA1c levels among African Americans, Hispanics, and Asian/Pacific Islanders compared to non-Hispanic whites.²¹ Studies that have compared HbA1c levels across racial and ethnic groups within organized systems of health care and have carefully adjusted for processes of care have demonstrated persistent though attenuated differences in HbA1c.^{22–28}

Hemoglobin A1c has been described by race and ethnicity among patients with impaired glucose tolerance enrolled in the Diabetes Prevention Program (DPP).²⁹ There were 3819 individuals eligible to participate in the DPP who were ≥ 25 years of age and had BMI ≥ 24 kg/m² (≥ 22 kg/m² for Asians). Fasting plasma glucose was

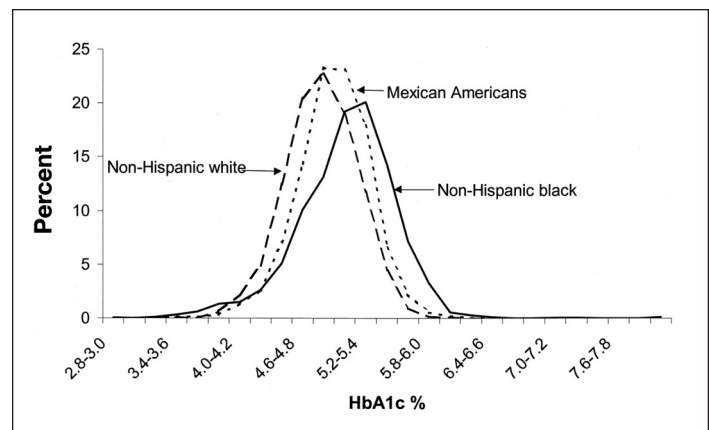


Figure 1. Hemoglobin A1c distribution by ethnicity in U.S. children and young adults ages 5–24 NHANES-3, 1988–1994.

between 95 and 125 mg/dl (<125 mg/dl for American Indians), and 2 h plasma glucose was between 140 and 199 mg/dl. Hemoglobin A1c levels were higher among blacks, Hispanics, American Indians, and Asian Americans compared to whites both before and after using multiple linear regression to adjust for factors that differed among groups and might affect glycemia. These included age, sex, education, marital status, adiposity (BMI and waist circumference), blood pressure, fasting and postload glucose, glucose area under the curve, β -cell function, insulin resistance, and hematocrit. Table 2 summarizes these results. In fully adjusted models, up to 8% of the variance in HbA1c was associated with race or ethnicity, a proportion similar to that explained by age and fasting glucose level.

In another analysis of patients with recent-onset, drug-naïve type 2 diabetes, we found that HbA1c adjusted for age, sex, and BMI was significantly higher among blacks and Hispanics compared to whites, despite similar fasting plasma glucose levels and similar or lower postglucose load glucose levels.³⁰ In another study of type 2 diabetes patients failing oral antidiabetes therapies, it was found that HbA1c levels were significantly higher in African Americans, Hispanics, Asians, and other races and ethnicities compared to whites before and after adjusting for age, gender, BMI, duration of diabetes, oral medication use, mean fasting plasma glucose, mean postprandial glucose, insulin resistance, and β -cell function.³¹ In the ADAG Study, although there were no significant differences in the slope or intercept for the regression equations for any of the subgroup comparisons, including age, sex, type of diabetes, or smoking status, there was a suggestion ($p = .07$) that the regression line was different for African Americans compared to Caucasians, such that for a given

Table 2.
Differences in HbA1c by Race and Ethnicity among Patients with Impaired Glucose Tolerance in the Diabetes Prevention Program

	White	Black	Hispanic	American Indian	Asian
<i>n</i>	2117	752	609	174	167
HbA1c (%)	5.80 ± 0.44	6.19 ± 0.59	5.89 ± 0.46	5.96 ± 0.46	5.96 ± 0.45
Adjusted HbA1c (%) ^a	5.78	6.18	5.93	6.12	6.00

^a Adjusted for age; sex; education; marital status; BMI; waist circumference; blood pressure; fasting, 30 min, and 120 min plasma glucose; glucose area under the curve; fasting insulin; corrected insulin response; homeostasis model assessment of insulin resistance; and hematocrit. All differences $p < .0001$ adjusted for multiple comparison. Results were not changed using common criteria for BMI and fasting plasma glucose.

mean glucose level, African Americans had a slightly higher HbA1c level.¹⁰ A study in children with type 1 diabetes found substantial variation in the relationship between mean glucose and HbA1c and questioned the advisability of transforming HbA1c into calculated mean glucose values.³²

The reasons for these racial and ethnic differences in HbA1c remain to be explained. Heritable differences in intraerythrocyte 2, 3-diphosphoglycerate, which catalyzes production of HbA1c,³³ and intraerythrocyte fructosemine 3-kinase, which deglycates intracellular fructosemines,³⁴ have been proposed to account for interindividual and intergroup variation in HbA1c. Their role has not, however, been established. Indeed, since fructosemine 3-kinase mediates deglycation at amine side chains such as on lysine, it is unlikely to affect HbA1c, which involves glycation of an N-terminal valine.

Enthusiasm is growing for greater use of HbA1c for both screening and diagnosis of diabetes.³⁵ Although HbA1c provides an integrated measure of glycemia that is less susceptible to short-term modulation than fasting glucose and is useful for tracking therapy within individuals with diabetes, it is imperative that the nonglycemic factors that affect assays be more clearly defined. Additional studies are needed to determine which factors account for intraindividual variability in HbA1c and for differences among racial and ethnic groups. Even more important, it must be determined whether differences among individuals and groups correlate with susceptibility to complications or merely variation in hemoglobin glycation.

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